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Original article

Targeting the effect of sofosbuvir on selective oncogenes expression level of hepatocellular carcinoma Ras/Raf/MEK/ERK pathway in Huh7 cell line

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ABSTRACT

Direct acting antiviral agents are emerging line of treatment to eradicate Hepatitis C virus. Recent controversy over whether direct acting antiviral agents increase rate of hepatocellular carcinoma in HCV patients or prevent it, has increased the need to elaborate underlying mechanisms on molecular basis. This work was aimed to investigate the effect of sofosbuvir on the expression of selected oncogenes from the Ras/Raf/MEK/ERK pathway in Huh7 cell line. Results found concrete molecular evidence that sofosbuvir has significantly altered the expression of selected genes when huh7 cell line was treated with sofosbuvir. Nine genes related to HCC were found to be affected by sofosbuvir in a mixed effect manner. The relative expression of growth factors (VEGF, PDGFRB and HGF) was increased in sofosbuvir treated cell lines. The kinase family genes H-RAS, B-RAF, MET except MAPK1 were downregulated. Similarly, DUSP1 was upregulated and SPRY2 was slightly downregulated; both were negative feedback inhibitors of ERK signalling cascade. Sofosbuvir upregulated the growth factors and MAPK1 which suggests it to be a carcinogen. The downregulation of kinases and upregulation of DUSP1 make it an anticancer drug. Hence, the results from this study are important to prove that sofosbuvir neither reduce nor induce hepatocellular carcinoma.

1. Introduction

Chronic HCV infection often leads to cirrhosis, fibrosis and hepatocellular carcinoma (HCC) which is second major cause of cancer related mortalities (Goossens and Hoshida, 2015). HCV induced HCC is major concern of scientific community working on HCV eradication. The clear-cut mechanism of HCV linked HCC has not been elucidated in previous studies. The bouts of recurrent hepatitis cause the production of reactive oxygen species. These reactive oxygen species induce inflammation, necrosis and ultimately cell proliferation which promotes hepatocellular carcinoma, cirrhosis and fibrosis (Navab et al., 2008).

Molecular analyses revealed that alterations in several cellular signaling pathways are associated with hepatocellular carcinoma. The deregulation of these pathways severely affects the cellular growth, apoptosis, cell cycle and several other cellular processes. Mounting evidence are there which depicts the role of these pathways in hepatocarcinogenesis. The better understanding of changes in these pathways is necessary for the development, efficacy and safety of novel therapeutics. Selumetinib, Salirasib and Sorafenib are anticancer drugs which target several kinases of Ras/Raf/MEK/ERK pathway (Yang and Liu, 2017). Ras/Raf/MEK/ERK signaling pathway is observed to be deregulated in multiple sorts of cancer (Guo et al., 2020).

The activating mutations in H-RAS and B-RAF are most common in hepatocellular carcinoma. These mutations lead to hyperstimulation of MEK/ERK cascade, thus increasing hepatocyte proliferation and metastasis (Sui et al., 2012; Aravalli et al., 2018; Gnoni et al., 2019). The overexpression of MAPK1 (ERK) is strongly linked with HCC (Guégan et al., 2012). The activating mutations of several growth factors and their receptors were found in HCC. The overexpression of hepatocyte growth factor (HGF) and its receptor c-Met promotes metastasis of hepatocarcinoma (Wang et al., 2020). Activated mutations in vascular endothelial growth factor (VEGF) is

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crucial for angiogenesis of hepatocarcinoma cells (Zhang et al., 2012). Platelet derived growth factor receptor B (PDGFRB) is linked with regeneration and recurrence of hepatocellular carcinoma. The overexpression of PDGFRB was linked with decreased survival rate of those patients who undergone resection of HCC (Patel et al., 2011).

The HCV treatment strategies aim to achieve sustained virological response (SVR). The DDAs alone or in combination of other drugs have increased the SVR up to 90 percent and decreased the treatment duration up to 12 weeks (Asselah et al., 2016). Debate has been emerging about the occurrence and recurrence of hepatocellular carcinoma in HCV patients receiving DAAs therapy (Tayyab et al., 2020; Ogawa et al., 2020; Luna-Cuadros et al., 2022). Over the time, a series of clinical studies reported the efficacy of DAAs therapy in reducing the risk of HCC development in cirrhotic HCV patients. Researchers have also reported that the DAAs therapy cannot eradicate the risk of HCC recurrence permanently (Kanwal et al., 2017; Li et al., 2018; Nahon et al., 2018; Singer et al., 2018; Singh et al., 2018; Ioannou et al., 2018; Nakano et al., 2019; Pons et al., 2020). Few reports claimed that DAAs therapy rapidly increased the risk of HCC in HCV patients (Reig et al., 2016; Nakao et al., 2018; Renzulli et al., 2018; Renzulli et al., 2018). However, considerable body of literature found no such complaint of HCC recurrence after achieving SVR through DAAs. Rather, it has been demonstrated that DAAs therapy has reduced the liver related complications and chances of HCC ocurrence and recurrence (Prenner et al., 2017; Huang et al., 2018; Lledó et al., 2018; Park et al., 2019; Young et al., 2019; Dang et al., 2020).

However, in this scenario, it is much difficult to make an unequivocal conclusion about the subject matter. The reason for this difficulty is the heterogeneous population and statistical methodology adopted by these studies. Such population based observational, cross-sectional and evidence-based studies lacked the molecular biology approach. Notwithstanding the ongoing debate, two contradictory hypotheses were emerged. The first hypothesis is that the recurrence of hepatocellular carcinoma in HCV patients is the indirect effect of DAAs therapy. The off-target effects of DAAs (sofosbuvir) might include some immunological changes such as decreased natural cell activation linked with the inhibition of cytotoxic activity (Giovannini et al, 2020). The second hypothesis is the hepatoprotective and anticancer activity of direct acting agents. The proposed mechanism for this assumption is the positive impact of these agents on the expression level of HCC linked signaling proteins. The present study is important to highlight the investigations on impact of sofosbuvir on gene expression of human hepatoma cell line. The study also signifies hepatocarcinogenesis related to Ras/Raf/MEK/ERK signaling pathway in hepatocytes. The study is first report to elaborate effect of sofosbuvir on gene expression in hepatocellular carcinoma.

2. Materials and methods

2.1. Cell culturing and sofosbuvir treatment

Standard sofosbuvir drug was purchased from local pharmaceutical market under the brand name Sovaldi. Cryopreserved human hepatoma Huh-7 cells were harbored at Molecular Biochemistry Laboratory, Government College University, Faisalabad, Pakistan.

2.1.1. Maintenance of cell culture

The cells were maintained on Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco Life Science Technologies, USA), streptomycin (100 μg/ml) and penicillin (100 U/ml) The cells were routinely checked for mycoplasma contamination (Cervello et al. 2013).

2.1.2. Cell viability and sofosbuvir toxicity analysis

Cell viability assay was performed to optimize the dose of sofosbuvir. Trypan blue exclusion assay was used to find percent cell viability. Huh7 cell culture was treated with sofosbuvir (0–80 μM) dissolved in 0.1% solution of dimethyl sulfoxide for 24 h. The control group was treated with only 0.1% solution of dimethyl sulfoxide for the period of 24 h in 96 well plates (Strober, 2015).

2.2. Gene expression analysis

2.2.1. RNA extraction and assessment

The total RNA was extracted from Huh-7 cells. Extracted RNA was subjected to the qualitative and quantitative assessment. Denaturing agarose gel (1%) electrophoresis was used to assess the quality and integrity of total RNA. Formaldehyde agarose gel 1 % (w/v) was used to check the integrity of RNA. Nanodrop-1000 (Thermo Fisher Scientific) spectrophotometer was used to quantify the extracted RNA. cDNA was synthesized by using reverse transcriptase kit (Thermo Fisher) (Rio et al. 2010).

2.2.2. Criteria for selection of genes

All the genes selected for expression analysis were part of Ras/Raf/MEK/ERK signaling pathway and their upregulation or downregulation was linked with HCC. The genes like H-RAS, B-RAF, MAPK1 and MET belong to kinase family while VEGF, PDGFRB and HGF are growth factors. The upregulation of these genes is related with HCC. The other two genes i.e., DUSP1 and SPRY2 were negative feedback inhibitors of Ras/Raf/MEK/ERK pathway, and their downregulation is found in several cases of cancers. TFG was used as reference/housekeeping gene because it was reported as the most stable and efficient gene in hepatoma cell lines treated with certain drugs (Liu et al., 2017).

2.2.3. Selection of primers for gene expression analysis

The primers for target genes were selected from previous qPCR gene expression studies. The selected primers were again verified by using different software and databases. The primers were verified based on their specificity, complementarity, melting temperature and GC content percentage. The primers used in this study matched to different exons. Moreover, the GC contents of all primers were between 50-60 percent, melting temperature was almost 62 °C and there were no more than four repeats of bases. Primers were not self-complementary, thus the chance of primer dimerization during reaction were zero (Yasumura et al., 2004; Charette et al., 2010; Xu et al., 2010; Yang et al., 2015; Singh et al., 2017; Xu et al., 2017a, 2017b; Gu et al., 2018; Liu et al., 2018; Kalimutho et al., 2019). Table 1.

2.2.4. qPCR amplification for gene expression analysis

The SYBR green qPCR (BioRad) was selected for gene expression analysis. The qPCR master mix was prepared with various concentrations of cDNA by taking 10 μL of IQTM SYBR Green (BioRad), 6 μL of PCR graded water and 10 μM of primers of TFG and target genes. The reaction was executed in iQ^{TM}5 Multi-color Real-Time PCR Detection System (BioRad). The qPCR product size was between 80 and 120 bp. The efficiency of qPCR was measured by the Pfaffl linear equations (Pfaffl, 2001).

2.3. Statistical analysis

The relative fold expression of each gene was measured by Pfaffl method. The p-value ($p < 0.05$) was calculated by 'Wilcoxon Signed Rank Test' using GraphPad Prism software.

Table 1
Primer used in qPCR for gene expression analysis.

Gene symbol	Official full name of gene	Forward primer sequence 5́ to 3́ Reverse primer sequence 5́ to 3́	Locus	References
H-RAS	H-RAS proto-oncogene, GTPase	GCTGGTGGCGTAGGCAAGAG CTCCTCTTGACCTGCTGTGTCG	Exon 7	(Charette et al., 2010)
B-RAF	B-RAF protooncogene serine/threonine kinase	CCTCATTACCTGGCTCACTAAC CATCCTCAGAAGACAGGAATCG	Exon 22	(Gu et al., 2018)
MAPK1	mitogen-activated protein kinase 1	AGAGAACCCTGAGGGAGATAAA TATTCGAGCACCAACCATCG	Exon 9	(Xu et al., 2017b)
MET	MET proto-oncogene receptor tyrosine kinase	CCTGGGCACCGAAAGATAAA GTTTACCTTGGTGCAGAGGAG	Exon 24	(Xu et al., 2017a)
VEGF	vascular endothelial growth factor A	TGGTGTCTTCACTGGATGTATTT GAAGAGGAGGAGATGAGAGACT	Exon 9	(Liu et al., 2018)
PDGFR-B	platelet derived growth factor receptor beta	GCTCACCATCATCTCCCTTATC GGAAGGTGATTGAGTCTGTGAG	Exon 24	(Kalimutho et al., 2019)
HGF	hepatocyte growth factor	GGTAAAGGACGCAGCTACAA GATACCACACGAACACAGCT	Exon 20	(Yang et al., 2015)
DUSP1	dual specificity phosphatase 1	CAGCACATTCGGGACCAATA GAAAGGA CTCAGTGTGTGATCC	Exon 4	(Singh et al., 2017)
SPRY2	sprouty RTK signaling antagonist 2	GAGGACAACTGTGCTGACAA TTATGGTGTTACCTTCCAGCC	Exon 4	Xu et al., 2010
TFG	trafficking from ER to Golgi regulator	CAGTACCAGGCGAGCAATTA CTCTCAACCTGGAATGGCTC	Exon 10	(Yasumura et al., 2004)

3. Results

The present research work reports the effect of sofosbuvir on selective oncogenes expression levels of hepatocellular carcinoma Ras/Raf/MEK/ERK pathway in Huh7 cell line to evaluate whether direct acting agents like sofosbuvir are involved in changing the gene expression to cause hepatocellular carcinoma or prevent it.

3.1. Cell culturing, cell viability and sofosbuvir toxicity analysis

Human hepatoma cell line (Huh-7) was used for gene expression analysis. The thawing of suspended cells at 37 °C kept the viability of almost 95% cells. The non-toxic concentration was regarded as that concentration at which more than 80% cells can survive. Results indicated that 36 μM concentration of sofosbuvir was nontoxic to Huh7 cells (Table 2). Therefore, 30 μM dose of sofosbuvir was selected as optimum dose and further used for gene expression analysis.

3.2. Gene expression analysis

3.2.1. Determination of qPCR efficiency

The percentage qPCR efficiency was between 99.19 and 100.02. The slope values were nearly ideal and fell under the normal range (Table 3). These values not only assessed qPCR efficiency but also gave the idea about the quality of cDNA.

Table 2
Analysis of toxicity of sofosbuvir in Huh-7 cells.

Total number of cells/mL	Sofosbuvir concentration (μM)	Viable cells/mL	Percentage (%)
6.125×10^6	0 μM	6.064×10^6	99
6.125×10^6	12 μM	5.758×10^6	94
6.125×10^6	24 μM	5.268×10^6	86
6.125×10^6	36 μM	4.961×10^6	81
6.125×10^6	48 μM	4.471×10^6	73
6.125×10^6	60 μM	4.104×10^6	67
6.125×10^6	72 μM	3.614×10^6	59
6.125×10^6	80 μM	2.695×10^6	44

3.2.2. Real time PCR data analysis

The study showed that sofosbuvir has significantly altered the expression level of selected genes. Sofosbuvir had affected the expression of selected genes in mixed effect manner (Fig. 1). Results of this study showed that the expression of H-RAS was relatively reduced after sofosbuvir treatment. The fold change value of H-RAS depicts that it was relatively half expressed. B-RAF gene was also slightly downregulated by the sofosbuvir. However, the Ct values of treated samples still are good enough for the transcription of this gene (Table 4). This gene was highly expressed in control samples, proving its efficiency in hepatoma cell lines. The Ct values of treated samples suggest that sofosbuvir is neither a potent inhibitor of B-RAF nor an inducer of this gene. Hence, the role of sofosbuvir in hepatocarcinogenesis needs more verification. MAPK1 was overexpressed under sofosbuvir treatment, but the value of its expression fold change was not too significant (Table 4). The results suggest that sofosbuvir might induce hepatocellular carcinoma by continuous overexpression of MAPK1 gene if taken for long time.

The relative expression of MET gene was slightly decreased after sofosbuvir treatment. This suggest that sofosbuvir suppress the activity of this gene. However, Ct value of treated sample indicates that sofosbuvir is not potent inhibitor of MET gene (Table 4). Thus, sofosbuvir is not enough for treating both HCV and hepatocellular carcinoma. Sofosbuvir has upregulated the VEGF gene and contributed to cell proliferation. It is interesting to note that sofosbuvir has upregulated all the growth factors analyzed in this work. However, it has downregulated the receptor tyrosine kinase gene, whose expression is necessary for tumorigenesis (Fig. 1).

The Ct value showed higher expression of PDGFRB in hepatoma cell lines. The fold change value for this gene suggested that sofosbuvir is potent enough to upregulate this gene. The relative gene expression of HGF was also increased (Table 4). Although, overexpression of cellular growth factors is mostly linked with carcinoma, but it is not true always. Though, sofosbuvir has increased the expression level of growth factors in hepatoma cell lines but it is early to say that sofosbuvir is linked with hepatocellular carcinoma.

Dual specificity phosphate 1 is negative feedback inhibitor of ERK signaling cascade. The relative expression of DUSP1 was increased 1.48-fold in sofosbuvir treated samples. This suggest that sofosbuvir might reduce the chances of HCC. The results indicated

Table 3
Ct value of TFG along with different concentration of cDNA.

cDNA amount (μL)	H-RAS	B-RAF	MAPK1	MET	VEGF	PDGFRB	HGF	DUSP1	SPRY2
Ct values (Target)									
1	24.01	23.69	24.14	25.83	24.51	23.59	23.02	26.23	25.99
0.33	26.28	26.7	27.68	28.95	26.23	26.37	26.61	29.16	28.78
0.11	29.87	29.72	30.42	33.12	31.5	29.35	30.07	33.58	32.94
0.037	33.95	33.62	33.42	37.34	34.98	34.07	34.13	37.96	37.41
0.012	38.53	38.48	39.51	39.88	38.39	38.11	37.52	40.08	39.91
Ct values (Reference)									
1	25.17	25.71	25.19	25.06	25.99	25.16	24.98	25.97	25.84
0.33	28.02	28.71	28.15	28.13	27.04	28.03	27.84	28.34	28.21
0.11	31.84	32.54	32.01	31.71	32.66	31.66	31.48	32.52	32.4
0.037	35.95	36.45	35.92	35.93	36.63	35.91	35.69	36.84	36.7
0.012	39.46	40.08	39.56	39.43	39.43	39.51	39.33	39.98	39.84
Efficiency (Target)	1.9919	1.9998	2.0006	2.0002	1.9994	1.9908	1.9991	1.9998	2.0010
Efficiency (Reference)	1.9994	2.0006	1.9994	1.9983	2.0010	1.9968	1.9979	1.9991	2.0002

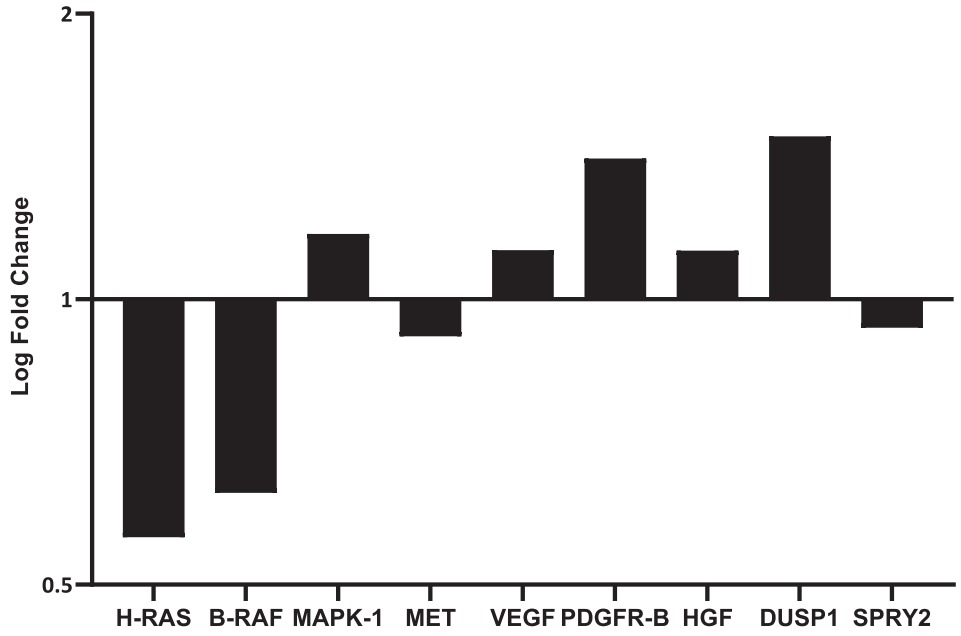

Fig. 1. Log fold change of selected genes. Fold change was calculated by Pfaffl equation. The increase in relative gene expression of growth factors (VEGF, PDGFRB, HGF) and MAPK1 suggested that sofosbuvir promoted HCC. However, sofosbuvir negatively affected the HCC development by downregulating the expression of kinases (H-RAS, B-RAF and MET). The negative feedback inhibitors of ERK signalling cascade DUSP1 and SPRY2 were upregulated and downregulated, respectively.

Table 4
Average cycle threshold values (ΔCT) of selected oncogenes.

	Gene targeted	ΔCt (Target)	ΔCt (Reference)	Fold change
H-RAS	Control	24.21	26.02	1
	Treated	27.06	28.02	0.560920192
B-RAF	Control	23.79	25.82	1
	Treated	26.54	27.89	0.624695833
MAPK1	Control	24.38	25.03	1
	Treated	26.21	27.09	1.171536481
MET	Control	26.65	25.41	1
	Treated	27.09	25.72	0.913551232
VEGF	Control	24.5	25.95	1
	Treated	25.93	27.55	1.126373128
PDGFRB	Control	23.42	25.16	1
	Treated	24.25	26.48	1.406850549
HGF	Control	23.69	24.98	1
	Treated	23.41	24.87	1.125038493
DUSP1	Control	28.45	26.02	1
	Treated	27.24	25.38	1.48480563
SPRY2	Control	26.79	25.85	1
	Treated	27.05	26.01	0.932931714

a minor downregulation of SPRY2 gene in sofosbuvir treated hepatoma cell line (Huh-7). However, it is clearly indicated from Ct value of both control and sofosbuvir treated samples, that expression of SPRY2 was not inhibited. The expression was relatively lower than the normal, but it does not indicate that sofosbuvir had inhibited the gene (Table 4).

4. Discussion

Chronic HCV infection has been found as major cause of hepatocellular carcinoma. The occurrence and reoccurrence of HCC in HCV patients receiving DAAs therapy has sparked a large debate (Tayyab et al., 2020; Ogawa et al., 2020; Luna-Cuadros et al., 2022). The clinical studies have shown that the DAAs therapy is an effective tool in reducing the risk of HCC development in cirrhotic HCV patients, however, it cannot eradicate the risk of HCC recurrence permanently (Singer et al., 2018; Singh et al., 2018; Ioannou et al., 2018; Nakano et al., 2019; Pons et al., 2020; Dang et al., 2020). The recurrence of hepatocellular carcinoma in HCV patients is the indirect off target effect of DAAs therapy.

The reported work in present study highlights important investigations on controlling the gene expression levels of important proteins, enzymes and hormones in Ras/Raf/MEK/ERK signaling pathway in human hepatoma cell line.

H-RAS, a proto-oncogene GTPase enzyme, is upstream regulator of RAS/RAF/MEK/ERK pathway. The hyper-activation of this pathway is linked with HCC (Li et al., 2016). The H-RAS along with its other isoforms is activated by upstream growth factors. Interestingly, the Ras activation in HCC is not linked with Ras mutation which is contrary to other human carcinomas (Delire and Stärkel, 2015). The inhibition of Ras will not only suppress the RAS/RAF/MEK/ERK pathway, but it will also suppress the PI3K/AKT proliferation pathways. Moreover, the inhibition of Ras is linked with reduced cell proliferation and apoptosis in hepatocarcinogenic cells (Bantel and Canbay, 2018). Sofosbuvir has downregulated the H-RAS but remained unable to inhibit this gene. The overexpression of RAF kinases especially B-RAF is found in hepatocellular carcinoma (Gnoni et al., 2019). It encodes a protein which belongs to the serine threonine kinase family. It affects cell division, secretions and differentiations. The most common mutation in this gene is V600E type of mutation which is frequently been identified in most types of cancers. However, this mutation has not proved to be linked with hepatocellular carcinoma (Lakhani and Fox, 2017). The expression of B-RAF decreased slightly but the Ct value of the treated sample was still sufficient to upregulate the gene. The role of sofosbuvir in liver cancer requires more validation.

MAPK1 is widely known as ERK because it encodes a protein MAP kinase or extracellular signal regulated kinase. It is an integrated gene which acts to integrate several biochemical signals linked to multiple pathways. It is activated by upstream kinases, and after activation, it is translocated to the nucleus of activated cells where it catalyzes the phosphorylation of nuclear targets. The overexpression of this gene found to be linked with multiple carcinomas, especially HCC (Guégan et al., 2012). One plausible reason for the overexpression of MAPK1 is the higher phosphorylation of EGFR because the phosphorylated EGFR activates a wide range of genes including MAPK1. Meanwhile, Bojkova et al. (2020) has reported the increased phosphorylation of EGFR in sofosbuvir treated cell lines.

MET encodes tyrosine kinase proteins which is receptor of many growth factors and acts an activator of many cell proliferation pathways. The overexpression of this gene is also linked with several kind of carcinomas especially hepatocellular carcinoma (Asaoka et al., 2020). The guanine derivative antiviral drugs were found best against c-Met during computational drug repurposing approach (Sherin and Manojkumar, 2021). Although, sofosbuvir is uracil derivative but it has lowered the relative expression of c-Met in huh7 cell lines. Hepatocyte growth factor (HGF) is the major ligand of the product of this gene. The binding of HGF induces dimerization and activation of receptor tyrosine kinase. It plays a vital role in survival of cellular machinery, invasion, and embryogenesis (Xu et al., 2017a). The mutation and overexpression of HGF gene is linked with hepatocellular carcinoma (Bouattour et al., 2018). The HGF encodes hepatocyte growth factor which regulates the growth, motility and morphology of various cells and type of tissues. The product of this gene is a multifunctional cytokine which act on epithelial cells. The protein encoded by this gene is secreted by mesenchymal cells. It plays a significant role in tumorigenesis, angiogenesis and regeneration of tissues.

Vascular endothelial growth factor (VEGF) initiates the growth and translocation of vascular endothelial cells. This gene is found to be overexpressed in many known tumors especially hepatocellular carcinoma (Zhang et al., 2012). It is among those growth factors which upregulate Ras/Raf/MEK/ERK pathway and multiple other integrated pathways involved in cell proliferation (Liu et al., 2018). The result of this study regarding VEGF complies with the already published literature. Villani et al. (2016) reported that DAAs therapy increased the serum level of VEGF. The elevated level of VEGF in serum after sofosbuvir treatment was also reported (El-Ghandour et al., 2021).

The platelet derived growth factor receptor (PDGFRB) is among those genes which promote recurrence of hepatocellular carcinoma after resection (Patel et al., 2011). The study first time reports effect of sofosbuvir on expression level of PDGFRB. Interestingly, sofosbuvir increased the expression of growth factors in this study. This rather contradictory result may be due to the fact that growth factors are usually required for HCV removal (Goto et al., 2020). Previous study also suggested that increased level of growth factor is associated with HCV clearance (Villani et al., 2016).

The DUSP1 is dual specificity phosphatase for tyrosine and threonine and negative feedback inhibitor of Ras/Raf/MEK/ERK pathways. The encoded protein of this gene catalyzes the dephosphorylation of MAPK1. The dephosphorylation results in the downregulation of Ras/Raf/MEK/ERK pathway. It is an important protein which respond to the environmental factors and negatively regulate the cell proliferation (Chen et al., 2019). The role of downregulated DUSP1 in carcinogenesis is dubious. Shen et al. (2016) reported tumorigenesis role of DUSP1 in prostate, breast, lungs and gastric cancer. It targets JNK induced apoptosis and produce a resistant solid tumor which is unable to treat by chemotherapy and radiotherapy (Shen et al., 2016). However, there are some evidence which exhibits its role in inhibiting hepatocellular carcinoma (Tsujita et al., 2005). DUSP1 cooperates with Hcr1 and reduce hepatocarcinogenesis (De Miglio et al., 2001). Referring to these studies, the results of this study related to DUSP1 are promising and require more deeper study at metabolomic and epigenetic level. The protein encoded by SPRY2 belongs to the sprouty family which have cysteine rich domain at its carboxylic end that perform its inhibitory activity. It is also a negative feedback inhibitor of Ras/Raf/MEK/ERK signaling pathway. It inhibits the upstream receptor tyrosine kinase signaling proteins. This inhibition results in accumulation of growth factors and downregulation of multiple integrated cell proliferation pathways. The upregulation of this protein negatively affects the hepatocarcinogenesis (Lee et al., 2010). It is important to note that overexpression or upregulation of a single inhibitor of cellular proliferation pathways is not enough to reduce the growth of tumor cells.

This research is a first step towards a more profound understanding of drug induced hepatocellular carcinoma. The results

suggest promising biomarker and evidenced based studies. The results of this work unraveled and shed light on the understanding of the DAAs linked hepatocarcinoma. However, the study does not clearly show that to what extent the studied genes promote cancer in hepatic cells. Several interesting aspects may be explored further by extending this work to larger subset of oncogenes.

5. Conclusion

The present study was designed to determine the effect of sofosbuvir on expression of nine oncogenes. The outcome of various experimentation led to the conclusion that sofosbuvir has significantly changed the relative expression of selected genes, but it was not potent enough to inhibit or mute the expression of selected genes. Sofosbuvir upregulated the expression of growth factors which in turn cause progression of cancer cells. The relative expression level of kinases was decreased except MAPK1 which suggest that sofosbuvir can suppress the Ras/Raf/MEK/ERK signaling pathway. One of the more significant findings was the overexpression of DUSP1, a negative feedback inhibitor of ERK signaling cascade. However, the expression SPRY2, also a negative feedback inhibitor, was slightly reduced. Overall, this drug repurposing study strengthens the idea that sofosbuvir neither eliminate nor induce the hepatocellular carcinoma. Rather it has mixed effect on hepatocellular carcinoma. Therefore, further research is required to investigate the effect of sofosbuvir on hepatocellular carcinoma.

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7. Note

Muhammad Atif and Muhammad Abdul Mustaan have equal contribution in this work and should be considered as first authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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