δ-Tocotrienol feeding modulates gene expression of EIF2, mTOR, protein ubiquitination through multiple-signaling pathways in chronic hepatitis C patients

Asaf A. Qureshi 1*, Dilshad A. Khan 2, Shahida Mushtaq 2, Shui Qing Ye 1,3,4, Min Xiong 1,3 and Nilofer Qureshi 1,5

Abstract

Background: δ-Tocotrienol is a naturally occurring proteasome inhibitor, which has the capacity to inhibit proliferation and induce apoptosis in several cancer cells obtained from several organs of humans, and other cancer cell lines. Moreover, results of plasma total mRNAs after δ-tocotrienol feeding to hepatitis C patients revealed significant inhibition in the expression of pro-inflammatory cytokines (TNF-α, VCAM1, proteasome subunits) and induction in the expression of ICAM1 and IFN-γ after post-treatment. This down-regulation of proteasome subunits leads to autophagy, apoptosis of immune cells and several genes. The present study describes RNA-sequence analysis of plasma total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients on gene expression regulated by proteasome.

Methods: Pooled specimens of plasma total mRNAs of pre-dose versus post-dose of δ-tocotrienol treatment of hepatitis C patients were submitted to RNA-sequence analyses. The data based on > 1 and 8-fold expression changes of 2136 genes were uploaded into “Ingenuity Pathway Analyses (IPA)” for core analysis, which describes possible canonical pathways, upstream regulators, diseases and functional metabolic networks.

Results: The IPA of “molecules” indicated fold change in gene expression of 953 molecules, which covered several categories of biological biomarkers. Out of these, gene expression of 220 related to present study, 12 were up-regulated, and 208 down-regulated after δ-tocotrienol treatment. The gene expression of transcription regulators (ceramide synthase 3 and Mohawk homeobox) were up-regulated, and gene expression of 208 molecules were down-regulated, involved in several biological functions (HSP90AB1, PSMC3, CYB5R4, NDUFB1, CYP2R1, TNFRF1B, VEGFA, GPR65, PIAS1, SFPQ, GPS2, EIF3F, GTPBP8, EIF4A1, HSPA14, TLR8, TUSSC2). IPA of “causal network” indicated gene regulators (676), in which 76 down-regulated (26 s proteasomes, interleukin cytokines, and PPAR-ligand-PPAR-Retinoic acid-RXRα, PPAR-ligand-PPAR-Retinoic acid-RARα, IL-21, IL-23) with significant P-values. The IPA of “diseases and functions” regulators (85) were involved with cAMP, STAT2, 265 proteasome, CSF1, IFNγ, LDL, TGFA, and microRNA-155-5p, miR-223, miR-21-5p. The IPA of “upstream analysis” (934) showed 57 up-regulated (mainly 38 microRNAs) and 64 gene regulators were down-regulated (IL-2, IL-5, IL-6, IL-12, IL-13, IL-15, IL-17, IL-18, IL-21, IL-24, IL-32), interferon β-1a, interferon γ, TNF-α, STAT2, NOX1, prostaglandin J2, NF-kB, 1kB, TCF3, and also miRNA-15, miRNA-124, miRNA-218-5P with significant activation of Z-Score (P < 0.05).

Conclusions: This is first report describing RNA-sequence analysis of δ-tocotrienol treated plasma total mRNAs obtained from chronic hepatitis C patients, that acts via multiple-signaling pathways without any side-effects. These studies may lead to development of novel classes of drugs for treatment of chronic hepatitis C patients.

Keywords: δ-Tocotrienol, Chronic hepatitis C, RNA-sequence, Gene expression of biomarkers, Causal network, Diseases and functions, Up-stream regulators, Canonical pathways

* Correspondence: qureshia@umkc.edu
1Department of Biomedical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA

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Background
We have recently reported that δ-tocotrienol is a potent anti-cancer agent (liver, pancreas, prostrate, breast cancer cell lines, Hela, melanoma, B lymphocytes and T-cells), and also a modulator of proteasome function, as compared to other outstanding proteasome inhibitors (thiostrepton, 2-methoxyestradiol, and quercetin) [1]. Moreover, plasma total mRNAs obtained from δ-tocotrienol treated hepatitis C patients showed significant inhibition in the expression of pro-inflammatory cytokines (TNF-α and VCAM-1), and induction in expression of ICAM-1, IFN-γ, whereas proteasome subunits X, Y, Z, LMP7, LMP2, LMP10 (22–44%) were significantly inhibited compared to pre-dose values, and this down-regulation of proteasome subunits leads to autophagy and apoptosis of cells [1]. The present study is an extension of these findings to study the effect of δ-tocotrienol (Fig. 1) treatment of chronic hepatitis C patients in their plasma mRNAs using RNA-Sequencing by Ingenuity Pathway Analysis (IPA). The viral infection with hepatitis C is responsible for a vast majority of chronic hepatitis cases over 180 million people worldwide, which is further supported by epidemiological and clinical studies have also demonstrated a causative role of viral infection of hepatitis C in the development of hepatocellular carcinoma [2]. These figures are alarming, as patients currently asymptomatic with relatively mild disease may eventually progress to complications of chronic liver diseases, like cirrhosis, and hepatocellular carcinoma [3]. The mechanisms of liver disease are not fully understood.

The mechanisms that contribute to the pathogenesis of hepatitis virus-related liver infections are diverse and very complex. Investigation of altered cellular mechanisms through gene profiling techniques has improved the clear understanding of various disease processes and development of novel therapeutic targets [4]. Earlier, techniques applied for studying gene expression profiling included microarrays, which analyzes quantitative expression of thousands of genes, and time consuming real-time PCR assays that gives only small number of expression of genes. These tools have been used previously for identification of differentially expressed genes in hepatitis C virus associated cirrhosis and carcinoma [5]. In summary, these changes in gene expression were associated with immune response, fibrosis, cellular growth, proliferation, and apoptosis [5–7]. Nowadays, similar estimation carried out by RNA-sequence procedure, which will provide very accurate gene expression of several virus important biological functions and biomarkers.

The genotype hepatitis C is an important determinant of the response to treatment, and differences found in clinical outcomes of the disease with respect to infection of various genotypes [6–8]. The genotype 3 is the most prevalent genotype around the world compared to other genotype infection [8]. In the present study we will identify altered cellular processes in chronic hepatitis C patients after treatment with δ-tocotrienols. The main purpose of this preliminary study was to isolate plasma total mRNAs from a few participants after δ-tocotrienol treatment of chronic hepatitis C patients, and to carry out RNA-sequence analysis, which quantified mRNA expression of a large number of genes in pooled specimens of pre-dose versus post-dose of δ-tocotrienol treatment of chronic hepatitis C patients. The gene expression data was analyzed by “Ingenuity Pathway Analysis”, which would reveal the cellular and biological mechanisms at the molecular level in plasma total mRNAs obtained from chronic hepatitis C patients.

Methods
Materials
DeltaGold 125 mg softgels from annatto seeds (typical composition 90% δ-tocotrienol and 10% γ-tocotrienol) were supplied by American River Nutrition, Inc. (Hadley,
Impact of δ-tocotrienol in chronic hepatitis C patients
The study was carried out in Pakistan Ordinance Factory (POF) Hospital, Wah Cantonment, Rawalpindi, Pakistan; in collaboration with department of biomedical Sciences, University of Missouri-Kansas City, MO, USA. The study protocol was registered (IRB # 129–2015) was approved by Institutional Review Board of POF, Rawalpindi, Pakistan. The study was carried out under a FDA approved IND number 36906. The hepatitis C antibody test was purchased from Sigma Chemical Co., St. Louis, USA. The second diagnosing hepatitis C test is RNA PCR test was obtained from the EDTA treated fresh whole blood by using total RNA purification kit # 17200 (NORGEN Biotech Corporation, Thorold, ON, Canada).

RNA-Sequence Analyses of plasma total RNAs obtained from EDTA treated whole blood after feeding δ-tocotrienol for 6-weeks to hepatitis C patients
The details of study design, inclusion/exclusion criteria, experimental design, and physical characteristics of hepatitis C patients were same as reported [1]. In short, the total mRNA was extracted from plasma of EDTA treated fresh whole blood of each hepatitis C patients (n = 14) fed δ-tocotrienol (500 mg/d) for 6 weeks by total RNA purification kit (NORGEN Biotech Corporation, Thorold, ON, Canada). The purity of total RNAs (stored – 80 °C) was estimated by the ratios of 260/280 (2.02–2.08) of all samples, which was determined using Thermo Scientific NanoDrop 1000 Spectrophotometer. The mRNAs samples from Pakistan were brought in person (by Dr. Dilshad A. Khan in dry ice to avoid any degradation of RNAs) to UMKC, Medical School after approval by (Compliance officer Mr. Christopher Winders, and Chemical/Biological Safety officer Mr. Mike Philips) members of University of Missouri Kansas City institutional review board.

The results of most important cytokines and other biomarkers associated with the present investigation were estimated by real-time RT-PCR by using plasma total RNAs purified from pre-dose versus post-dose samples after feeding δ-tocotrienol for 6-weeks to chronic hepatitis C patients has been published recently [1], therefore present manuscript lacks in vitro estimations of RT-PCR data. The same plasma total RNAs were used in the present study.

The RNA-Sequence analyses were carried out at Division of Experimental and Translational Genetics, Children’s Mercy Hospital, Kansas City, MO. Five randomized samples selected of total RNAs of hepatitis C patients, and combined. Total mRNAs of combined samples were purified by Biostic Blood Total RNA Isolation Kit (MOBIO Laboratories, Inc). The purified total mRNAs were further purified and concentrated to 10.0 μl by using by Gene Jet RNA Clean up and Concentration Micro Kit (Thermo Scientific, EU, Lithuania). The purity of these RNAs was further determined in the Division of Experimental and Translational Genetic & Core of Omic Research (The Children Mercy Hospital, Kansas City, MO) by their own instruments for quality control and quantity of each sample to make sure that each sample is up to standard before putting into a NGS run. The concentrated total mRNAs of each set was converted to cDNA, and total RNA-Seq carried out. Gene expression level and fold change (post vs pre-dose) of FPKM were calculated at > 1, > 2, or > 5 levels at 2-fold, 4-fold, and 8-fold after filtering several million fold up-regulated and down-regulated genes (Table 1).

Statistical analyses
These data were analyzed by IPA program of treatment-mediated effects as post-dose versus pre-dose. The statistical significance level was set at 5% (P < 0.05).

Results
Genome-wide profiling experiment of plasma mRNAs obtained from pre-dose and post-dose δ-tocotrienol treatment of hepatitis C patients
The RNA-Sequence analysis was based on FPKM >1 and 8-fold change of 2136 genes (0 values replaced with 0.001; Table 1) ratios of post-dose over pre-dose treatment of δ-tocotrienol to hepatitis C patients were uploaded into “Ingenuity Pathway Analyses (IPA)” for core analysis (Ingenuity Systems, Redwood City, CA). The various genes associated with different biological

| # | RNA-Seq expression unit | Number of genes | Genes based on 2-fold | Genes based on 4-fold | Genes based on 8-fold |
|---|------------------------|-----------------|----------------------|----------------------|----------------------|
| 1 | FPKM > 1               | 12614           | 9480                 | 5369                 | 2136                 |
| 2 | FPKM > 2               | 7426            | 1366                 | 696                  | 527                  |
| 3 | FPKM > 5               | 3233            | 379                  | 285                  | 268                  |

1The gene expression level and fold change (post-dose vs pre-dose) of FPKM were calculated at more than 1, 2, or 5 at 2-fold, 4-fold, and 8-fold after filtering million-fold up-regulation and down-regulation. The RNA-seq analyses data based on FPKM >1 and 8-fold change of 2136 genes (0 values were replaced with 0.001) of ratios of post-dose over pre-dose treatment of δ-tocotrienol to hepatitis C patients was submitted into “Ingenuity Pathway Analyses (IPA)” for core analysis (Ingenuity Systems, Redwood City, CA)
functions and biomarkers are from “Ingenuity Knowledge Base” generated molecular networks, according to biological as well as molecular functions. These include canonical pathways, upstream regulatory analysis, and disease-based functional network, which helped discovering the list of several biomarkers. The core analysis was carried out with the settings of indirect and direct relationship between focused molecules based on experimentally observed data and human databases in the “Ingenuity Knowledge Base” were considered as the data sources in these analyses and pathways.

“Molecules” affected by δ-tocotrienol feeding to hepatitis C patients

The IPA of “molecules section” indicates fold changes in gene expression of 953 genes, which covered several categories of biological biomarkers, which are presented in the heat-map of this section (Fig. 2). Out of these, expression of 220 genes were related to present study, and only 12 genes were up-regulated (Table 2), and remaining 208 genes of various biomarkers were down-regulated after δ-tocotrienol treatment (Table 3). The ceramide synthase 3 and Mohawk homeobox were only two up-regulated genes involved as transcription regulators. The down-regulated gene expression of 208 molecules are involved in several biological functions (Additional file 1: Table S1, Additional file 2: Table S2 and Additional file 3: Table S3). The functions of these regulators are ATPase Na⁺/K⁺ transporting subunit α1, apolipoprotein B, proteasome 26S subunits, NADH ubiquinone oxidoreductase subunits B1, B9, cytochrome b5 reductase 4, autophagy related 4−5, cytochrome P450 family, TNF receptor superfamily 1B, RAS P21 protein activator 2, ubiquitin conjugating enzyme B2 J1, several other types of ubiquitin proteasome subunits, and protein inhibitor of activated STAT1 (Table 3). Similarly, gene regulator of G-protein signaling 2, nuclear factor of activated T-cells 2 interacting protein, TNF-α induced protein 8, C-X-C motif chemokines ligand 1, RNA polymerase II subunit H, tumor suppressor candidate 2, splicing factor 3b subunit 5, and several miRNAs (877, 1250, 140), RNAs, tRNAs are reported in Table 3. The summary of most important down-regulated biomarkers are HSP90AB1, IL-16, autophagy, TNFSF1B, VEGFA, NFIL3, UBP1, USP25, RASA3, USP15, UBE4A, UBE19, PSMD3, IL-27RA, SCP2, IFNGR1, ID2, TUSC2, IL-1R2, IL-18RP, IRF2, PCNA1250,77,40 and several tRNAs (Table 3).

“Causal Networks” affected by δ-tocotrienol feeding to hepatitis C patients

The down-regulation of several biomarkers of “causal network” of IPA of RNA samples obtained after treatment with δ-tocotrienol of chronic hepatitis C patients is described in Tables 4 and 5.

There were 676 gene regulators identified in this section, and only 98 regulators were associated with present study, indicating significant P-values for all regulators (Tables 4 and 5). The fold change gene expression of 24 was up-regulated (Table 4) and 74 down-regulated (Table 5). This section includes down-regulated gene expression of 26S proteasomes, interleukin cytokines, and PPAR-ligand-PPAR-Retinoic acid-RXRα, PPARγ-ligand-PPARγ-Retinoic acid-RARα, IL-7R, CD80, IRS, IL-2, IL-2RG, IL-5, IL-15, IL-21,
IL-23 and several types of microRNAs (miRNAs) as shown in Table 5. The activation Z-Score, \( P \)-values, network bias-corrected and causal network values were in descending order of all these gene biomarkers (Tables 4 and 5).

“Diseases and functions” affected by \( \Delta \)-tocotrienol feeding to hepatitis C patients

The IPA of RNAs obtained from effect of \( \Delta \)-tocotrienol treatment of chronic hepatitis C patients on relative percentage relationship of gene regulators (70) of “diseases and functions” reported in Table 6. In this section, percentage relationships of main regulators were AP1, cAMP, EIF2AK2 2RL1, IL-17A, IL-1RN, KITLG, miRNA-155-5p, STAT2 (48%; 43/90), 26S proteasome, CSF1, IFNG, IL-17A, IRF4, LDL, RELA, TGFA (43%; 17/40); mir-223 (0%; 0/2), IL-15 (100%; 1/1), IL-17A (0%; 0/1), and miR-21-5p (100%; 1/1) (Table 6). The consistency score of these regulators varied from 1.73 ~ 36.34, total regulars (1–9), total node (5–57), diseases and functions total varied 1–10 as shown in Table 6.

“Upstream analysis” affected by \( \Delta \)-tocotrienol feeding to hepatitis C patients

The most interesting results of present IPA was “upstream analysis” of \( \Delta \)-tocotrienol treated hepatitis C patients. There were 934 gene regulators identified in this section. The 57 genes regulator correspond to present study were up-regulated (Table 7), and 64 gene regulators down-regulated (Table 8). There were several miRNAs (38), which were up-regulated and remaining other important biomarkers gene were down-regulated (Table 8). The activation Z-Scores (3.79–1.26) and \( P \)-values (5.39E-8 – 1.26) were significant from each biomarkers. The down-regulated biomarkers included several cytokines (IL-2, IL-5, IL-6, IL-7, IL-12, IL-13, IL-15, IL-17, IL-17A, IL-18, IL-21, IL-24, IL-27, IL-32), as well as miRNA-15, miRNA-124, miRNA-218-5P, interferon \( \beta \)-1a, interferon \( \gamma \), TNF-\( \alpha \), STAT2, NOX1, prostaglandin J2, NF-\( \kappa \)B, I\( \kappa \)B, and TCF3 (transcription regulator), with significant activation Z-Score (−4.56–2.531), and \( P \)-values were 9.17–14.00; \( P < 0.05 \), respectively (Table 8).

“Diseases or functions annotation” affected by \( \Delta \)-tocotrienol feeding in hepatitis C patients

The effect of \( \Delta \)-tocotrienol on gene expression in “diseases or functions annotation” of IPA of mRNAs sample of chronic hepatitis C patients resulted in determining 500 types of diseases and functions. Out of these 11 type genes of diseases and functions were up-regulated, while 49 were down regulated (Table 9A and B). The gene expression of 49 were down-regulated after \( \Delta \)-tocotrienol treatment of chronic hepatitis C patients. These genes are involved in cellular development, cellular growth, proliferation hematolog, infectious diseases, cell-to-cell signaling/interaction, cardiovascular disease, antimicrobial response, cell morphology, inflammatory response, neurological disease, humoral immune response, free radical scavenging, immunological diseases, lipid metabolism, gene expression, cancer, RNA post-transcriptional modification and many other diseases as outlined in Table 9B.

The results described so far are summarized in Table 10. The data were divided into 12 categories, each

### Table 2 Effect of \( \Delta \)-tocotrienol on up-regulation of fold change gene expression of “Molecules” section (12) of IPA analysis in hepatitis C patients

| # | Symbol | Entrez Gene Name | Expr Fold Change | Type(s) |
|---|---|---|---|---|
| 1 | HIST1H2AD | histone cluster 1 H2A family member d | 1804955.068 | other |
| 2 | HHIPL2 | HHIP like 2 | 28.710 | other |
| 3 | RPP38 | ribonuclease P/MRP subunit p38 | 24.946 | enzyme |
| 4 | CERS3 | ceramide synthase 3 | 19.082 | transcription regulator |
| 5 | HBG1 | hemoglobin subunit gamma 1 | 17.945 | other |
| 6 | MT-TQ | tRNA | 14.252 | other |
| 7 | AKR1D1 | aldo-keto reductase family 1 member D1 | 14.056 | enzyme |
| 8 | TSPAN15 | tetraspanin 15 | 11.523 | other |
| 9 | HBG2 | hemoglobin subunit gamma 2 | 11.413 | other |
| 10 | MKX | mohawk homeobox | 9.573 | transcription regulator |
| 12 | P4HA3 | prolyl 4-hydroxylase subunit alpha 3 | 8.686 | enzyme |
Table 3 Effect of δ-tocotrienol on down-regulation of fold change gene expression of "Molecules" section (64) of IPA analysis in hepatitis C patients

| #  | Symbol     | Entrez Gene Name                      | Expr Fold Change | Type(s)                     |
|----|------------|---------------------------------------|------------------|-----------------------------|
| 1  | ATP1A1     | ATPase Na+/K+ transporting subunit alpha 1 | -8.014           | transporter                 |
| 2  | HSP90AB1   | heat shock protein 90 alpha family class B member 1 | -8.049           | enzyme                      |
| 3  | APOBEC3A   | apolipoprotein B mRNA editing enzyme catalytic subunit 3A | -8.163           | enzyme                      |
| 4  | CXCR2      | C-X-C motif chemokine receptor 2       | -8.208           | G-protein coupled receptor  |
| 5  | IL16       | interleukin 16                         | -8.239           | cytokine                    |
| 6  | PSMC3      | proteasome subunit, ATPase 3           | -8.346           | transcription regulator     |
| 7  | NDUFB9     | NADH:ubiquinone oxidoreductase subunit B9 | -8.354           | enzyme                      |
| 8  | CYB5R4     | cytochrome b5 reductase 4              | -8.367           | enzyme                      |
| 9  | AGT3       | autophagy related 3                    | -8.367           | enzyme                      |
| 10 | CREB1      | cAMP responsive element binding protein 1 | -8.452           | transcription regulator     |
| 11 | NDUFAB1    | NADH:ubiquinone oxidoreductase subunit B1 | -8.566           | enzyme                      |
| 12 | PDE3B      | phosphodiesterase 3B                   | -8.568           | enzyme                      |
| 13 | IGF2R      | insulin like growth factor 2 receptor  | -8.66            | transmembrane receptor      |
| 14 | CYP2R1     | cytochrome P450 family 2 subfamily R member 1 | -8.682           | enzyme                      |
| 15 | NDUFA11    | NADH:ubiquinone oxidoreductase subunit A11 | -8.686          | enzyme                      |
| 16 | IGSF6      | immunoglobulin superfamily member 6    | -8.712           | transmembrane receptor      |
| 17 | TNFRSF1B   | TNF receptor superfamily member 1B     | -8.746           | transmembrane receptor      |
| 18 | PRPF18     | pre-mRNA processing factor 18          | -8.777           | transporter                 |
| 19 | SERP1      | stress associated endoplasmic reticulum protein 1 | -8.872         | other                       |
| 20 | UBE2J1     | ubiquitin conjugating enzyme E2 J1     | -8.933           | enzyme                      |
| 21 | VEGFA      | vascular endothelial growth factor A    | -9.027           | growth factor               |
| 22 | GYS1       | glycogen synthase 1                    | -9.054           | G-protein coupled receptor  |
| 23 | GPRA       | G protein-coupled receptor 65          | -9.105           | transcription regulator     |
| 24 | ILF2       | interleukin enhancer binding factor 2  | -9.201           | transcription regulator     |
| 25 | OSBPL11    | oxysterol binding protein like 11      | -9.31            | peptidase                   |
| 26 | PSMAS       | proteasome subunit alpha 5             | -9.326           | transcription regulator     |
| 27 | PIAS1      | protein inhibitor of activated STAT 1   | -9.341           | enzyme                      |
| 28 | TRAF7      | TNF receptor associated factor 7        | -9.447           | other                       |
| 29 | COX14      | COX14, cytochrome c oxidase assembly factor | -9.456         | enzyme                      |
| 30 | RPS26      | ribosomal protein S26                  | -9.469           | other                       |
| 31 | SFQ        | splicing factor proline and glutamine rich | -9.515          | transcription regulator     |
| 32 | ATP4       | activating transcription factor 4       | -9.552           | other                       |
| 33 | PECAM1     | platelet and endothelial cell adhesion molecule 1 | -9.56            | transcription regulator     |
| 34 | GPS2       | G protein pathway suppressor 2          | -9.66            | enzyme                      |
| 35 | NFIL3      | nuclear factor, interleukin 3 regulated | -9.668           | transcription regulator     |
| 36 | PSMB8      | proteasome subunit beta 8              | -9.709           | peptidase                   |
| 37 | UBP1       | upstream binding protein 1 (LBP-1a)     | -9.718           | transcription regulator     |
| 38 | RAP2C      | RAP2C, member of RAS oncogene family    | -9.792           | enzyme                      |
| 39 | PRF1       | progesterone immunomodulatory binding factor 1 | -9.876         | other                       |
| 40 | USP25      | ubiquitin specific peptidase 25         | -9.911           | peptidase                   |
| 41 | FRS2       | fibroblast growth factor receptor substrate 2 | -9.962          | kinase                      |
| 42 | PSMB4      | proteasome subunit beta 4               | -10.119          | peptidase                   |
category has 5 topics (total 60), and out of these 60 topics, only 13 topics were further investigated in detail for their functions related to present studies. For example, the “diseases and disorder” category (III) includes infectious diseases, immunological diseases, cancer, and organismal injury/abnormalities and tumor morphology (Table 10). The “molecular and cellular functions” category (IV) includes cellular development, cellular growth and proliferation, death/survival, cell-to-cell signal ligand interaction and cellular function and maintenance. Table 10 also includes a list of expression log ratio of 10 up-regulated genes (SNORD15A, SNORA32, SNORA56, SNORA9, SNORA3B, SNORA3A, HIST1H2AD, LINC00305, HHIPL2), and 10 down-regulated genes (HMGN1P3, SNHG25, SNORA67, RPL17-C18orf32, ISY1-RAB43, ARHGEF18, KLR44-KLRK1/KLRK1, HIST1H3J, MTHFS, SNORA16A) were related to present investigation. At the end, out of 360 “canonical pathways” of IPA of total mRNAs samples of effects of δ-tocotrienol treatment to hepatitis C patients, 33 pathways are selected, which are associated with various signaling and biomarkers relative to present results (Table 11). The heat map (Fig. 2) also depicts same diseases and functions as outlined in Tables 9A, B and 10.

Table 3 Effect of δ-tocotrienol on down-regulation of fold change gene expression of “Molecules” section (64) of IPA analysis in hepatitis C patients (Continued)

| # | Symbol | Entrez Gene Name                                      | Expr Fold Change | Type(s)       |
|---|--------|-------------------------------------------------------|------------------|---------------|
| 44| USP15  | ubiquitin specific peptidase 15                       | -10.16           | peptidase     |
| 45| UBA52  | ubiquitin A-52 residue ribosomal protein fusion product 1 | -10.176          | enzyme        |
| 46| UBE4A  | ubiquitination factor E4A                              | -10.189          | enzyme        |
| 47| GTPBP8 | GTP binding protein 8 (putative)                       | -10.19           | other         |
| 48| USP19  | ubiquitin specific peptidase 19                        | -10.713          | peptidase     |
| 49| TNFAIP8| TNF alpha induced protein 8                            | -10.974          | other         |
| 50| HSPA14 | heat shock protein family A (Hsp70) member 14          | -10.978          | peptidase     |
| 51| TLR8   | toll like receptor 8                                  | -11.975          | transmembrane receptor |
| 52| IL27RA | interleukin 27 receptor subunit alpha                  | -12.004          | transmembrane receptor |
| 53| SCP2   | sterol carrier protein 2                              | -13.672          | transporter   |
| 54| IFNGR2 | interferon gamma receptor 2                            | -13.844          | transmembrane receptor |
| 55| ID2    | inhibitor of DNA binding 2, HLH protein                | -14.133          | transcription regulator |
| 56| TUSC2  | tumor suppressor candidate 2                           | -15.922          | other         |
| 57| IL2R   | interleukin 2 receptor subunit gamma                   | -16.787          | transmembrane receptor |
| 58| IL1R   | interleukin 1 receptor type 2                          | -19.547          | transmembrane receptor |
| 59| IRF2   | interferon regulatory factor 2                         | -22.655          | transcription regulator |
| 60| PTG52  | prostaglandin-endoperoxide synthase 2                  | -25.841          | enzyme        |
| 61| mir-877| microRNA 877                                          | -4497.07         | microRNA      |
| 62| mir-1250| microRNA 1250                                         | -4755.79         | microRNA      |
| 63| mir-140| microRNA 140                                          | -5688.259        | microRNA      |
| 64| KLR44-KLRK1/KLRK1 | killer cell lectin like receptor K1 | -1565687.642 | transmembrane receptor |

Discussion

The fold-change gene expression data analyzed by Ingenuity Pathway Analysis describes cellular and biological mechanisms at the molecular level on the effect of δ-tocotrienol in chronic hepatitis C patients. It involves metabolic and cellular processes, mainly associated with catalytic activity of structural molecules. It also reveals an insight of correlation of signaling pathways and transcriptional factors, and subsequently describes inhibition or activation of anti- and pro-inflammatory genes. The results of these functional genomics produced a huge amount of data analyzed by biological networks using differentially gene expression after treatment with δ-tocotrienol to chronic hepatitis C patients. It predicts possible canonical pathways, upstream regulators, diseases and functional metabolic networks. The differential gene expressions of several biological functions illustrated in the heat map is shown in Fig. 2.

The present data revealed that genes responsible for replication of virus, infection by RNA viruses, infection of tumor cell lines, HIV infection and replication of influenza virus were all down-regulated, while cell death processes were all up-regulated. Moreover, as mentioned
Table 4 Effect of δ-tocotrienol on up-regulation (24) of fold change gene expression in "causal networks" section of IPA analysis in hepatitis C patients

| #  | Master Regulator | Molecule Type | Part. regulators\(^1\) | Depth | Pred Acti State\(^2\) | Act. Z-Score\(^3\) | P-Value Over\(^4\) | Network Bi-Corr\(^5\) | Causal Net\(^6\) | Target-Con-Re\(^7\) |
|----|-----------------|---------------|-------------------------|-------|-----------------------|-------------------|------------------|-------------------|-----------------|------------------|
| A  | Up-regulation   |               |                         |       |                       |                   |                  |                   |                 |                  |
| 1  | leuprolide      | biologic drug | 26s Proteasome,AKT1     | 3     | Activated             | 2.104             | 8.5E-10          | 0.0032            | 217 (71)        | 69               |
| 2  | HLA-DR complex  | complex       | 26s Proteasome,ARAATR   | 3     | Activated             | 5.458             | 3.44E-09         | 0.0145            | 260 (87)        | 86               |
| 3  | PRDX1 enzyme    | 26s Proteasome,ABL1 | 3     | Activated             | 7.084             | 1.73E-08         | 0.0047            | 250 (76)        | 75               |
| 4  | alefacept       | bilologic drug | alefacept, AP1,CD2      | 3     | Activated             | 2.278             | 2.50E-07         | 0.0222            | 85 (20)         | 20               |
| 5  | juglone         | chemical taxicant | CASP3,FO5,juglone,JUN | 2     | Activated             | 2.449             | 0.00000682       | 0.0272            | 54 (9)          | 9                |
| 6  | mir-148         | microRNA      | mir-148                 | 1     | Activated             | 2.000             | 0.00103          | 0.0055            | 4 (1)           | 1                |
| 7  | 26s Proteasome complex | 26s Proteasome | 1     | Activated             | 2.840             | 0.00167          | 0.0476            | 15 (1)          | 1                |
| 8  | mir-122         | microRNA      | mir-122                 | 1     | Activated             | 3.317             | 0.00189          | 0.022             | 11 (1)         | 1                |
| 9  | mir-19          | microRNA      | mir-19                  | 1     | Activated             | 2.236             | 0.002            | 0.0185            | 5 (1)           | 1                |
| 10 | mir-9           | microRNA      | mir-9                   | 1     | Activated             | 2.000             | 0.00473          | 0.0208            | 4 (1)           | 1                |
| 11 | IL2RG transmembrane | IL2RG         | 1     | Activated             | 0.000             | 0.00181          | 0.0188            | 8 (1)           | 1                |
| 12 | miR-2682-5p (other miRNAs w/seed AGGC) | mature microRNA | miR-2682-5p (miRNAs) | 1     | Activated             | 1.414             | 0.00584          | 0.0073            | 2 (1)           | 1                |
| 13 | alpha-tocopherol succinate | chemical drug | alpha-tocopherol succinate | 1 | Activated             | 0.000             | 0.00597          | 0.0316            | 4 (1)           | 1                |
| 14 | mir-199         | microRNA      | mir-199                 | 1     | Activated             | 1.732             | 0.00849          | 0.0258            | 3 (1)           | 1                |
| 15 | mir-138         | microRNA      | mir-138                 | 1     | Activated             | 0.000             | 0.0113           | 0.0239            | 2 (1)           | 1                |
| 16 | miR-330-5p (other miRNAs w/seed CUCU) | mature microRNA | miR-330-5p (and other miRNAs w/seed CUCU) | 1 | Activated             | 1.414             | 0.0113           | 0.0209            | 2 (1)           | 1                |
| 17 | mir-326         | microRNA      | mir-326                 | 1     | Activated             | 1.414             | 0.0113           | 0.0191            | 2 (1)           | 1                |
| 18 | mir-32          | microRNA      | mir-32                  | 1     | Activated             | 1.414             | 0.0113           | 0.0304            | 2 (1)           | 1                |
| 19 | LAMP2 enzyme    | LAMP2         | 1                        | 1     | Activated             | 0.000             | 0.0113           | 0.0251            | 2 (1)           | 1                |
| 20 | mir-218         | microRNA      | mir-218                 | 1     | Activated             | 1.732             | 0.0183           | 0.0398            | 3 (1)           | 1                |
| 21 | UBA7 enzyme     | UBA7          | 1                        | 1     | Activated             | 1.414             | 0.0183           | 0.0416            | 2 (1)           | 1                |
| 22 | miR-147a (miRNAs w/seed UUGUGGG) | mature microRNA | miR-147a (other miRNAs) | 1 | Activated             | 1.000             | 0.0448           | 0.0417            | 1 (1)           | 1                |
| 23 | miR-504-5p (other miRNAs w/seed GACCC) | mature microRNA | miR-504-5p (miRNAs) | 1 | Activated             | 1.000             | 0.0448           | 0.0417            | 1 (1)           | 1                |
| 24 | BI 2536         | chemical drug | 26s Proteasome,ABL1     | 3     | Activated             | 1.331             | 2.06E-12         | 0.0034            | 249 (50)        | 49               |

\(^1\)Part. Regulators = Participating Regulators; \(^2\)Pred Acti state = Predicted Activation State; \(^3\)Act. Z-Score = Activation Z-Score; \(^4\)P-Value Over = P-Value Overlap; \(^5\)Network Bi-Corr = Network Bias-Corrected P-Values; \(^6\)Causal Net = Causal Network; \(^7\)Target-Con-Re = Target Connected regulators
| #  | Master Regulator          | Molecule Type                  | Part. regulators | Depth | Pred Acti State | Act. Z-Score | P-Value Over | Network Bi-Corr | Causal Net | Target-Con-Re |
|----|--------------------------|--------------------------------|-----------------|-------|----------------|--------------|--------------|-----------------|------------|---------------|
| 25 | JAK1/2                    | group                          | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -7.511       | 2.54E-14     | 0.0008          | 295 (81)   | 80            |
| 26 | PPAR ligand-PPAR-Retinoic acid-RXR | complex                      | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -4.549       | 3.31E-13     | 0.0131          | 306 (61)   | 60            |
| 27 | LXR ligand-LXR-Retinoic acid-RXR | complex                      | 26s ProteasomeAktAR  | 3     | Inhibited      | -4.815       | 4.17E-13     | 0.0085          | 290 (58)   | 57            |
| 28 | PPARy ligand-PPARy-Retinoic acid-RA Ra | complex                    | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -4.230       | 4.23E-13     | 0.0121          | 306 (66)   | 65            |
| 29 | PXR ligand-PXR-Retinoic acid-RXR | complex                      | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -4.432       | 3.33E-12     | 0.0221          | 294 (58)   | 58            |
| 30 | RAR ligand-RARA-Retinoic acid-RXR | complex                      | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -5.396       | 3.52E-12     | 0.039           | 297 (57)   | 56            |
| 31 | Vegf Receptor             | group                          | 26s ProteasomeABL1,Akt | 3     | Inhibited      | -5.056       | 1.56E-11     | 0.0052          | 276 (93)   | 90            |
| 32 | FXR ligand-FXR-Retinoic acid-RXR | complex                      | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -5.100       | 1.96E-11     | 0.0484          | 291 (56)   | 55            |
| 33 | hydrogen sulfide          | chemical - endogenous mammalian | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -4.222       | 2.15E-11     | 0.0013          | 237 (92)   | 89            |
| 34 | NIX                      | kinase                         | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -3.429       | 8.72E-11     | 0.0375          | 248 (50)   | 45            |
| 35 | CD80                     | transmembrane receptor         | CD28,CD80,JFCJL4  | 2     | Inhibited      | -6.267       | 1.32E-10     | 0.003           | 132 (8)    | 8             |
| 36 | Pdgfria-Pdgfrb           | complex                        | 26s ProteasomeAktAR  | 3     | Inhibited      | -7.878       | 1.37E-10     | 0.0184          | 285 (93)   | 89            |
| 37 | Klr7 (includes others)   | transmembrane receptor         | 26s ProteasomeAktAR  | 3     | Inhibited      | -7.445       | 1.44E-10     | 0.0324          | 291 (93)   | 93            |
| 38 | FLT4                     | transmembrane receptor         | 26s ProteasomeAktAR  | 3     | Inhibited      | -5.020       | 1.46E-10     | 0.0177          | 280 (80)   | 78            |
| 39 | Vegfr dimer              | complex                        | 26s ProteasomeAktAR  | 3     | Inhibited      | -7.071       | 1.59E-10     | 0.0178          | 242 (61)   | 58            |
| 40 | lipopolysaccharide       | chemical drug                  | lipopolysaccharide | 1     | Inhibited      | -7.668       | 2.75E-10     | 0.0045          | 120 (1)    | 1             |
| 41 | TEK                      | kinase                         | 26s ProteasomeADRB2 | 3     | Inhibited      | -4.954       | 3E-10        | 0.0124          | 274 (93)   | 93            |
| 42 | LAT1                     | kinase                         | 26s ProteasomeARF4A | 3     | Activated      | 4.680        | 3.43E-10     | 0.0322          | 250 (56)   | 54            |
| 43 | NYAP1                    | other                          | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -6.264       | 3.54E-10     | 0.0304          | 281 (86)   | 85            |
| 44 | MYO16                    | other                          | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -6.264       | 3.54E-10     | 0.0304          | 281 (86)   | 85            |
| 45 | NYAP2                    | other                          | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -6.264       | 3.54E-10     | 0.0304          | 281 (86)   | 85            |
| 46 | IRS                      | group                          | 26s ProteasomeADRB2 | 3     | Inhibited      | -5.548       | 1.63E-09     | 0.0456          | 269 (77)   | 74            |
| 47 | FAK-Src                  | complex                        | 26s ProteasomeABL1,Akt | 3     | Inhibited      | -6.839       | 2.41E-09     | 0.043           | 273 (90)   | 86            |
| 48 | Ptk                      | group                          | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -2.500       | 2.77E-09     | 0.0425          | 219 (55)   | 50            |
| 49 | G-protein beta           | group                          | 26s ProteasomeADORA2A | 3     | Inhibited      | -5.647       | 3.22E-09     | 0.0309          | 283 (103)  | 99            |
| 50 | ADRA1B                   | G-protein coupled receptor     | 26s ProteasomeADRA1B | 3     | Inhibited      | -6.238       | 4.49E-09     | 0.0406          | 278 (86)   | 85            |
| 51 | IL2                      | cytokine                       | IL2             | 1     | Inhibited      | -4.619       | 8.23E-09     | 0.0004          | 48 (1)     | 1             |
| 52 | propolis                 | biologic drug                  | 26s ProteasomeAktAKT1 | 3     | Inhibited      | -2.829       | 1.78E-08     | 0.0482          | 231 (76)   | 73            |
Table 5 Effect of δ-tocotrienol on down-regulation (74) of fold change gene expression in “causal networks” section of IPA analysis in hepatitis C patients (Continued)

| #  | Master Regulator | Molecule Type | Part. regulators | Depth | Pred Acti State  | Act. Z-Score | P-Value Over   | Network Bi-Corr | Causal Net | Target-Con-Re |
|----|------------------|---------------|-----------------|-------|-----------------|-------------|---------------|----------------|------------|---------------|
| 53 | exenatide        | biologic drug | 26s Proteasome,Akt,AMPK | 3     | -1.432          | 2.36E-08    | 0.0088        | 236 (88)       | 88         |               |
| 54 | imidazole        | chemical - endogenous mammalian | 26s Proteasome,ADORA2A | 3     | 1.091           | 2.79E-08    | 0.05          | 243 (75)       | 70         |               |
| 55 | LETM1            | other         | Akt,AMPK,APP,AR | 3     | -1.023          | 0.0000000069| 0.036         | 215 (64)       | 63         |               |
| 56 | IL-2R            | complex       | IL-2R,IL2RA,IL2RG,JAK1 | 2     | Inhibited       | -3.491      | 0.00000012   | 0.0103         | 84 (14)    | 13            |
| 57 | IL23             | complex       | IL2B,IL23,JAK2,MTOR | 2     | Inhibited       | -7.155      | 0.000000165  | 0.0112         | 80 (9)     | 9             |
| 58 | IL15             | cytokine      | IL15            | 1     | Inhibited       | -2.121      | 0.0000000551 | 0.0009         | 32 (1)     | 1             |
| 59 | TH17 Cytokine    | group         | IL17A,IL21,IL22,TH17 | 2     | Inhibited       | -4.323      | 0.000000813  | 0.0037         | 39 (4)     | 4             |
| 60 | IL4R             | transmembrane receptor | IL4,IL4R,IRS1,IRS2,JAK | 2     | Inhibited       | -4.503      | 0.00000102   | 0.0252         | 75 (13)    | 12            |
| 61 | IL21             | cytokine      | IL21            | 1     | Inhibited       | -2.985      | 0.000000527  | 0.0028         | 22 (1)     | 1             |
| 62 | SATB1            | transcription regulator | SATB1          | 1     | 1.528           | 0.000000669 | 0.0011        | 21 (1)         | 1           |               |
| 63 | cyclosporin A    | biologic drug | cyclosporin A   | 1     | 1.441           | 0.0000108   | 0.0163        | 39 (1)         | 1           |               |
| 64 | IL12RB2         | transmembrane receptor | IL12 (family),IL12RB2 | 2     | Inhibited       | -4.116      | 0.00000233   | 0.0103         | 34 (4)     | 3             |
| 65 | mir-26          | microRNA      | Akt,mir-26      | 2     | 0.192           | 0.0000247   | 0.0126        | 27 (2)         | 2           |               |
| 66 | mir-221         | microRNA      | Akt,mir-221     | 2     | -0.192          | 0.0000247   | 0.0129        | 27 (2)         | 2           |               |
| 67 | IL5             | cytokine      | IL5             | 1     | Inhibited       | -4.914      | 0.00000541   | 0.0136         | 28 (1)     | 1             |
| 68 | ropivacaine     | chemical drug | Akt,NO53,PKc(s) | 2     | -1.029          | 0.0000544   | 0.0289        | 34 (5)         | 4           |               |
| 69 | UCP3            | transporter   | IRS1,IRS2,PKc   | 2     | -1.961          | 0.0000657   | 0.0231        | 26 (4)         | 3           |               |
| 70 | IGF1            | other         | IGF1,Akt,BAD    | 2     | -1.177          | 0.0000657   | 0.0211        | 26 (3)         | 3           |               |
| 71 | IFN Beta        | group         | IFN Beta        | 1     | Inhibited       | -2.138      | 0.00082       | 0.043          | 14 (1)     | 1             |
| 72 | PDGF             | growth factor | PDGF           | 1     | -0.577         | 0.000838   | 0.0044        | 3 (1)          | 1           |               |
| 73 | PARP9           | enzyme        | PARP9          | 1     | Inhibited       | -2.236      | 0.00123       | 0.0073         | 5 (1)      | 1             |
| 74 | PPP1R14B        | phosphatase   | PPP1R14B       | 1     | -1.732         | 0.000162   | 0.005         | 3 (1)          | 1           |               |

1Part. Regulators = Participating Regulators; 2Pred Acti state = Predicted Activation State; 3Act. Z-Score = Activation Z-Score; 4P-Value Over. = P-Value Overlap; 5Network Bi-Corr = Network Bias-Corrected P-Values; 6Target-Con-Re. = Target Connected regulators
| ID | Consistency | Node Total | Total | Regulator Conditions | Regulators | Target | Disease & Functions | Diseases & Functions | Known Regulator-Disease/Function Relationship |
|----|-------------|------------|-------|----------------------|------------|---------|---------------------|---------------------|---------------------------------------------|
| 1  | 36.338      | 57 9       | Ap1,CAMP,BF2AK2,IL17A,L1R,mIR-155-5,STAT2   | 38 10      | activation of phagocytes | 48% (43/90) |
| 2  | 32.199      | 69 13      | 26s Proteasome,ANGPT2,Ap1,BCL2,CAMP,CEBPA,TGFA | 45 11      | activation of antigen presenting cells | 40% (57/143) |
| 3  | 30.414      | 57 12      | 26s Proteasome,CAMP,CSF2,F2RL1,L17A,mIR-21-5,TGFA | 37 8       | activation of myeloid cells | 32% (31/99) |
| 4  | 30.375      | 97 13      | Ap1,CAMP,CL5,EF2AK2,F2RL1,FGF10,L17A       | 64 20      | accumulation of I cells, leukopoiesis | 38% (99/260) |
| 5  | 28.605      | 56 10      | 26s Proteasome,BCL2,CAMP,STAT3,TGFA,TGM2   | 37 9       | adhesion of blood cells | 36% (32/90) |
| 6  | 25.456      | 49 8       | 26s Proteasome,F2RL1,L1RN,IF4,KLF3,STAT3,TGFA | 32 9       | adhesion of immune cells | 26% (19/72) |
| 7  | 25.126      | 127 20     | ANGPT2,Ap1,CAMP,CSF2,ETS1,F2RL1,IFNL1,GFX1,L17A  | 92 15      | cell movement of granulocytes | 40% (121/300) |
| 8  | 24.82       | 53 8       | 26s Proteasome,BCL2,CSF1,F2RL1,L1RN,STAT3,TGFA | 32 9       | adhesion of blood cells | 41% (23/56) |
| 9  | 23.333      | 50 7       | CAMP,F2RL1,L17A,mir-10,NRG1,TGFA,Ifn         | 36 7       | cell viability of tumor cell lines | 63% (31/49) |
| 10 | 23.026      | 36 7       | 26s Proteasome,BCL2,CREB1,F2RL1,IFNA2,L1RN,TGFA | 22 7       | binding of leukocytes | 24% (12/49) |
| 11 | 22.687      | 55 11      | 26s Proteasome,Calcineurin protein(s),CD38,BF4E,F2RL1, | 22 7       | cell viability of tumor cell lines | 53% (16/30) |
| 12 | 21.651      | 23 5       | CITAEB3,IL17,IL17F,PARP9,PDCD1               | 12 6       | activation of lymphatic system cells | 53% (16/30) |
| 13 | 21.355      | 41 6       | F2RL1,L1RN,mIR-155-5p,(mRNAs w/seed UAAUGCU), | 28 7       | cell viability of mononuclear leukocytes | 36% (15/42) |
| 14 | 20.788      | 42 5       | F2RL1,L1RN,PKc(s),TNFSF11,VEGFA              | 28 9       | adhesion of immune cells | 47% (21/45) |
| 15 | 20.175      | 50 7       | BTNL2,CITAEB3,IL17,SYN1,VEGFA                | 33 10      | activation of leukocytes | 20% (14/70) |
| 16 | 19.856      | 54 8       | Ap1,CAMP,CSF2,BF2AK2,F2RL1,L1RN,mIR-155-5p   | 39 7       | chemotaxis of granulocytes | 38% (21/56) |
| 17 | 19.73       | 30 3       | CAMP,mIR-155-5p,(mRNAs w/seed UAAUGCU)       | 19 8       | cell death of connective tissue cells | 33% (8/24) |
| 18 | 19.1        | 50 8       | F2,F2RL1,L17A,miR-10,PPIR1,REL,TGFA          | 35 7       | cell viability of lymphatic system cells | 46% (26/56) |
| 19 | 18.764      | 67 13      | Ap1,CAM,BCR(complex),CAMP,CSF2,L12(complex),L12STAT1, | 48 6       | synthesis of reactive oxygen species | 41% (32/78) |
| 20 | 18.475      | 41 7       | F2RL1,L17A,DL,miR-1,PRCR1,REL,REL           | 27 7       | cell viability of mononuclear leukocytes | 39% (19/49) |
| 21 | 18.429      | 75 8       | CCL5,F2RL1,L1RN,mIR-155-5p,PSMD10,STAT4,TGFA | 49 18      | apoptosis of fibroblast cell lines | 31% (45/144) |
| 22 | 17.098      | 34 6       | F2RL1,lgm1LRN,IL65,STAT3,VEGFA               | 23 5       | binding of myeloid cells | 37% (11/30) |
| 23 | 16.585      | 33 7       | CEBPA,EGF,FLT3L,IL17A,MIIF,miR-1,REL         | 21 5       | NK cell proliferation | 37% (13/35) |
| 24 | 16.44       | 50 7       | CAMP,F2RL1,L17A,LN,DL,NRG1,TLG1               | 37 6       | activation of antigen presenting cells, cell accumulation of cells | 51% (21/42) |
| 25 | 15.167      | 50 7       | CAMP,ETS1,F2,F2RL1,L17A,MIIF                   | 36 7       | chemotaxis of kidney cell lines | 43% (17/40) |
| 26 | 14.732      | 52 8       | 26s Proteasome,CSF1,IFNG,IL17A,IR4,L1DL,REL,TLG1 | 39 5       | cellular homeostasis | 48% (12/25) |
| 27 | 14.467      | 47 5       | 26s Proteasome,AKT1,DL,TLG1,TFGM2             | 37 5       | translation of mRNA | 44% (24/55) |
| 28 | 12.928      | 70 11      | 26s Proteasome,APP,CREB1,CSF1,F1A2,IFNG,IL17A,TGFA | 54 5       | quantity of IgG, recruitment of cells | 31% (14/45) |
| 29 | 12.667      | 50 5       | CEBPA,F2RL1,L1RN,TNFSF11,VEGFA               | 36 9       | homing of neutrophils, recruitment of cells | 40% (17/42) |
| 30 | 12.33       | 50 7       | CAMP,EF2AK2,F2RL1,HRAS,IL17A,L1RN,STAT2       | 37 6       | production of reactive oxygen species | 45% (19/42) |
| 31 | 12.221      | 76 6       | CD40LG,GAST,mIR-155-5p,TNFSF11               | 63 7       | infiltration by myeloid cells | 29% (23/80) |
| 32 | 11.939      | 32 6       | CAMP,ETS1,L17A,KTLG,miR-155-5,miR-21-5p   | 22 4       | cell viability of tumor cell lines | 53% (16/30) |
| ID  | Consistency | Node | Regulator | Regulators | Target | Disease & Functions | Known Regulator-Disease/Function |
|-----|-------------|------|-----------|------------|--------|---------------------|-------------------------------|
| 33  | 11.839      | 34   | 4         | BTNL2,hib-b2,Ifnar1,TRM24 | 24     | diabetes mellitus, hypersensitive reaction | 8% (2/24) |
| 34  | 10.818      | 46   | 5         | CEBPA,EGF,FLOT3,IL17A,MIF | 35     | cell viability of tumor cell lines | 43% (13/30) |
| 35  | 9.707       | 21   | 5         | F2,F2RL1,IL1RN,IL6,VEGFA | 13     | migration of antigen presenting cells | 60% (9/15) |
| 36  | 8.693       | 13   | 4         | CD3,F2RL1,IL1RN,VEGFA | 7      | binding of myeloid cells | 25% (2/8) |
| 37  | 8.521       | 22   | 5         | 26s Proteosome,FOXO3,IL18,Pkc(s),TNFSF11 | 15     | response of lymphatic system cells | 60% (6/10) |
| 38  | 8.01        | 74   | 8         | A2M,CD40LG,GAST,mir-17,mir-17-5p,other miRNAs | 58     | anemia,binding of tumor cell lines | 28% (18/64) |
| 39  | 7.649       | 36   | 5         | GAST,PARP9,PIK3R1,SOX4,VEGFA | 26     | anemia, autophagy, organismal death | 16% (4/25) |
| 40  | 7.464       | 87   | 13        | CD40LG,GEP300,ERG,IL7,miR-19a-3p,miR-29a-3 | 69     | cell death of fibroblast cell lines | 28% (18/66) |
| 41  | 7.181       | 14   | 6         | CSF2,EDN1,F2,IL18,KITLG,SP1 | 7      | migration of granulocytes | 33% (2/6) |
| 42  | 6.791       | 26   | 5         | EDN1,F2,KRCA,TNFSF11,VEGFA | 17     | Nephritis, synthesis of eicosanoid | 40% (8/20) |
| 43  | 6.333       | 17   | 3         | IRF5,mir-155-5p,miRNAs w/seed UAAUCGU,PSMD10 | 11     | apoptosis of connective tissue cells | 0% (0/9) |
| 44  | 6.379       | 18   | 3         | GFI1 | 12 | quantity of hematopoietic progenitor cells | 100% (6/6) |
| 45  | 6.306       | 22   | 3         | mir-155-5p,miRNAs w/seed UAAUCGU,mir-21-5p | 17 | cell death of connective tissue cells | 17% (1/6) |
| 46  | 6.183       | 27   | 3         | CREB1,IFNA2,POCD1 | 22 | activation of leukocytes | 67% (4/6) |
| 47  | 5.667       | 19   | 1         | GFI1 | 9 | HIV infection, proliferation of blood cells | 75% (3/4) |
| 48  | 5.345       | 34   | 4         | IL5 | 14 | inflammation of body cavity | 50% (2/4) |
| 49  | 5.292       | 17   | 3         | CAMP,CSF2,IFNG,IL12 (complex) | 28 | synthesis of leukotriene | 75% (6/8) |
| 50  | 4.907       | 17   | 3         | EGF,PRDM1,SMARCA4 | 12 | endocytosis, phagocytosis of cells | 17% (1/6) |
| 51  | 4.276       | 18   | 2         | GFI1,Pkc(s) | 14 | differentiation of mononuclear leukocytes | 50% (2/4) |
| 52  | 4.199       | 37   | 3         | IL2,IL21,IL4 | 30 | apoptosis of connective tissue cells | 42% (5/12) |
| 53  | 4.16        | 17   | 3         | CAMP,CSF1,Immunoglobulin | 13 | mobilization of Ca2+ | 67% (2/3) |
| 54  | 3.889       | 12   | 2         | mir-9,mir-92a-3p (and other miRNAs w/seed AUUGCAC) | 8 | cell cycle progression | 0% (0/4) |
| 55  | 3.13        | 8    | 1         | FOXO1 | 5 | hyperplasia of lymphoid organ, | 0% (0/2) |
| 56  | 3.024       | 11   | 3         | IgM,Interferon alpha,STAT1 | 7 | apoptosis of kidney cell lines | 0% (0/3) |
| 57  | 3           | 13   | 3         | CEBPA,JNF Beta,mir-223 | 9 | production of protein | 33% (1/3) |
| 58  | 2.236       | 8    | 1         | mir-223 | 5 | Bacterial infection, production of protein | 0% (0/2) |
| 59  | 1.789       | 7    | 1         | EZF1 | 5 | cell death of fibroblasts | 100% (1/1) |
| 60  | 1.789       | 7    | 1         | IL15 | 5 | cytotoxicity of natural killer cells | 100% (1/1) |
| 61  | 1.789       | 7    | 1         | IL18 | 5 | binding of lymphatic system cells | 100% (1/1) |
| 62  | 1.732       | 5    | 1         | CD28 | 3 | hyperplasia of lymphoid organ | 0% (0/1) |
| 63  | 1.508       | 13   | 1         | TP53 | 11 | catabolism of protein | 100% (1/1) |
| 64  | 0.802       | 17   | 2         | HRAS,TCR | 14 | expression of miRNA | 0% (0/2) |
| 65  | 0.577       | 32   | 4         | IFNA2,IF7,TFGB1,TFN | 27 | systemic lupus erythematosus | 25% (1/4) |
### Table 6: Effects of δ-tocotrienol treatment on "Regulator Effects" section (70) of IPA analysis of "Diseases and Functions" in hepatitis C patients (Continued)

| ID  | Consistency | Node | Regulator | Regulators | Target | Disease & Functions       | Known Regulator-Disease/Function |
|-----|-------------|------|-----------|------------|--------|---------------------------|---------------------------------|
| 66  | -2.714      | 13   | 1         | IL4        | 11     | infection of cells        | 100% (1/1)                      |
| 67  | -4.082      | 8    | 1         | miR-21-5p (and other miRNAs w/seed AGCUUAU) | 6      | cell death                | 100% (1/1)                      |
| 68  | -6.5        | 6    | 1         | TCF7L2     | 4      | apoptosis of fibroblast cell lines | 0% (0/1)                        |
| 69  | -16.748     | 5    | 1         | TRAP1      | 3      | synthesis of reactive oxygen species | 100% (1/1)                      |
| 70  | -23.519     | 58   | 1         | APP        | 56     | cancer                    | 100% (1/1)                      |
Table 7  Effect of δ-tocotrienol on up-regulation of fold change expression in “upstream regulator” section (57) of IPA analysis in hepatitis C patients

| Upstream Regulator | Molecule Type | Predicted Activation State | Activation Z-Score | P-value of overlap | Mechanistic Network |
|--------------------|---------------|----------------------------|--------------------|-------------------|---------------------|
| 1 mir-17-5p (and other miRNAs w/seed AAAGUGC) | mature microrna | Activated | 3.798 | 5.39E-08 | 127 (7) |
| 2 mir-155-5p (miRNAs w/seed UAAUGCU) | mature microrna | Activated | 4.518 | 9.04E-06 | 137 (7) |
| 3 mir-19b-3p (and other miRNAs w/seed GUGGAAA) | mature microrna | Activated | 2.198 | 0.00017 | |
| 4 mir-92a-3p (and other miRNAs w/seed AUUGCAU) | mature microrna | Activated | 2.187 | 0.00744 | |
| 5 mir-214-3p (and other miRNAs w/seed CAGCAGG) | mature microrna | | 0.0113 | | |
| 6 mir-291a-3p (and other miRNAs w/seed AAGUGCU) | mature microrna | Activated | 2.994 | 0.017 | |
| 7 mir-21-5p (and other miRNAs w/seed AGCUAAU) | mature microrna | Activated | 2.595 | 0.0159 | |
| 8 mir-330-5p (and other miRNAs w/seed CUCUGGG) | mature microrna | | 0.0113 | | |
| 9 mir-122-5p (miRNAs w/seed GGAGUGU) | mature microrna | Activated | 2.586 | 0.0279 | |
| 10 mir-2682-5p (and other miRNAs w/seed AGGCAGU) | mature microrna | | 0.00584 | | |
| 11 mir-205-5p (and other miRNAs w/seed CCGUAAU) | mature microrna | | 0.0325 | | |
| 12 mir-200b-3p (and other miRNAs w/seed AAUACUG) | mature microrna | | 1.960 | 0.0273 | |
| 13 mir-542-3p (miRNAs w/seed GUGCAAG) | mature microrna | | 0.0363 | | |
| 14 mir-221-3p (and other miRNAs w/seed GCUCAUU) | mature microrna | | 1.957 | 0.0349 | |
| 15 mir-147a (miRNAs w/seed UGUGUGG) | mature microrna | | 0.0448 | | |
| 16 mir-450a-5p (and other miRNAs w/seed UUUGCGA) | mature microrna | | 0.0448 | | |
| 17 mir-216a-5p (miRNAs w/seed AAUCUCA) | mature microrna | | 0.0448 | | |
| 18 mir-504-5p (and other miRNAs w/seed GACCUAG) | mature microrna | | 0.0448 | | |
| 19 mir-657 (miRNAs w/seed GCAGGUU) | mature microrna | | 0.0448 | | |
| 20 mir-17 | microrna | Activated | 2.581 | 0.00091 | |
| 21 mir-122 | microrna | Activated | 3.300 | 0.00189 | |
| 22 mir-19 | microrna | Activated | 2.204 | 0.002 | |
| 23 mir-1 | microrna | Activated | 2.72 | 0.00354 | 128 (6) |
| 24 mir-214 | microrna | | 0.00906 | | |
| 25 mir-326 | microrna | | 0.0113 | | |
| 26 mir-138 | microrna | | 0.0113 | | |
| 27 mir-32 | microrna | | 0.0113 | | |
| 28 mir-155 | microrna | | 1.965 | 0.00691 | 173 (8) |
| 29 mir-148 | microrna | | 1.997 | 0.00103 | |
| 30 mir-199 | microrna | | 0.0028 | 164 (7) | |
| 31 mir-218 | microrna | | 0.0183 | | |
Table 7  Effect of δ-tocotrienol on up-regulation of fold change expression in "upstream regulator" section (57) of IPA analysis in hepatitis C patients (Continued)

| #  | Upstream Regulator | Molecule Type          | Predicted Activation State | Activation Z-Score | P-value of overlap | Mechanistic Network |
|----|--------------------|------------------------|-----------------------------|--------------------|--------------------|---------------------|
| 32 | mir-515            | microrna               |                             |                    | 0.0225             |                     |
| 33 | mir-132            | microrna               |                             |                    | 0.0349             |                     |
| 34 | mir-10             | microrna               | Activated                   | 2.786              | 0.0366             |                     |
| 35 | mir-8              | microrna               | Activated                   | 2.128              | 0.0344             |                     |
| 36 | mir-25             | microrna               |                             | 1.972              | 0.0349             |                     |
| 37 | mir-622            | microrna               |                             |                    | 0.0448             |                     |
| 38 | mir-181            | microrna               |                             | 0.988              | 0.0498             |                     |
| 39 | Immunoglobulin complex | Activated             |                             | 2.345              | 0.000024           | 283 (16)            |
| 40 | prednisolone      | chemical drug          |                             | 1.763              | 0.00025            | 235 (13)            |
| 41 | 26s Proteasome     | complex                | Activated                   | 2.921              | 0.00093            | 326 (16)            |
| 42 | IgG                | complex                |                             | 1.003              | 0.00824            | 295 (16)            |
| 43 | TRAP1              | enzyme                 | Activated                   | 2.236              | 0.0169             |                     |
| 44 | IL1RN              | cytokine               | Activated                   | 3.235              | 0.0275             |                     |
| 45 | prostaglandin A1   | chemical - endogenous non-mammalian | Activated | 0.686              | 0.00249            | 159 (8)             |
| 46 | AGTR1              | g-protein coupled receptor |                    | 1.067              | 0.0291             |                     |
| 47 | MAPK1              | kinase                 |                             | 1.017              | 0.0361             |                     |
| 48 | Ubiquitin          | group                  |                             |                    | 0.039              |                     |
| 49 | IL18RAP            | transmembrane receptor |                             |                    | 0.0363             |                     |
| 50 | TAB1               | enzyme                 |                             | 1.258              | 0.0349             |                     |
| 51 | eIF2B              | complex                |                             |                    | 0.0448             |                     |
| 52 | SNRPN              | other                  |                             |                    | 0.0448             |                     |
| 53 | SNORD21            | other                  |                             |                    | 0.0448             |                     |
| 54 | SOS2               | other                  |                             |                    | 0.0448             |                     |
| 55 | IL1RL2             | transmembrane receptor |                             |                    | 0.0469             |                     |
| 56 | IL18BP             | other                  |                             |                    | 0.0469             |                     |
| 57 | IL10RA             | transmembrane receptor | Activated                   | 2.688              | 0.229              |                     |
| #  | Upstream Regulator          | Molecule Type             | Predicted Activation State | Activation z-score | p-value of overlap | Mechanistic Network |
|----|----------------------------|---------------------------|----------------------------|--------------------|--------------------|---------------------|
| 1  | interferon beta-1a         | biologic drug             |                             |                    |                    |                     |
| 2  | IL2                        | cytokine                  | Inhibited                   | -4.562             | 2.23E-09           | 297 (17)            |
| 3  | IL15                       | cytokine                  | Inhibited                   | -2.247             | 1.37E-08           | 299 (19)            |
| 4  | FAS                        | transmembrane receptor    |                             | -1.461             | 3.94E-08           | 263 (17)            |
| 5  | TNF                        | cytokine                  | Inhibited                   | -5.914             | 0.00000294         | 378 (19)            |
| 6  | IL21                       | cytokine                  | Inhibited                   | -2.747             | 0.00000339         | 264 (15)            |
| 7  | GATA1                      | transcription regulator    |                             | -0.822             | 0.00000497         | 243 (11)            |
| 8  | IRF1                       | transcription regulator    | Inhibited                   | -3.223             | 0.000011           | 245 (13)            |
| 9  | EGF                        | growth factor             | Inhibited                   | -5.15              | 0.0000204          | 303 (15)            |
| 10 | TGFB1                      | growth factor             | Inhibited                   | -3.491             | 0.000004           | 350 (17)            |
| 11 | IL6                        | cytokine                  | Inhibited                   | -3.043             | 0.0000566          | 284 (15)            |
| 12 | IL5                        | cytokine                  | Inhibited                   | -4.866             | 0.00000654         | 243 (13)            |
| 13 | Interferon alpha group     |                           | Inhibited                   | -4.069             | 0.000154           | 150 (9)             |
| 14 | STAT4                      | transcription regulator    | Inhibited                   | -4.536             | 0.0000489          | 111 (6)             |
| 15 | IL7                        | cytokine                  | Inhibited                   | -2.665             | 0.000064           | 243 (18)            |
| 16 | IL13                       | cytokine                  | Inhibited                   | -1.516             | 0.000806           | 295 (16)            |
| 17 | STAT1                      | transcription regulator    | Inhibited                   | -4.582             | 0.000877           | 241 (14)            |
| 18 | IL1B                       | cytokine                  | Inhibited                   | -4.367             | 0.000982           | 330 (17)            |
| 19 | STAT2                      | transcription regulator    | Inhibited                   | -2.219             | 0.00105            | 173 (9)             |
| 20 | PARP9                      | enzyme                    | Inhibited                   | -2.200             | 0.00123            | 142 (6)             |
| 21 | FOXC1                      | transcription regulator    | Inhibited                   | -1.961             | 0.002              |                     |
| 22 | IL2RG                      | transmembrane receptor    |                             | -0.113             | 0.00233            |                     |
| 23 | IL12 (complex)             | complex                   | Inhibited                   | -2.378             | 0.00251            | 246 (17)            |
| 24 | TGF-A                      | growth factor             | Inhibited                   | -2.888             | 0.00327            | 283 (17)            |
| 25 | CD14                       | transmembrane receptor    |                             | -1.768             | 0.00332            | 298 (16)            |
| 26 | TNFSF10                    | cytokine                  | Inhibited                   | -1.376             | 0.00477            | 297 (17)            |
| 27 | mir-223                    | microrna                  | Inhibited                   | -2.060             | 0.00527            | 167 (7)             |
| 28 | IL27                       | cytokine                  | Inhibited                   | -2.937             | 0.00527            | 317 (16)            |
| 29 | beta-estradiol             | chemical - endogenous mammalian | Inhibited | -4.574             | 0.00546 | 358 (17) |
| 30 | IL10                       | cytokine                  |                             | -0.803             | 0.00582            | 247 (17)            |
| 31 | ADORA2A                    | g-protein coupled receptor | Inhibited                   | -2.365             | 0.00599            | 175 (9)             |
| #  | Upstream Regulator | Molecule Type                           | Predicted Activation State | Activation z-score | p-value of overlap | Mechanistic Network |
|----|-------------------|----------------------------------------|---------------------------|--------------------|--------------------|---------------------|
| 32 | IFNL1             | cytokine                               | Inhibited                | -2.925             | 0.00622            | 224 (11)            |
| 33 | IL18              | cytokine                               | Inhibited                | -2.26              | 0.00701            | 326 (19)            |
| 34 | NOX1              | ion channel                            |                           | -1.951             | 0.00741            | 263 (14)            |
| 35 | SOX4              | transcription regulator                 | Inhibited                | -3.033             | 0.00834            |                     |
| 36 | prostaglandin J2 | chemical - endogenous non-mammalian   |                           | -1.432             | 0.0115             |                     |
| 37 | E2F1              | transcription regulator                 | Inhibited                | -2.081             | 0.0142             |                     |
| 38 | CREB1             | transcription regulator                 | Inhibited                | -3.766             | 0.0143             |                     |
| 39 | IGF1              | growth factor                          | Inhibited                | -2.385             | 0.0158             |                     |
| 40 | IL12 (family)     | group                                  |                           | -0.500             | 0.016              |                     |
| 41 | IRF5              | transcription regulator                 | Inhibited                | -2.155             | 0.0162             |                     |
| 42 | FOXO4             | transcription regulator                 |                           | -1.98              | 0.0179             |                     |
| 43 | PGF               | growth factor                          |                           | -1.959             | 0.0237             |                     |
| 44 | BTG2              | transcription regulator                 |                           | 1.165              | 0.0239             |                     |
| 45 | miR-15            | microrna                               |                           | -0.927             | 0.0279             |                     |
| 46 | STAT5A            | transcription regulator                 |                           | -0.896             | 0.0294             |                     |
| 47 | NFE2L2            | transcription regulator                 | Inhibited                | -3.644             | 0.0295             |                     |
| 48 | MIF               | cytokine                               | Inhibited                | -2.642             | 0.0304             |                     |
| 49 | FGF10             | growth factor                          | Inhibited                | -2.200             | 0.0305             |                     |
| 50 | miR-26a-5p (and other miRNAs w/seed UCAAAGUA) | mature microrna |                           | 1.916              | 0.0309             |                     |
| 51 | NOX4              | enzyme                                 |                           | -1.941             | 0.0309             |                     |
| 52 | NFKBIB            | transcription regulator                 |                           | -1.400             | 0.0331             |                     |
| 53 | IFNA1/IFNA13      | cytokine                               |                           | -1.77              | 0.0331             |                     |
| 54 | FLT3LG            | cytokine                               | Inhibited                | -2.411             | 0.0331             |                     |
| 55 | IL17F             | cytokine                               |                           | -1.917             | 0.0349             |                     |
| 56 | IL32              | cytokine                               |                           | -1.15              | 0.0416             |                     |
| 57 | CCL5              | cytokine                               | Inhibited                | -2.621             | 0.042              |                     |
| 58 | IL17A             | cytokine                               | Inhibited                | -3.075             | 0.0422             |                     |
| 59 | MIR124            | group                                  |                           | 1.941              | 0.0435             |                     |
| 60 | miR-218-5p (and other miRNAs w/seed UGUGCUU) | mature microrna |                           |                    | 0.0443             |                     |
| #  | Upstream Regulator | Molecule Type                  | Predicted Activation State | Activation z-score | p-value of overlap | Mechanistic Network |
|----|-------------------|--------------------------------|----------------------------|--------------------|--------------------|---------------------|
| 61 | CXCR4             | g-protein coupled receptor     |                           | -0.842             | 0.0447             |                     |
| 62 | CD38              | enzyme                         | Inhibited                 | -3.429             | 0.0482             |                     |
| 63 | IL24              | cytokine                       | Inhibited                 | -0.277             | 0.0498             |                     |
| 64 | TCF3              | transcription regulator         | Inhibited                 | -2.530             | 0.231              |                     |
| # | Categories | Diseases or Functions Annotation | P-value | Predicted Activation | Act Z-Score | Molecules | # Molecules |
|---|------------|----------------------------------|---------|---------------------|-------------|-----------|------------|
| 1 | Up-regulated (11) | cell death | 3.94E-21 | Increased | 2.645 | ABCD1, ABL1, ACO2 | 349 |
| 2 | Cell Death and Survival | cell death | 4.75E-21 | Increased | 3.412 | ABL1, B2M, BCL2L11 | 76 |
| 3 | Cancer, Cell Death and Survival | function of lymphatic system cells | 2.1E-16 | 0.273 | ARHGEF6 | 60 |
| 4 | Cancer, Cell Death and Survival | function of leukocytes | 1.25E-15 | 0.051 | B | 77 |
| 5 | Gene Expression, Protein Synthesis | translation of mRNA | 1.6E-12 | Increased | 2.941 | BTG2, DNX1, EIF5 | 36 |
| 6 | Metabolic Disease | glucose metabolism disorder | 2.76E-08 | Increased | 1.558 | ABHD16A, ALOX5AP, ANAPC13 | 136 |
| 7 | Organismal Survival | organismal death | 0.00000495 | Increased | 11.544 | ABL1, ADORA2A, APT | 210 |
| 8 | Cancer, Hematological Disease | lymphoproliferative malignancy | 0.00000592 | Increased | 1.725 | ABL1, ADORA2A, AIMP1 | 203 |
| 9 | Neurological Disease, Organismal Injury | brain | 0.00000781 | Increased | 1.538 | ABL1, ABL1, ADORA2A | 76 |
| 10 | Organismal Injury | organismal death | 0.00000854 | Increased | 0.711 | ABL1, ABL1, ADORA2A | 749 |
| 11 | Down-regulated (49) | proliferation of immune cells | 1.29E-24 | Decreased | -2.128 | ABL1, ADORA2A, ARHGEF6 | 128 |
| 12 | Cellular Development, Cellular Proliferation | proliferation of mononuclear leukocytes | 6.29E-24 | Decreased | -2.073 | ABL1, ADORA2A, ARHGEF6 | 123 |
| 13 | Infectious Diseases | Viral Infection | 6.4E-24 | Decreased | -5.928 | ABL1, ADORA2A, AGO4 | 207 |
| 14 | Cellular Growth and Proliferation | proliferation of lymphatic system cells | 8.63E-24 | Decreased | -2.019 | ABL1, ADORA2A, ARHGEF6 | 129 |
| 15 | Immunological Disease | systemic autoimmune syndrome | 2.37E-23 | Increased | 11.544 | ABL1, ADORA2A, APT | 210 |
| 16 | Hematological System Development | quantity of mononuclear leukocytes | 6.64E-19 | Decreased | -4.691 | ABL1, ADORA2A, ARHGEF6 | 113 |
| 17 | Lymphoid Tissue Structure | quantity of lymphatic system cells | 1.46E-18 | Decreased | -4.679 | ABL1, ADORA2A, ARHGEF6 | 115 |
| 18 | Hematological System Development | quantity of blood cells | 6.22E-16 | Decreased | -4.724 | ABL1, ADD3, ADORA2A | 134 |
| 19 | Cell-To-Cell Signaling and Interaction | activation of cells | 2E-15 | Decreased | -5.698 | ADORA2A, APARBB | 127 |
| 20 | Connective Tissue Disorders | inflammation of joint | 2.16E-13 | Decreased | -1.573 | ABL1, ADORA2A, APT | 128 |
| 21 | Cardiovascular Disease, Developmental | Diamond-Blackfan anemia | 4.55E-11 | Decreased | -1.395 | APOB, C3, ATG5, BCL10 | 44 |
| 22 | Antimicrobial Response, Inflammatory | antimicrobial response | 8.55E-09 | Decreased | -3.395 | APOB, C3, ATG5, BCL10 | 44 |
| 23 | Embryonic Development, Hematological | formation of lymphoid tissue | 1.45E-08 | Decreased | -2.618 | ABL1, B2M, BCL2L11 | 48 |
| 24 | Free Radical Scavenging | metabolism of reactive oxygen species | 1.56E-08 | Decreased | -2.89 | ABL1, ATG5, ATP7A | 63 |
| 25 | Neurological Disease, Skeletal | neuromuscular disease | 5.12E-07 | Decreased | -0.200 | ABL1, ADORA2A, ALS | 95 |
| 26 | Cell Morphology | morphology of blood cells | 737E-07 | Decreased | -2.058 | ABL1, ABL1, ADD3 | 52 |
| 27 | Inflammatory Response, Neurological | inflammation of central nervous system | 0.00000109 | Decreased | -1.099 | ADORA2A, B2M, CAR1 | 48 |
| 28 | Humoral Immune Response, Protein | production of antibody | 0.00000114 | Decreased | -1.497 | B2M, BCL10, BCL2L11 | 40 |
| 29 | Endocrine System Disorders | diabetes mellitus | 0.00000166 | Decreased | -2.058 | ABL1, ABL1, ADD3 | 52 |
| 30 | Digestive System Development | morphology of Peyer's patches | 0.00000208 | Decreased | -2.058 | ABL1, ABL1, ADD3 | 52 |
| #  | Categories                              | Diseases or Functions Annotation                           | P-Value     | Predicted Activation | Act Z-Score | Molecules                                      | # Molecules |
|----|----------------------------------------|-----------------------------------------------------------|-------------|----------------------|-------------|------------------------------------------------|-------------|
| 32 | Cellular Compromise, Inflammatory       | degranulation of cells                                    | 0.0000021   | Decreased            | -3.08       | C3AR1,C5AR1,CAMP                                | 31          |
| 33 | Cell Signaling, Molecular Transport     | mobilization of Ca2+                                      | 0.00000212  | Decreased            | -2.95       | ADORA2A,ARRB2,B2M                               | 42          |
| 34 | Cell-To-Cell Signaling and Interaction  | binding of leukocytes                                     | 0.00000273  | Decreased            | -4.799      | ABL1,ADORA2A,ARRB2                              | 46          |
| 35 | Immunological Disease                  | allergy                                                   | 0.00000286  | Decreased            | -1.655      | ABL1,ACO2,ADORA2A                               | 49          |
| 36 | Humoral Immune Response, Protein       | quantity of immunoglobulin                                | 0.00000494  | Decreased            | -1.731      | B2M,BCL10,BCL2L11                              | 37          |
| 37 | RNA Post-Transcriptional Modification  | processing of RNA                                         | 0.0000059   | Decreased            | -0.670      | ADAT1,AFF2,CFL1                                 | 36          |
| 38 | Hematological System Development       | quantity of thymocytes                                     | 0.00000592  | Decreased            | -3.599      | ABL1,B2M,BCL10                                 | 36          |
| 39 | Immunological Disease                  | abnormal morphology of immune                            | 0.00000593  |                     |             | ABCD1,ABL1,B2M                                 | 37          |
| 40 | Cancer, Hematological Disease          | mature B-cell lymphoma                                    | 0.00000888  |                     |             | ABL1,B2M,BCL10                                 | 38          |
| 41 | Digestive System Development           | abnormal morphology of Peyer's                            | 0.00000906  |                     |             | DDX58,JD2,GXK                                   | 11          |
| 42 | Lipid Metabolism, Small Molecule       | synthesis of eicosanoid                                    | 0.00000989  | Decreased            | -3.209      | ALOX5AP,ATPSJ,C5AR1                             | 29          |
| 43 | Cellular Growth and Proliferation      | expansion of cells                                         | 0.0000113   | Decreased            | -1.716      | ALOX5AP,B2M,BMI1                               | 37          |
| 44 | Lipid Metabolism, Small Molecule       | synthesis of leukotriene C4                               | 0.0000148   | Decreased            | -2.753      | ALOX5AP,C5AR1,COTL1                             | 18          |
| 45 | Gene Expression                        | activation of DNA endogenous                              | 0.000016    | Decreased            | -3.846      | ARR2B1,ATF4,BMI1                               | 111         |
| 46 | Antigen Presentation, Inflammatory     | antigen presentation                                       | 0.0000715   | Decreased            | -1.556      | ARL8B,CD74,CST3                                | 14          |
| 47 | Cell Death and Survival, Organismal    | cell death of kidney cells                                | 0.0000715   |                     | -1.863      | ATG5,ATP1A1,BCL10                               | 39          |
| 48 | Cellular Movement, Hematological       | chemotaxis of granulocytes                                | 0.0000723   | Decreased            | -2.233      | ADORA2A,BST1,C3AR1                              | 24          |
| 49 | Cancer, Hematological Disease          | large-cell lymphoma                                       | 0.0000741   |                     |             | B2M,BCL2L11,CAMLG                               | 34          |
| 50 | Cell-To-Cell Signaling and Interaction  | binding of mononuclear leukocytes                         | 0.0000753   | Decreased            | -3.212      | CD47,CD48,CD58                                 | 21          |
| 51 | Cellular Movement, Embryonic           | chemotaxis of embryonic cell lines                        | 0.0000767   | Decreased            | -2.587      | ARR2B1,CAMP,CXCL1                               | 13          |
| 52 | Cellular Movement, Hair and Skin       | chemotaxis of epithelial cell lines                       | 0.0000767   | Decreased            | -2.587      | ARR2B1,CAMP,CXCL1                               | 7           |
| 53 | Cell Death and Survival, Skeletal      | cell death of smooth muscle cells                         | 0.0000775   |                     | -0.332      | ARR2B1,CAMP,CASP3                               | 16          |
| 54 | Cell Death and Survival                | cell viability of phagocytes                              | 0.0000775   | Decreased            | -2.939      | BCL2A1,CD48,CEBP8                               | 16          |
| 55 | Cell Death and Survival                | killing of lymphatic system cells                         | 0.0000789   | Decreased            | -2.016      | BCL2L11,CD47,CD58                               | 10          |
| 56 | Cell Death and Survival, B2M           | cell viability of mononuclear leukocytes                  | 0.0000805   | Decreased            | -3.491      | ATG3,BCL10,BCL2L11                             | 25          |
| 57 | Cellular Development, Cellular Growth  | differentiation of myeloid leukocytes                     | 0.0000809   |                     | -1.081      | ABL1,CAMP,CD47                                 | 31          |
| 58 | Cell-To-Cell Signaling and Interaction  | binding of lymphatic system cells                         | 0.0000847   | Decreased            | -3.360      | CD47,CD48,CD58                                 | 23          |
| 59 | RNA Post-Transcriptional Modification  | unwinding of mRNA                                         | 0.000086    |                     |             | EIF4A1,EIF4A2,EIF4B                             | 3           |
| 60 | Cell Death and Survival, Organismal    | cell death of epithelial cells                            | 0.000136    |                     | -1.105      | ARR2B1,ATG5,BCL10                               | 51          |
Table 10 Summary of IPA analyses of RNAs obtained from δ-tocotrienol treatment of hepatitis C patients

| # | Subjects                        | P-Value overlap | Overlap     | # | Subjects                        | P-Value overlap | Overlap     | # Molecules |
|---|--------------------------------|----------------|-------------|---|--------------------------------|----------------|-------------|-------------|
| I | Top Canonical Pathways         |                |             | VII | Cardiotoxicity                 |                |             |             |
| 1 | EIF2 Signaling                 | 1.28E-37       | 30.3 % 67/221 | 31 | Cardiac Infarction             | 3.62E-01 - 5.40E-04 |             | 23          |
| 2 | Regulation of eIF4 and p70S6K Signaling | 5.38E-140       | 21.0 % 33/157 | 32 | Cardiac Necrosis/Cell Death | 1.65E-01 - 2.56E-03 |             | 23          |
| 3 | mTOR Signaling                 | 1.28E-13       | 18.4 % 37/102 | 33 | Cardiac Dysfunction            | 4.31E-01 - 2.63E-03 |             | 11          |
| 4 | B Cell Receptor Signaling      | 8.35E-08       | 14.2 % 27/190 | 34 | Cardiac Fibrosis               | 1.77E-01 - 5.68E-03 |             | 14          |
| 5 | Signaling                      | 1.72E-06       | 16.2 % 18/111 | 35 | Cardiac Transformation         | 1.10E-02 - 1.10E-02 |             | 2           |
| II | Top Upstream Regulators        |                |             | VIII | Hepatotoxicity                 |                |             |             |
| 6 | ST 1926                        | 5.62E-20       | Activated   | 36 | Liver Proliferation            | 2.15E-01 - 5.85E-05 |             | 26          |
| 7 | Sirolimus                      | 2.32E-18       | Activated   | 37 | Liver Necrosis/Cell Death      | 6.13E-01 - 6.59E-05 |             | 29          |
| 8 | CD 437                         | 1.45E-17       | Activated   | 38 | Liver Damage                   | 4.69E-01 - 1.81E-04 |             | 35          |
| 9 | RICTOR                         | 1.64E-17       | Activated   | 39 | Liver Inflamma/Hepatitision    | 4.52E-01 - 5.02E-04 |             | 36          |
| 10 | MYCN                           | 3.22E-15       | Inhibited   | 40 | Liver Cirrhosis                | 4.19E-02 - 1.65E-03 |             | 21          |
| III | Diseases and Disorder         |                |             | IX | Nephrotoxicity                 |                |             |             |
| 11 | Infectious Diseases           | 1.14E-04 - 1.29E-24 |         | 41 | Renal Necrosis/Cell Death      | 3.32E-01 - 7.15E-05 |             | 46          |
| 12 | Immunological Disease         | 7.41E-05 - 2.37E-23 |         | 42 | Renal Inflammation             | 3.74E-01 - 1.69E-03 |             | 33          |
| 13 | Cancer                        | 1.25E-04 - 4.75E-22 |         | 43 | Renal Nephritis                | 3.70E-01 - 1.69E-03 |             | 33          |
| 14 | Organismal Injury and Abnormalities | 1.36E-04 - 4.75E-21 |         | 44 | Renal Damage                   | 5.15E01 - 3.12E-03 |             | 21          |
| 15 | Tumor Morphology              | 1.19E-04 - 4.75E-21 |         | 45 | Glomerular Injury              | 1.00E-00 - 1.47E-02 |             | 22          |
| IV | Molecular and Cellular Functions |                |             | X | Top Regulator Effect Networks  |                |             |             |
| 16 | Cellular Development          | 1.24E-04 - 1.29E-24 |         | 46 | Ap1,CAMP,F2RL,IL17A,IL1RN,KITLG,mir10,NRG1,SELP (+2 >) | Activation of antigen presenting cells (+11 >) |             | 40848       |
| 17 | Cellular Growth and Proliferation | 1.24E-04 - 1.29E-24 |         | 47 | AP1,CAMP,BF2AK2,F2RL,IL17A,IL1RN, KITLG (+2 >) | Activation of phagocytes (+9 >) |             | 36338       |
| 18 | Cell Death and Survival       | 1.36E-04 - 3.94E-21 |         | 48 | 26s Proteasome,ANGPT2,AP1,BCL2,CAMP,CEBPA,F2RL (+6 >) | Activation of antigen presenting cells (+10 >) |             | 32199       |
| 19 | Cell-To-Cell Signaling and Interaction | 1.34EE-18-04 - 7.04 |         | 49 | 26s Proteasome,CAMP,CSF1,IL17A,JUN,LDL (+5 >)F2RL (+6 >) | Activation of antigen presenting cells (+7 >) |             | 30414       |
| 20 | Cellular Function and Maintenance |                |             | 50 | Accumulation of leukocytes (+19 >) |             |             | 30375       |
Table 10 Summary of IPA analyses of RNAs obtained from δ-tocotrienol treatment of hepatitis C patients (Continued)

| # Subjects | P-Value overlap | Overlap | # Molecules | P-Value overlap | Overlap | # Molecules |
|------------|----------------|---------|-------------|----------------|---------|-------------|
| V Physiological System Development and Function | 1.02E-04 - 2.10E-16 | # Molecules | XI Top Networks (Associated Network Functions) | Score |
| 1 Physiological System Development and Function | 1.34E-04 - 1.29E-24 | 255 | 51 Developmental Disorder, Hereditary Disorder, Metabolic Diseases | 46 |
| 2 Lymphoid Tissue Structure and Development | 1.33E-04 - 1.29E-24 | 194 | 52 Cancer, Cell Death and Survival, Organismal Injury and Abnormalities | 44 |
| 3 Tissue Morphology | 1.19E-04 - 2.45E-19 | 184 | 53 Post-Translational Modification, Cell Cycle, Cellular Development | 44 |
| 4 Immune Cell Trafficking | 1.34E-04 - 7.04E-18 | 160 | 54 Cancer, Hematological Disease, Immunological Disease | 41 |
| 5 Hematopoiesis | 1.02E004 - 6.87E-14 | 130 | 55 Protein Synthesis, RNA Post-Transcriptional Modification, Gene Expression | 39 |
| VI Top Tox Functions (Clinical Chemistry and Hematology) | # Molecules | XII Top Toxicology Lists | p-value | Overlap |
| 6 Increased Levels of Albumin | 2.38E-01 - 1.24E-02 | 4 | 56 Renal Necrosis/Cell Death | 1.58E-05 | 8.60 % 46/538 |
| 7 Increased Levels of Alkaline Phosphatase | 2.12E-01 - 4.42E-02 | 6 | 57 Liver Proliferation | 1.80E-05 | 11.0 % 26/236 |
| 8 Decreased Levels of Hematocrit | 5.71E-02 - 5.71E-02 | 2 | 58 Liver Necrosis/ Cell Death | 8.35E-05 | 9.6 % 29/303 |
| 9 Increased Levels of Hematocrit | 6.20E-02 - 6.20E-02 | 8 | 59 Mechanism of Gene regulation by Peroxisome | 2.74E-04 | 13.7 % 13/95 |
| 10 Increased Levels of Potassium | 5.36E-01 - 8.64E-02 | 2 | 60 Increases Liver Damage | 7.40E-04 | 11.4 % 15/132 |
| A Gene Expression Fold Change (Up-regulated) | Expression Value | 8 Gene Expression Fold Change (Down-regulated) | Expression Value |
| 1 SNORD15A | 581.151 | 1 HMGN1P3 | -381.06 |
| 2 SNORA32 | 390.353 | 2 SNHG25 | -350.055 |
| 3 SNORA56 | 185.194 | 3 SNORA67 | -148.69 |
| 4 SNORA9 | 124.698 | 4 RPL17-C18orf32 | -67.253 |
| 5 SNORS3B | 102.91 | 5 ISY1-RAB43 | -51.147 |
| 6 SNORA3A | 93.09 | 6 ARHGEF18 | -41.381 |
| 7 HIST1H2AD | 20.784 | 7 KLC4-KLRK1/1KLK1 | -205.78 |
| 8 SNORD3D | 17.157 | 8 HIST1H3J | -19.795 |
| 9 LINC00305 | 4.853 | 9 MTHFS | -18.71 |
| 10 HHIP12 | 4.844 | 10 SNORA16A | -18.285 |
Table 11 Effect of δ-tocotrienol on canonical pathways (33) of IPA ingenuity canonical pathways analysis (360) in hepatitis C patients

| #  | Ingenuity Canonical Pathways (Fold Change Expression) | -log (p-value) | Ratio | Z-Score | Molecules |
|----|-------------------------------------------------------|----------------|-------|---------|-----------|
| 1  | EIF2 Signaling; Eukaryotic translation initiation factors (221) | 36.900 | 0.303 | -5.692 | RPL7A,EIF3G,RPL13A,RPL32,RPS24,RPL37A,RPL23,RPL26,RPS13 |
| 2  | Regulation of eIF4 and p70S6K signaling (157) | 13.300 | 0.210 | 0.000 | PPP2R5E, EIF3G, RPS26 |
| 3  | Protein ubiquitination pathway (266) | 3.130 | 0.091 | NUMI | UBE2J1, USP19, UBA52 |
| 4  | mTOR signaling; Mammalian target of rapamycin (201) | 12.900 | 0.184 | -2.138 | PPP2R5E, EIF3G, RPS26 |
| 5  | Type I Diabetes Mellitus Signaling (111) | 5.760 | 0.162 | -2.496 | NFKB1,MAP3K5,AK2,HLA-DOB1,TFGFR2,TNFRSF1B,PIAS1,TRADD |
| 6  | Th1 and Th2 Activation Pathway (185) | 5.640 | 0.130 | NUMI | NFKB1,AK2,NOTCH1,HLA-DOB1,TFGFR2,PIK3R1,HLA-ORA |
| 7  | Interferon Signaling (36) | 4.700 | 0.250 | -2.333 | IFNGR1,OSAS1,AK2,IFIT1,TFGFR2,IFIT2,PIAS1,PSMB8 |
| 8  | Role of IL-17F (44) | 3.960 | 0.205 | -3.000 | NFKB1,AK2,MAP3K5,TNFRSF1B,PIK3R1,HLA-ORA |
| 9  | IL-8 Signaling (197) | 3.320 | 0.102 | -4.123 | NFKB1,GNAI3,GNB4,RACK1,TFGFR2,AK2,PIK3R1,ARRB2,NCF2 |
| 10 | NF-kB Signaling (181) | 2.940 | 0.171 | -2.449 | GSK3B,SOX9,NFKB1,CSNK2B,TNFRSF1B,PIK3R1,HLA-ORA |
| 11 | IL-17A Signaling in Fibroblasts (35) | 2.400 | 0.171 | NUMI | GSK3B,NFKB1,CSEB1,CEBPB,MAP3K5,PIK3R1,TRA6 |
| 12 | IL-6 Signaling (128) | 2.360 | 0.102 | -3.051 | NFKB1,AK2,CSN2B,TNFRSF1B,VEGF,AK2,PIK3R1,CSNK1B,FRS2 |
| 13 | Induction of Apoptosis by HIV1 (61) | 2.280 | 0.131 | -2.828 | CSNK4A,NFKB1,MAP3K5,TNFRSF1B,PIAS1,CSNK2,BLCKL11,VEGF |
| 14 | HMGB1 Signaling (133) | 2.220 | 0.098 | -3.606 | OSM,NFKB1,TFGFR2,TNFRSF1B,PIK3R1,SP1,CSNK1B,FRS2 |
| 15 | PPAR Signaling (95) | 2.040 | 0.105 | 1.897 | NFKB1,TNFRSF1B,PTGS2,IL18R,PIK3R1,AK2,MAP3K5,CSNK2,BLCKL11 |
| 16 | IL-10 Signaling (69) | 1.960 | 0.116 | NUMI | NFKB1,IL18R,AK2,MAP3K5,PIK3R1,HLA-ORA |
| 17 | INOS Signaling (45) | 1.860 | 0.133 | -2.449 | IFNGR1,NFKB1,IFIT1,TFGFR2,PIK3R1,AK2,MAP3K5,PIK3R1 |
| 18 | Insulin Receptor Signaling (141) | 1.650 | 0.085 | -1.508 | GSK3B,PPP1CC,PTEN,AK2,AK1,PIK3R1,CSNK1B,FRS2 |
| 19 | p35 Signaling (111) | 1.600 | 0.090 | 0.000 | GSK3B,AK2,PIK3R1,CSNK1B,FRS2,AK2,PTGS2 |
| 20 | Role of IL-17A in Arthritis (69) | 1.490 | 0.101 | NUMI | NFKB1,CSNK2B,PIK3R1,AK2,MAP3K5,CSNK1B,FRS2 |
| 21 | Toll-like Receptor Signaling (76) | 1.300 | 0.092 | -1.000 | SIGIRR,TLR8,UBA52,NFKB1,MAP3K5,PIK3R1,CSNK1B,FRS2 |
| 22 | IL-1 Signaling (62) | 1.300 | 0.087 | -2.449 | GNAQ,NFKB1,GNB4,RACK1,MAP3K5,PIK3R1,CSNK2 |
| 23 | Apoptosis Signaling (90) | 0.987 | 0.078 | -0.378 | NFKB1,MAP3K5,BCL2L11,BCL2A1,TNFRSF1B,PIK3R1,CSNK2 |
| 24 | PDGF Signaling (90) | 0.987 | 0.078 | -2.646 | ABL1,AK2,CSNK2B,MAP3K5,FPS2,PIK3R1,AK2,MAP3K5 |
| 25 | Type II Diabetes Mellitus Signaling (128) | 0.944 | 0.070 | -2.333 | NFKB1,MAP3K5,TNFRSF1B,PIK3R1,CSNK2,FPS2,PIK3R1,AK2 |
| 26 | IL-15 Signaling (76) | 0.904 | 0.107 | NUMI | NFKB1,AK2,TFX |
| 27 | autophagy (62) | 0.859 | 0.081 | NUMI | CTSW,ATG3,ATG5,ATG7 |
| 28 | IL-2 Signaling (64) | 0.818 | 0.078 | -2.000 | CSNK2B,FPS2,PIK3R1,CSNK2B,FPS2 |
| 29 | PPARa/RXRα Activation (180) | 0.759 | 0.061 | 3.000 | TGFBR2,NFKB1,AK2,IL18R,MAP3K5,PIK3R1,CSNK2 |
| 30 | TNFα (32) | 2.210 | 0.140 | -2.646 | NFKB1,MAP3K5,PIK3R1,CSNK2,PIK3R1,CSNK2B |
| 31 | STAT3 Pathway (74) | 0.641 | 0.068 | -1.342 | TGFBR2,AK2,MAP3K5,PIK3R1,CSNK2B |
| 32 | Nitric Oxide Signaling in the Cardiovascular System (113) | 0.633 | 0.062 | -2.646 | IFNGR2,VEGF,VEGF,AK2,PIK3R1,CSNK2B,PIK3R1,CSNK2B |
| 33 | Osteoarthritis Pathway (210) | 3.370 | 0.100 | -2.524 | NFKB1,CREB1,NOTCH1,TNFRSF1B,VEGF,AK2,PIK3R1,CSNK2B,PIK3R1,CSNK2B |
earlier, that Table 10 includes a list of expression log ratio of 10 up-regulated and 10 down-regulated genes. The forgoing information is mainly from “Ingenuity Knowledge Base” including as the information source for these facts and pathways.

The first up-regulated gene, SNORD15 is a non-coding RNA (ncRNA) gene which involves in the modification of other small nuclear RNAs (snRNAs), located in the nucleus of the eukaryotic cell, which is a major site of snRNA biogenesis, and known as small nuclear RNA (snRNA) [9]. It belongs to C/D box class of snRNA, which function in directing site-specific 2-O-methylation of substrate RNAs [9]. In humans, there are two closely related copies of the U15 snRNA (called SNORD15A and SNORD15B) [10]. Histone H2A type 1-D encoded by HIST1H2AD gene in humans. Histones are basic nuclear proteins that are responsible for the nucleosome structure of chromosomal fiber in eukaryotes. LINC00305 is associated with atherosclerotic plaques and monocytes [11]. Overexpression of LINC00305 promoted the expression of inflammation-associated genes in THP-1 cells and reduced the expression of contractile markers in co-cultured human aortic smooth muscle cells. LINC00305 overexpression activated NF-κB and inhibition of NF-κB abolished LINC00305-mediated activation of cytokine expression [12]. HHIPL-2 identified as a candidate gene involved in iron-related modulation of osteoblast markers. The excess of iron limits HHIPL-2 gene expression and decreases osteoblastic activity in human MG-63 cell [13].

Whereas, the “High Mobility group Nucleosome Domain 1 Pseudogene 3” (HMGN1P3) is a down-regulated pseudogene 3, and belongs to NURSA nuclear receptor

Figure 3: Effect on eukaryotic translation initiation factors (EIF2) signaling pathway in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. EIF2 was down-regulated by δ-tocotrienol treatment, which is involved in protein synthesis, requires a large number of polypeptides. EIF2 is a GTP-binding protein, which initiates specific form of met-tRNA onto the ribosome.
signaling pathways expression of HMGN1P3 gene, and involves in all type of cancers (from breast, prostate, pancreas, colon kidney, lung, ovary, uterus) [14, 15]. The small nuclear RNA (SNORA67) is also a down-regulated non-coding RNA molecule that belongs to the H/ACA class of snoRNA, which guide the sites of modification of uridines and pseudouridines [16]. The ISY1-RAB43 is the naturally occurring read-through transcription gene, which act between the neighboring ISY1 (splicing factor homolog) and RAB43 (member RAS oncogene family) gene on chromosome 3. The read-through transcript encodes a protein that shares sequence identity with the upstream gene product, but its C-terminus is distinct due to a frameshift relative to the downstream gene [17]. The Rho/Rac guanine nucleotide exchange factor 18 (ARHGEF18) is GTP binding proteins that regulate a number of cellular functions such as, cytoskeletal rearrangements, gene transcription, cell growth and motility [18].

The KLRC4-KLRK1 gene represents also naturally occurring down-regulated read-through transcription gene, which acts between the neighboring KLRC4 (killer cell lectin-like receptor subfamily C, member 4) family. This protein and its ligands are therapeutic targets for the treatment of immune diseases and cancers [19]. Histone H3.1 is a protein that in human encoded by the HIST1H3J gene [20, 21]. Histones are basic nuclear proteins that are responsible for the nucleosomes fiber in eukaryotes. The methenyltetrahydrofolate synthetase (MTHFS) is down-regulated encoded an enzyme that catalyzes the conversion of 5-formyltetrahydrofolate to 5, 10-methenyltetrahydrofolate, and helps regulate carbon flow through the folate-dependent one-carbon metabolic

Fig. 4 Effect on protein ubiquitination signaling pathway in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. The protein ubiquitination pathway was down-regulated by δ-tocotrienol treatment. It plays a major role in the degradation of regulatory proteins, including a variety of cellular processes, such as cell cycle, cell proliferation, DNA repair, apoptosis, transcription regulation, cell surface receptors, ion channel regulation and antigen presentation.
network [22, 23]. The small nucleolar RNA, H/ACA box 16A (SNORA16A) gene provides a unified query environment for genes defined by sequence [24].

The study also provides an insight of correlation of signaling pathways and transcriptional factors and subsequently describes the modulation of anti- as well as pro-inflammatory genes. It described the effects δ-tocotrienol in chronic hepatitis C patients on gene expression of liver cancer, liver hyperplasia, cell proliferation, cell growth, cell death/survival, infections, inflammatory diseases, and apoptosis. Collectively, the effects of δ-tocotrienol on "canonical pathways" observed in IPA of total mRNA sample of hepatitis C patients resulted in modulation of over 360 pathways, which are associated with multiple signaling pathways. It is conceivable that some or most of these pathways may be controlled by the proteasome, since the protein ubiquitination pathway was down-regulated by δ-tocotrienol treatment as described previously [1].

The important signaling pathways modulated by tocotrienols are as follows: at the top of the list is "eukaryotic translation initiation factors" (EIF2) signaling pathway (Fig. 3). This is involved in protein synthesis, and requires a large number of polypeptides. EIF2 is a GTP-binding protein, which initiates specific forms of met-tRNA onto the ribosome. Its important function is to deliver charged initiator met-tRNA to the ribosome, it also identifies the translational starting site [9]. This is followed by protein ubiquitination pathway, which plays a major role in the degradation of short-lived or regulatory proteins. It plays a role in a variety of cellular processes, such as cell cycle, cell proliferation, apoptosis, DNA repair, transcriptional regulation, cell surface receptors, ion channels regulation and antigen presentation, as outlined in Fig. 4 [10]. We have discussed the importance of ubiquitination in our several earlier publications [11–15].

δ-Tocotrienol treatment of chronic hepatitis C patients also affects several other regulators in canonical pathways,
we will limit our discussion to only important signaling and biomarkers associated with present investigation. The toll-like receptor signaling (TLRs) belongs to the family of pathogen-associated pattern recognition receptors, and bind to specific molecular patterns in bacteria and viruses. The pathogen-associated ligands include bacterial flagellin, viral DNA, lipopolysaccharide (LPS) and CpG DNA motifs. TLRs form a complex with different combinations of adaptor molecules like MYD88, TRAF6 and TIRAP to initiate signal transduction upon ligand binding. This binding triggers a cascade of signaling events via the TLR-adapter complex, and downstream signaling molecules like p38MAPK, JNK. NF-κB activated and translocated into the nucleus, where they activate transcription regulators like c-Fos and c-Jun, leading to the induction of several pro-inflammatory cytokines, eventually leading to antibacterial and antiviral responses [25, 26]. Tocotrienol treatment causes a downregulation of the TLR pathways in hepatitis C patients. The toll-like receptor signaling pathways outlined in Fig. 5.

The signal transducers and activators of transcription (STATs) are a family of cytoplasmic proteins with Src homology-2 (SH2) domains. STATs acts as a signal messenger and transcription factors. It participates in normal cellular responses to cytokines and growth factors. STATs pathways activated via tyrosine phosphorylation cascade after ligand binding by stimulation of the cytokine receptor-kinase complex and growth factor-receptor complex. The IL-6 cytokine activates STAT3 and STAT1. STAT3 encoded in human gene. The STAT3 signaling pathway (Fig. 6) plays an important role in normal development, particularly hematopoiesis, and regulates cancer metastasis by regulating the expression of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and tumor immune evasion [27–29].

![Fig. 6 Effect on signal transducer and activators of transcription (STATs) signaling pathways in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. The STATs were down-regulated by δ-tocotrienol treatment, and belong to a family of cytoplasmic proteins with Src homology-2 (SH2) domains that acts as signal messenger and transcriptional factors and responses to cytokines and growth factors. The STAT pathways are activated via tyrosine phosphorylation cascade and play an important role in normal development of hematopoiesis, and regulates cancer metastasis by regulating the expression of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and tumor immune evasion.](image-url)
The nuclear factor kappa B (NF-κB) transcription factors are key regulators of gene expression and acts in response to stress and the development of innate and acquired immunity [30]. A multitude of extracellular stimuli (such as cytokines, infections, oxidative, DNA-damaging agents, UV light, osmotic shock) can lead to NF-κB activation. NF-κB activators mediate the site-specific phosphorylation of serine on IκB (inhibitor of NF-κB), resulting in IκB ubiquitination and subsequent proteasomal destruction [31]. The pathway highlights the important components of the NF-κB signaling pathway outlined in (Fig. 7). Inhibiting this pathway by proteasome inhibitors would possibly expected to cause cell death of infected hepatic cells.

The catalytic activity of iNOS is to kill or inhibit the growth of invading viruses and microorganisms. It produces nitric oxide from L-arginine [32, 33]. Nitric oxide is a free radical effector of the innate immune system that can directly inhibit pathogen replication. A variety of extracellular stimuli can activate signaling pathways that converge to initiate expression of iNOS. Moreover, components of cell wall of bacteria (lipopolysaccharide; LPS) or fungi trigger the innate immune signaling cascade leading to expression of iNOS [34–36]. This leads to activation of NF-κB and p38 MAPK signaling pathways [37]. NF-κB in the nucleus binds to NF-κB elements in the iNOS 5′ flanking region, triggering iNOS transcription. Cytokines released from the infected host

**Fig. 7** Effect on nuclear factor kappa B (NF-κB) in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. δ-Tocotrienol modulates NF-κB transcription factors, which are key regulators of gene expression and act in response to stress and the development of innate and acquired immunity. A number of NF-κB activators mediate the site-specific phosphorylation of serine on IκB (inhibitor of NF-κB), thereby marking IκB for ubiquitination and subsequent proteasomal destruction.
cell also activate nitric oxide production. IFNγ activates JAK family kinases to trigger JAK/STAT signaling, leading to synthesis of the transcription factor IRF1 and stimulation of a large number of iNOS mRNA transcription [38]. The iNOS signaling pathways (Fig. 8) shows all possible regulators of production of nitric oxide, and highlights the important molecular events leads to production in macrophages. Collectively, IFN-γ induced by δ-tocotrienols would be expected to modulate the JAK/STAT pathway and NO production.

Interleukin-6 (IL-6) is a regulator of acute phase responses and a lymphocyte stimulatory factor. The central role of IL-6 is for the management of infectious and inflammatory diseases [39]. IL-6 responses transmitted through glycoprotein 130 (GP130), which serves as the universal signal-transducing receptor subunit for all IL-6 related cytokines. Moreover, IL-6-type cytokines utilize tyrosine kinases of the Janus kinase (JAK) family and signal transducer/activators of STAT transcription family as major mediators of signal transduction [40]. In addition to the JAK/STAT pathway of signal transduction, IL-6 also activates the extracellular signal-regulated kinases (ERK1/2) of the mitogen activated protein kinase (MAPK) pathway (Fig. 9). The upstream regulators of ERK1/2 include RAS and the src homology-2 containing proteins GRB2 and SHC. The SCH protein activated by JAK2 and thus serves as a link between the IL-6 activated JAK/STAT and RAS-MAPK pathways shown in IL-6 signaling pathway Fig. 9 [41]. Furthermore, phosphorylation of MAPks in response to IL-6 activated RAS results in the activation of nuclear factor IL-6 (NF-IL-6), which in turn stimulates the transcription of the IL-6 gene. IL-6 gene transcription is also stimulated by TNF-α and IL-1 via activation of NF-κB

**Fig. 8** Effect on nitric oxide synthase (iNOS) in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. The iNOS was down-regulated by δ-tocotrienol treatment. It produces nitric oxide from L-arginine, a cytotoxic weapon generated by macrophages. The catalytic activity of iNOS is to kill or inhibit the growth of invading microorganisms. Nitric oxide is a free radical effector of the innate immune system that inhibits pathogen replication. A variety of extracellular stimuli (components of bacteria and fungi) can activate signaling pathways that help to initiate expression of iNOS.
The tumor necrosis factor receptor (TNFR1) belongs to a family of 20 in mammalian cells. TNF-α, an important cytokine involves in cell proliferation, differentiation, and apoptosis modulate immune responses and induction of inflammation [44]. TNF-α functions through two receptors, TNFR1 TNFR2. TNFR1 is expressed in human tissue and TNFR2 expressed in immune cells (Fig. 10) [44, 45]. δ-Tocotrienol also inhibits expression of IL-6 and TNFR induction in chronic hepatitis C patients.

Autophagy is a basic catabolic mechanism that involves cellular degradation of unnecessary or dysfunctional cellular components through the actions of liposome [46, 47]. Autophagy is generally activate by condition of nutrient deprivation but has also been associated with physiological as well as pathological processes such as development, differentiation, neurodegenerative diseases, stress, infection, and cancer [47–49]. The mammalian target of rapamycin (mTOR) kinase is a critical regulator of autophagy induction, with activated mTOR (AKT and MAPK signaling) suppressing autophagy, and negative regulation of mTOR (AMPK and p53 signaling) promoting it [48]. The autophagy pathway (Fig. 11) highlights the key molecular events involved in triggering autophagy. Inhibiting the proteasome activity also causes the onset of autophagy, as observed with δ-tocotrienol treatment.

Whereas, apoptosis is a coordinated energy-dependent process that involves the activation of a group of cysteine proteases called caspases and a cascade of events that link the initiating stimuli to programmed cell death [50]. The two main pathways of apoptosis are the intrinsic and extrinsic pathways. Each pathway requires specific triggers to initiate a cascade of molecular events that converge at the stage of caspase-3 activation [50]. The activation of caspase-3 in turn triggers an execution pathway resulting in characteristic cytomorphological features including cell shrinkage, membrane blabbing, chromatin condensation and DNA fragmentation [51]. Further details of intrinsic and extrinsic pathways were found in the attached Ingenuity Apoptosis Signaling Pathway (Fig. 12), which highlights the key molecular events involved in trigging apoptosis.
Beside these, other regulators were also affected by \(\delta\)-tocotrienol treatment of hepatitis C patients, and they are interferon signaling, IL-2 signaling, and HMGB1 signaling, Cardiac hypertrophy signaling, Th1 and Th2 activation pathway, production of nitric oxide and reactive oxygen species in macrophages, Osteoarthritis pathway, PPAR signaling, type1 diabetes mellitus signaling, Type II diabetes mellitus, and insulin receptor signaling. In summary, EIF2 signaling regulator is at the top of the canonical pathway list but its fold change expression value is 221 as compared to protein ubiquitination pathway is 265 fold. On the other hand, osteoarthritis (210 fold), mammalian target of rapamycin (mTOR-201 fold), IL-8 (197 fold), Th1-Th2 (185 fold), PPAR\(\alpha/RXR\alpha\) activation (180 fold), NF-\(\kappa\)B (181 fold), IL-6 (128 fold), Type II diabetes mellitus signaling (128 fold), and nitric oxide signaling in cardiovascular system (113 fold), all have lower fold change expression compared to EIF2. This indicates the importance of \(\delta\)-tocotrienol on so many biological activities and signaling pathways (Table 11). The importance of most of these regulators was discussed in our several publications during course of the last decade [1, 11–15].

**Fig. 10** Effect on tumor necrosis factor receptor1 (TNFR1) regulator of gene expression in plasma of total mRNAs obtained from \(\delta\)-tocotrienol treatment of hepatitis C patients. The TNFR1 was down-regulated by \(\delta\)-tocotrienol treatment, and belongs to a family of 20 in mammalian cells. TNF-\(\alpha\) is an important cytokine involved in cell proliferation, differentiation, apoptosis, modulates immune responses and induction of inflammation. TNF-\(\alpha\) functions through two receptors, TNFR1 and TNFR2. TNFR1 is expressed in human tissue, and TNFR2 is expressed in immune cells.

**Conclusions**

Present results of fold-change expression data analyzed by “Ingenuity Pathway Analysis” describe the effect of \(\delta\)-tocotrienol in chronic hepatitis C patients on biological mechanisms at molecular level. It also revealed an insight of correlation of signaling pathways and transcriptional factors. Recently, two comprehensive reviews on the several biological activities of tocotrienols as hypocholesterolemic, anti-inflammatory, anticancer, antioxidant, neuroprotective, skin protection benefits, bone health and longevity have been published [52, 53]. These articles also cover the beneficial properties of different isomers of tocotrienols treatment along with possible mechanisms, signaling pathways in breast, prostate, pancreas, rectal cancers in cell lines and humans [52, 53]. Major signaling pathways that were affected by \(\delta\)-tocotrienol treatment in chronic hepatitis C subjects are summarized in the Table 12. The collective results indicate that tocotrienols inhibit cancer cell proliferation, promotes cell cycle arrest, decreases angiogenesis and acts via multiple signaling pathways [1]. Our present results are consistent with these conclusions and \(\delta\)-tocotrienol treatment of hepatitis C patients, acts by increasing cell death, and necrosis of
Fig. 11 Effect on autophagy in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. The autophagy modulated by δ-tocotrienol treatment of hepatitis C patients. Autophagy is a general term for the basic catabolic mechanism that involves cellular degradation of unnecessary or dysfunctional cellular components through the actions of lysosome. Autophagy is generally activated by conditions of nutrient deprivation but it has also been associated with physiological as well as pathological processes such as development, differentiation, neurodegenerative diseases, stress, infection, and cancer. The mammalian target of rapamycin (mTOR) kinase is a critical regulator of autophagy induction.

Fig. 12 Effect on apoptosis in plasma of total mRNAs obtained from δ-tocotrienol treatment of hepatitis C patients. Apoptosis modulated by δ-tocotrienol treatment of hepatitis C patients. Apoptosis is a coordinated energy-dependent process that involves the activation of a group of cysteine proteases called caspases and a cascade of events that link the initiating stimuli to programmed cell death. There are two main pathways of apoptosis, the intrinsic and extrinsic as shown here.
Table 12 Major signaling pathways affected by δ-tocotrienol treatment in chronic hepatitis C subjects

| Pathway                                      | Down-regulated by δ-tocotrienol treatment | Up-regulated by δ-tocotrienol treatment |
|---------------------------------------------|------------------------------------------|----------------------------------------|
| Proliferation of immune cells               | Cell death and survival                   | Cell death and survival                   |
| Proliferation of mononuclear leukocytes     | Necrosis of malignant tumor               | Necrosis of malignant tumor               |
| Viral infection                             | Gene expression                          | Gene expression                          |
| Free radical scavenging                     | Organismal Death                         | Organismal Death                         |
| Endocrine system disorder, Diabetes mellitus| Cell death of cancer cells                | Cell death of cancer cells                |
| Mobilization of Ca2+                        | Cell death of tumors                      | Cell death of tumors                      |
| Replication of virus                        |                                         |                                         |
| HIV infection, replication of Influenza virus|                                         |                                         |

malignant tumors, and by decreasing viral infection, cellular growth and proliferation, decreasing endocrine system disorders such as diabetes mellitus, and mobilization of calcium. Therefore, tocotrienols can safely be used for hepatitis C patients, without any side effects.

Additional files

Additional file 1: Table S1. Effect of d-tocotrienol on down-regulation of gene expression of "Molecules" (1-75) of IPA analyses in hepatitis C patients. (XLS 68 kb)

Additional file 2: Table S2. Effect of d-tocotrienol on down-regulation of gene expression of "Molecules" (76-150) of IPA analyses in hepatitis C patients. (XLS 68 kb)

Additional file 3: Table S3. Effect of d-tocotrienol on down-regulation of gene expression of "Molecules" (151-220) of IPA analyses in hepatitis C patients. (XLS 67 kb)

Abbreviations

EF2: Eukaryotic translation initiation factors; ICAM1: Intercellular adhesion molecule1; IL-6: Interleukin-6; IPA: Ingenuity Pathway Analysis; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappaB; TNF-α: Tumor necrosis factor-α; VCAM1: Vascular cell adhesion molecule1

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Author’s contributions

AAQ and DAK conceived and planned the study to carry out RNA-sequence analysis after feeding δ-tocotrienol to chronic hepatitis C patients; AAQ wrote the manuscript. DAK and SM carried out human study and prepared total mRNAs after feeding δ-tocotrienol to chronic hepatitis C patients. SQY and MX have carried out RNA-sequence analyses, including data analyses. NQ has edited the manuscript and also involves in data analyses of RNA-sequence. NQ, and DAK were also involved in proof reading of this manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study carried out at the Pakistan Ordinance Factory (POF) Hospital, Wah Cantonment, Rawalpindi, 64,000, Pakistan, in collaboration with the Department of Basic Medical Sciences, University of Missouri-Kansas City, MO, USA. The study protocol registered (IRB # 129–2015) and approved by Institutional Review Board of POF Hospital, Rawalpindi, 64,000, Pakistan. All subjects signed an informed-consent form, which approved by Institutional Board of POF Hospital, Rawalpindi, 64,000, Pakistan. The purified total RNA samples delivered at UMKC, School of Medicine after getting approval by the members “Compliance Officer (Christopher Winders)” and “Chemical Biological Safety Officer (Timothy Sturgis, RBP)” of Institution Board of UMKC School of Medicine, Kansas City, MO, USA.

Consent for publication

All contributing authors agree to the publication of this article.

Competing interests

The authors declare that they have no competing interests.

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Author details

1Department of Biomedical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA.
2Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), National University of Medical Sciences, Rawalpindi 64000, Pakistan.
3Division of Experimental and Translational Genetics, Department of Pediatrics, Children’s Mercy Hospital, 2401 Gillham Road, Kansas City, MO 64108, USA.
4Department of Biomedical and Health Informatics, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA.
5Pharmacology/Toxicology, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA.

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