DOES STAT3 KNOCKDOWN AFFECT HCV REPLICATION

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ABSTRACT

Hepatitis C virus (HCV) is considered as a major health problem, affecting more than 170 million individuals worldwide. Host genes were found to modulate viral infection. Studies from animal models have shown that STAT3 plays an important role in cell proliferation, survival, and transformation in the liver. STAT proteins play an important role in the regulation of inflammatory responses by Antigen presenting cells (APCs). This study aimed to evaluate the effect of STAT 3 silencing on HCV replication. Con1 cells were transfected with 5nM siSTAT3 using high perfect transfection reagent. STAT3 and HCV gene expression were determined using Quantitative real time RT-PCR. The STAT3 knockdown efficiency was 65.31% as compared to negative control siRNA treated cells. HCV normalized copy numbers did not show significant changes as compared to negative control siRNA treated cells. We concluded from this study that the role played by STAT3 in HCV replication could be accompanied by other roles from other pathways. Consequently, STAT3 knockdown alone is not enough to suppress HCV replication.

INTRODUCTION

Hepatitis C virus (HCV) is one of the leading causes of chronic liver disease, affecting more than 170 million individuals worldwide [1]. It is one of the main causes of liver-related morbidity and mortality. The virus establishes a persistent liver infection, leading to the development of chronic hepatitis, liver cirrhosis, and hepatocellular carcinomas. HCV belongs to the genus Hepacivirus of the family Flaviviridae. HCV is a single stranded, positive-sense RNA virus with a genome of approximately 9500 nucleotide [2].

Because of the effectiveness and specificity in gene silencing, siRNAs (small interfering RNA) and shRNAs (small hairpin RNA) are expected to be applicable to gene therapies for hard-to-cure diseases, such as HIV infection [3]. Both siRNA and shRNA induce cleavage and
degradation of RNA molecules in a sequence-specific manner\textsuperscript{[4]}. RNA interference (RNAi) occurs in a variety of organisms, including \textit{Caenorhabditis elegans}, \textit{Trypanosoma brucei}\textsuperscript{[5]}, plants\textsuperscript{[6]}, Drosophila, and mouse embryos\textsuperscript{[7]}. In most of these organisms, the injection of a double-stranded RNA (dsRNA) longer than 500 bp specifically suppresses the expression of the gene with the corresponding DNA sequence, but has no effect on genes with unrelated Sequences. Therefore, utilization of RNAi technology is appropriate as a novel therapy against HCV\textsuperscript{[8]}. Host genes modulate viral infection and are an underappreciated target for antiviral therapy. From the important host genes playing role in HCV infection are the transducer and activator of transcription (STAT3).

STAT-3 is an oncogenic transcription factor that is activated upon tyrosine phosphorylation in response to extracellular signals, such as cytokines and growth factors\textsuperscript{[9]}. Studies from animal models have shown that STAT3 plays an important role in cell proliferation, survival, and transformation in the liver\textsuperscript{[10]}; however, the role of STAT3 in human liver disease is less clear. STAT proteins play an important role in the regulation of inflammatory responses by Antigen presenting cells (APCs). In monocytes, STAT3 is a particularly critical transcription factor in limiting excessive inflammatory responses. It is commonly activated via canonical JAK-STAT signaling where a cytokine or extracellular factor binds its cognate receptor, resulting in the auto-phosphorylation of JAK and subsequent tyrosine phosphorylation of STAT3. In addition to STAT-3 activation by tyrosine phosphorylation, Ser727 phosphorylation mediated by mitogen-activated protein kinases (MAPKs) contributes to its maximal transcriptional activity\textsuperscript{[1]}. Knockout of STAT3 in macrophages dysregulates inflammatory responses and leads to severe colitis. In addition, macrophages and dendritic cells within the tumor microenvironment constitutively phosphorylate STAT3 and suppress the generation of potent anti-tumor T cell responses. Importantly, treatment with pharmacologic STAT3 inhibitors reverses the suppression. Recently, the activation of STAT3 has been shown to be responsible for inducing genes involved in the differentiation of myeloid-derived suppressor cells\textsuperscript{[11]}. 

**MATERIAL AND METHODS**

**Cell culture propagation:** Human Con1 cells (Huh-7 expressing HCV con1 I377/NS3-3’ replicon) (kind gift from Dr Charles Rice, USA) were used in siRNA transfection experiments. Cells were maintained in the proper conditions. The cells were cultured in DMEM medium supplemented with 100 IU/ml penicillin G sodium, 100 IU/ml streptomycin
sulfate, 1% L-glutamine, 1% G418 and 10 % fetal bovine serum (FBS) at 37 ºC in a humidified incubator with 5 % CO2. The cells was harvested after trypsinization and washed twice with Dulbecco's phosphate-buffered saline (DPBS). When the cell density reached approximately 80 %, cells were split for further culture. Experiments were conducted when the cells were in the logarithmic growth phase.

**siRNA transfection:** Cells were seeded in 24 well plate at a seeding density of 30,000-40,000 cells / well. Next day, cells were transfected with the siSTAT3 (siRNA targeting STAT3 which was provided by Qiagen (USA)) and the all star negative control siRNA (Qiagen, USA) at 5 nM concentrations. Hiperefect transfection reagent (Qiagen) was used for transfection. Briefly, siRNA was diluted using serum free media and then 3ul of transfection reagent was added and incubated for 15 mins to form the transfection complex, after that this complex was added to the cells. AllStars negative control siRNA (Qiagen) was used as control. The media was changed 24 hours after transfection and the cells were incubated with complete media for another 72 hours.

**RNA isolation, clean up and Quantitative real time RT-PCR:** RNA was isolated using Qiazol buffer (Qiagen, USA) according to manufacturer instruction. RNA was subsequently cleaned up using RNAeasy mini Kit (Qiag en, USA). HCV and STAT3, copy numbers were quantified using QuantiFast Sybergreen RT-PCR. The copy numbers were normalized to the house keeping beta actin gene. HCV primer sequence was as follow: Forward, 5’aaaactactgtttcaegcagaa 3’; reverse, 5’tgctcatggtgcacggtcta3’. The sequence for human Beta-actin was Forward, ccttcctgggcatggatctc; Reverse, ggagcaatgatcttgatcttc. Primers for STAT3 were provided by Qiagen, (USA). The RT and subsequent PCR cycling conditions were as follow, 50ºC for 10 mins, 95ºC for 5 mins, 60ºC for 30sec, then 95ºC for 15 seconds, the number of cycles were 40 cycles.

**RESULTS**
As demonstrated in fig (1), Con1 cell transfection with 5nM siSTAT significantly knocked down the STAT3 gene as compared to negative control siRNA treated cells. Knocking down efficiency was 65.31% as compared to negative control siRNA treated cells. The cells treated with siSTAT did not show significant change in HCV normalized copy numbers as compared to negative control siRNA treated cells, illustrated in fig (2).
**Fig (1): STAT3 gene knockdown.**

U, untreated Con1 cells; siNC, cells treated with all star negative control. siSTAT3, cells treated with siRNA targeting STAT3 gene. Data are represented as mean ± SEM, *Significant difference as compared to negative control treatment, P<0.05.

**Fig (2): The effect of STAT3 knockdown on HCV replication.**

U, untreated Con1 cells; NC, cells treated with all star negative control siRNA; siSTAT3, cells treated with siRNA targeting STAT3 gene. Data are represented as mean ± SEM.

**DISCUSSION**

Cellular stress response pathways modulate HCV replication, including the STAT3 and heat shock (Hsp70/HSPA1A). Previous studies have observed the activation of these pathways...
pathways during HCV replication, so it is likely that these pathways are responding, at least in part, to HCV infection [12].

Yahya, [13] found that HCV knockdown resulted in significant reduction in STAT3 gene expression in Con1 cells. This finding highlights the importance of this gene in HCV replication. Building on this finding, the current study was conducted to study the effect of STAT3 gene knockdown on HCV replication. The study revealed that STAT3 gene knockdown had no significant effect on HCV replication.

HCV core-induced STAT3 activation plays a critical role in the alteration of inflammatory responses by APCs which leads to impaired anti-viral T cell responses during HCV infection [11]. Once STAT-3 is activated in HCV-infected cells, it may regulate gene expression of survival factors to ensure an antiapoptotic environment in the cells, a situation favorable for oncogenesis [14]. STAT-3 exerts its growth-deregulating activity by activating the expression of cellular genes that are involved in cell cycle progression, such as fos, cyclin D1, myc, and pim-1, and by activating antiapoptotic proteins, such as Bcl-2 and Bcl-XL (9,14, 15, 16).

We concluded from this study that the role played by STAT3 in HCV replication could be accompanied by other roles played by other pathways. Consequently the silencing of STAT3 alone is not enough to knockdown the virus replication. Alternatively, we suggest further extensive studies to knockdown several host factors in order to knockdown HCV replication.

REFERENCES


