Host pharmacogenetic factors that may affect liver neoplasm incidence upon using direct-acting antivirals for treating hepatitis C infection

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ABSTRACT

Introduction: Direct-acting antivirals (DAAs) represent a breakthrough in hepatitis C virus (HCV) treatment as they directly inhibit HCV nonstructural (NS) proteins (NS3/4A, NS5A, and NS5B). However, ongoing debates exist regarding their relationship with hepatocellular carcinoma (HCC) whose incidence is widely debated among investigators. This study was conducted to identify host pharmacogenetic factors that may influence HCC incidence upon using HCV DAAs.

Materials and methods: Details regarding 16 HCV DAAs were collected from literature and DrugBank database. Digital structures of these drugs were fed into the pharmacogenomics/pharmacovigilance in-silico pipeline (PHARMIP) to predict the genetic factors that may underpin HCC development.

Results: We identified 184 unique genes and 40 unique variants that may have key answers for the DAA/HCC paradox. These findings could be used in different methods to aid in the precise application of HCV DAAs and minimize the proposed risk for HCC. All results could be accessed at: https://doi.org/10.17632/8ws8258hn3.2.

Discussion: All the identified factors are evidence related to HCC and significantly predicted by PHARMIP as DAA targets. We discuss some examples of the methods of using these results to address the DAA/HCC controversy based on the following three primary levels: 1 - individual DAA drug, 2 - DAA subclass, and 3 - the entire DAA class. Further wet laboratory investigation is required to evaluate these results.

1. Introduction

Hepatitis C is a liver disease caused by hepatitis C virus (HCV), which can cause both acute and chronic hepatitis. Worldwide, an estimated more than 70 million people have chronic hepatitis C infections [1]. HCV complications include liver cirrhosis and hepatocellular carcinoma (HCC), of which the latter is considered as the fourth most common neoplasm and the second commonest cause of cancer-related deaths in the world [2].

Several approaches have been applied in HCV treatment. The use of pegylated interferon plus ribavirin was the traditional approach, which achieved eradication of infection in 40%–50% of cases [3, 4]. In 2011, the FDA approved two drugs (boceprevir and telaprevir) that act directly on nonstructural (NS) HCV protein 3 and 4A (NS3/4A) protease [3]. This was an impetus for a new era of interferon-free direct-acting antiviral (DAA) treatment paradigms [6]. DAAs interfere with the life cycle of the virus by directly inhibiting HCV NS proteins (NS3/4A, NS5A, and NS5B), thus providing promising cure rates of >90% [7]. However, this dramatic increase in cure rates was not at no cost.

The shift to all-oral DAA-based regimens has significantly increased the cure rates of HCV to >90% in all patient groups. However, this shift has come at the expense of increasing some serious side effects [8, 9, 10]. As a new drug class, the side effects of DAAs are widely debated. One of the most controversial issues in this aspect is the relationship between the use of HCV DAAs and the incidence of de novo occurrence and recurrence of HCC and some other liver neoplasms [11].

Regarding the risk for HCC after DAA viral treatment, there are three significant developments as follows: 1 -DAA therapy reduced the incidence of HCC development in patients with chronic HCV with preexisting cirrhosis, but it did not eradicate the risk, implying that patients need...
ongoing surveillance for HCC after viral clearance [12], some reports have indicated that there is a high probability of HCC recurrence in cirrhotic patients who received DAAs [12] and HCC occurrence especially after the use of sofosbuvir without ribavirin regimens [13], and no difference exists in HCC incidence rates between different patient groups under DAA treatment [14]. Ongoing debates in favor of or against DAA links to HCC recurrence and/or de novo occurrence are frequently reported in the literature. Elucidating the entire profile of HCV DAAs/HCC relationship should consider the patient's (host's) genetic factors as approximately 80% of variability in drug efficacy and side effects are influenced by patient's pharmacogenomics [15].

Several studies have focused on host genetic factors as possible predictive markers for HCV DAA therapy response and side effects [16, 17]. For instance, variation in IFNL4 gene could affect the outcomes of ledipasvir/sofosbuvir treatment regimen [18]. However, variations in IFNA gene were found to be associated with decrease in hemoglobin levels related to treatment with sofosbuvir-containing regimen [19]. Moreover, polymorphism in IFNL3 gene (known also as IL28B) was found to influence the risk for hypercholesterolemia after clearance of HCV using DAA treatment [20].

Regarding HCC as a consequence of HCV infection, host genetics also play an essential role. For example, the single-nucleotide polymorphisms (SNPs) rs15957552, rs1012068, rs17047200, and rs2856723 of the genes MICA, DEPDC5, TLL1, and HLA-DBQ1, respectively, were found to be significantly associated with HCC development in patients with HCV [21].

On the other hand, studies focusing on the role of host genetics in the development of HCC as an adverse drug reaction (ADR) of HCV DAAs are still in their early infancy. For instance, a recent study reported no relationship between the SNPs rs12979860 in IFNL3 and rs4986791 in TLR4 and the development of HCC after sofosbuvir/daclatasvir combination regimen [22].

In this context, it is worth mentioning that HCV infection induces genome-wide epigenetic histone modifications that correlate with host gene expression reprogramming. This “epigenetic signature” persists after virus eradication by DAA treatment and has been associated with HCC progression [23, 24, 25, 26], which thus suggests using this epigenetic change as a biomarker for HCV infection [27]. Combining DNA methylation inhibitors (e.g. histone deacetylase inhibitors) with DAA could be a better approach to overcome the HCC risk after DAA treatment [28, 29, 30]. Moreover, “sonoporation via the microbubble” approach could help synergize the epigenetic treatment of HCC using DAAs and histone deacetylase inhibitors [31].

The scarcity of information and studies focusing on host pharmacogenetics role in DAAs/HCC relationship highlights the importance of the present study. The current gold standard for identifying pharmacogenomic associations of a drug is the expensive and labor-intensive genome-wide association studies (GWAS) [32, 33]. In a previous research, we introduced the pharmacogenomics/pharmacovigilance in-silico pipeline (PHARMIP) as a method that could be used to predict candidate genetic factors that underpin a certain ADR [34].

In the present study, PHARMIP was used with 16 approved HCV DAAs to predict candidate genetic factors that may affect HCC development upon their use. The genetic factors retrieved in this study could be helpful for further in-depth investigations focusing on the HCV DAA/HCC controversial relationship.

2. Materials and methods

2.1. HCV DAA drugs

A total of 16 DAAs, covering three DAA subclasses, were selected for this study (Table 1). In more detail, 8 NS3/4A, 6 NS5A, and 2 NS5B inhibitors were collected from literature [35] and DrugBank database [36]. Three of these DAAs (asunaprevir, bocaspruvir, and telaprevir) are withdrawn from the market. However, their results were retained to enrich the analyses of results. Digital structure files were retrieved from DrugBank in two primary formats, viz., the simplified molecular input line entry system (SMILES) [37] and structural data file (SDF) [38] (3D-SDF format was used when available), and used to run the PHARMIP pipeline.

2.2. Neoplastic case reports for the investigated drugs

To have a wider view on the problem addressed in our study, we retrieved neoplastic individual case study reports (ICSRs) for the 16 drugs from Vigibase [39]. On May 7, 2020, a total of 1594 neoplastic reports were retrieved for the 16 drugs, among which 972 reports (~61%) were for different liver neoplasms.

2.3. PHARMIP pipeline

Drug structure files were used as input for the PHARMIP pipeline to predict host off-label targets (OLTs) that are related to HCC. The pipeline comprises three primary steps, as simplified in Figure 1, and detailed as follows:

- Retrieving the drug in SMILES and SDF formats from DrugBank. The 3D-SDF format was used whenever possible.
- The drug in SMILES format was fed into SwissTargetPrediction [40], similarity ensemble approach server [41], and polypharmacology browser (PPB) [42] to predict possible OLTs using the similarity approach.
- The drug in SDF format was fed into PharmMapper [43] to predict possible OLTs using the pharmacophore mapping approach. The list of OLTs is obtained and filtered at a significance level of P value of <0.05 (one-tailed positive Z-score >1.645). Redundancies were removed using Excel.
- PharmMapper retrieves genes identified by their accession number in the Uniprot [44] database. We used the Uniprot retrieve/ID mapping tool to convert Uniprot accessions into gene names.
- The results obtained from similarity and pharmacophore mapping approaches were concatenated to generate the list of OLTs to be used in the next step.
- The lists of OLTs were used to feed DisGeNet [45] to retrieve genes and variants related ADRs (diseases).
- Results were downloaded in a tabulated text format (.tsv) and analyzed using Excel.
- DisGeNet results contain a column of disease “semantic type.” This column was filtered by “neoplastic process.” The results were further filtered by the column “disease” for diseases containing “liver” or “hep.”
- Visualization and basic analyses of gene lists were conducted using STRING [46].
- Comparisons were performed using InteractiVenn [47] to identify unique and intersected genetic factors.
- Human Genome Nomenclature Consortium (HGNC) [48] guidelines were followed for all gene symbols in this study.

3. Results

A total of 184 unique genes and 40 unique variants were obtained by the application of PHARMIP to 16 HCV DAAs. A general view and some basic analyses for this set of genes could be performed using the following STRING link: http://version-11-0.string-db.org/cgi/network.pl?networkId=ICEMxPPw86h.

According to our study, these results resemble candidate host pharmacogenetic factors that may influence the incidence of HCC upon using HCV DAAs. Results could be viewed and analyzed in different methods according to the researcher's interest in an individual DAA, in a DAA subclass, or in the entire DAA class. All the data obtained in this study can be found at: https://doi.org/10.17632/8ws8258hn3.2.
3.1. Results for individual drugs

As shown in Table 2, the highest number of retrieved genes was 53 for daclatasvir, and the lowest number was 25 for boceprevir. At the level of variants, the highest number was 14 for grazoprevir, and the lowest number was 2 for glecaprevir. Table 3 shows sample results for glecaprevir variants retrieved during the study.

In DisGeNet results, some genes were associated to synonymous diseases with different GDA scores. In such cases, we retained only the highest score and removed all other redundancies. For example, the gene F2 is associated to "adult hepatocellular carcinoma" with GDA = 0.01 and to "liver carcinoma" with GDA = 0.4. In this case, we retained the 0.4-GDA result and removed the others. It is worth mentioning that targets with low scores were retained as they could have synergetic effects with other high-score targets [49].

3.2. Results for drug subclasses

For investigators who may be interested in a certain DAA subclass rather than a certain drug, the results could be analyzed at the level of DAA subclasses. Figure 2 shows an example of the possible intersections between resulting genes of the six NS5A drugs included in this study (daclatasvir, elbasvir, ledipasvir, ombitasvir, pibrentasvir, and velpatasvir). Three genes (MAPK14, AKR1B1, and PTPN1) were found to be commonly predicted for these six drugs.

3.3. Results for the entire DAA class

We collected nonredundant hits for each subclass and obtained the intersection between the three subclasses. A total of 23 genes and 7 variants were found to be common between the three subclasses. Figure 3
summarizes the intersection results of genes between subclasses, and the intersections results of variants are depicted in Figure 4. The genetic map summarizes the intersection results of genes between subclasses, and the genetic map

Table 2. Number of genes and variants retrieved from the application of PHARMIP to the 16 investigated DAAs.

| No. | Drug          | No. of genes | No. of variants |
|-----|---------------|--------------|-----------------|
| 1.  | Asunaprevir   | 44           | 8               |
| 2.  | Boceprevir    | 25           | 7               |
| 3.  | Glecaprevir   | 29           | 2               |
| 4.  | Grazoprevir   | 37           | 14              |
| 5.  | Paritaprevir  | 37           | 5               |
| 6.  | Simeprevir    | 37           | 7               |
| 7.  | Telaprevir    | 31           | 9               |
| 8.  | Voxilaprevir  | 29           | 3               |
| 9.  | Daclatasvir   | 53           | 3               |
| 10. | Elbasvir      | 47           | 12              |
| 11. | Ledipasvir    | 26           | 4               |
| 12. | Ombitasvir    | 50           | 8               |
| 13. | Fibreantavir  | 39           | 8               |
| 14. | Velpatasvir   | 42           | 8               |
| 15. | Dasabuvir     | 45           | 5               |
| 16. | Sofosbuvir    | 51           | 13              |

By exploring the pharmacogenomics knowledge base (PharmGKB) [53] and the electronic pharmacogenomics assistant (ePGA) [54] for the genetic annotations of the 16 DAAs, only limited, if any, information was obtained regarding the possible explanation of the pharmacogenomics for their HCC relationship paradox. Most of the retrieved information was related to the role of interferon lambda 3 and 4 (IFNL3 and IFNL4) genetic polymorphism in the efficacy and safety of treatment using different DAAs. Using PHARMIP, 184 unique genes and 40 unique variants were successfully predicted as pharmacogenetic factors that may modulate HCC development in patients treated with HCV DAAs. These results could be analyzed in different manners based on the interests of researchers. As a discussion on all the results and possible analyses of this study is not feasible, we represent some of these possible analyses of the results and how they may help in resolving the DAA/HCC paradox.

4.2. Analysis of gene results

Considering GDA score, the MET proto-oncogene, a receptor tyrosine kinase (MET) gene, appeared as a very interesting hit. This gene was significantly predicted as an OLT for five DAAs (elbasvir, ombitasvir, pibrentasvir, simeprevir, and velpatasvir). As a well-investigated gene for its overexpression relationship with HCC [55, 56], it may be worth exploring its effect as a mediator for HCC development in DAA-treated patients. The same is applicable to HRas proto-oncogene, GTPase (HRAS), androgen receptor (AR), coagulation factor II, thrombin (F2), and peroxisome proliferator-activated receptor γ (PPARG). These candidate genes were significantly retrieved as DAA OLTs and related to HCC development with relatively high GDA scores. PPARG is an interesting hit from another point of view. Its prediction as a DAA OLT and its relationship with lipid metabolism [57] support our results and may explain the role of DAAs in lipid metabolism.

Considering these genes in a pharmacogenomic study design could disclose some key relationships of DAAs/HCC. However, genes with lower GDA scores could not be excluded as they may exert synergistic effects with genes with higher GDA scores.

From another point of view, the gene results could be analyzed depending on their repetition rather than their scores. For instance, protein tyrosine phosphatase nonreceptor type 1 (PTPN1) is significantly predicted as an OLT for all DAAs (except boceprevir). PTPN1 is upregulated in HCC, and its knockdown therapies are sex-linked (more effective in men than in women) [58, 59]. The mechanism of DAA interaction with this gene may deserve further investigation under wet laboratory settings, especially when patient sex is considered.

At the subclass level, 23 genes were found to appear at least once in each DAA subclass. Gene enrichment analysis of this gene set using
Table 3. Example of variant results showing two reference SNPs that may influence HCC development upon using glecaprevir.

| Variant   | Gene   | Gene_id | Chr | Consequence    | Alleles   | Class | Disease         | Disease_id  | Score_vda |
|-----------|--------|---------|-----|----------------|-----------|-------|-----------------|-------------|-----------|
| rs1057519958 | RXRA   | 6256    | 9   | missense variant | C/A,T     | snp   | Liver carcinoma | C2239176  | 0.7       |
| rs31223     | ITK    | 3702    | 5   | intron variant  | T/A,C     | snp   | Liver carcinoma | C2239176  | 0.01      |

Figure 2. Intersection results of genes between the six NSSA drugs included in the study.

Figure 3. Gene intersections between the three DAA subclasses.

Figure 4. Variant intersections between the three subclasses.
Enrichr [60] showed the best KEGG [61] pathways that may be enriched by these genes. As shown in Figure 6, “proteoglycans in cancer,” “progesterone-mediated oocyte maturation,” and “prolactin signaling” pathways have the best predicted P values. These pathways are evidence related to HCC [62, 63, 64, 65]. Modulation of these pathways by HCV DAAs could disclose some answers regarding their HCC mysterious relationship.

Alternatively, hits in a certain DAA subclass could be beneficial in other manners. For instance, F2 was retrieved for all the eight NS3/4A inhibitor DAAs included in this study. The functions of F2 polymorphism in HCC development have been extensively investigated throughout literature [66, 67, 68]. The effect of DAAs on the blood coagulation system has been debated [69, 70], which coincides with our results that some DAAs may target F2. A further in-depth investigation of DAA relationship with F2 may clarify their debated relationship with HCC development and coagulation system.

### 4.3. Variant results

Considering VDA scores, some variations are worth further investigation in wet laboratory settings. For example, the SNPs rs104894226, rs104894228, rs104894229, rs104894230, rs912193233, and

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**Figure 5.** Genetic map of the 23 common genes repeated at least once in each of the three DAA subclasses.

**Figure 6.** Gene enrichment analysis for 23 intersecting genes between DAA subclasses showing enriched KEGG pathways. Studying the modulation of these pathways by DAAs could disclose some answers about the DAA/HCC relationship.
rs28933406 in HRas proto-oncogene, GTPase (minus strand), HRAS, and leucine-rich repeat containing 56 (plus strand) LRRC56 are good candidates for further investigation on their possible role in DAA/HCC relationship. These genes are significantly predicted as DAA OLTs, and at the same time, their variants are evidence related to HCC [71].

Intersecting variants of the three subclasses returned seven variants that appeared at least once in each subclass. These variants (namely rs12338, rs13332, rs1870377, rs1979277, rs2071559, rs6147150, and rs8898) belong to four genes (CSTB, SHMT1, KDR, and ERBB4). Variants (rs12338, rs8898, and rs13332) of CSTB are associated with high risk and tumor size in HCC [72]. The variant rs1979277 of SHMT1 is linked to the risk for liver and colon cancer according to global DNA methylation [73]. Variants (rs2071559 and rs1870377) of KDR are related to overall survival and tumor resectability of patients with HCC [74]. The variant rs6147150 of ERBB4 is a polymorphism associated with high risk for HCC [75]. Considering these variants in pharmacogenetic studies could elucidate their possible role (risk or protective) in developing HCC in DAA-treated patients. This could help in the efforts to define DAA pharmacogenetic labels and in developing clinical pharmacogenetic guidelines for the precise use of DAs and according to the patient's genetic profile.

4.4. Future research

The rapid change in healthcare practice driven by high-throughput data technologies urges a rapid response in tools that aid implementing this change. Developing tools to help implement the patient's genetic profile in healthcare decision is becoming more interest, and there are research efforts across the world. In a previous study, we introduced PHARMIP as a tool to address some challenges in this area. In a future insight, we intend to develop a platform to automate PHARMIP to ease the process for non-specialists. Furthermore, we intend to develop a scoring system for candidate genetic factors that take into consideration the possible synergism between them.

In a more optimistic insight, we intend to use machine-learning techniques to help in detecting the action of drugs on candidate OLTs. Supervised binary classification algorithms could help predicting the drug effect (activator/inhibitor) on its predicted OLT, which based on its role will elucidate the role of the drug (risk/protection) in developing an ADR. Embedding this feature in PHARMIP will improve its use in daily activities related to precision medicine.

We also have some goals to use our technique earlier in a “pharmacogenetic-guided-drug-design” role rather than in an “ADR-explanation” role. For instance, three genes (MAPK14, AKR1B1, and PTPN1) appeared as OLTs for all the six NSSA inhibitors included in this study. Such results could be helpful in pharmacogenetic-guided NSSA development and precise design of new subclass members to minimize/maximize the risk/protective effect related to HCC development.

5. Conclusion

Our results demonstrated high prediction scores with several OLTs and variants that were markedly linked to HCC development. The predicted interactions may explain the unprecedented results with HCV treatment by DAs. It could be concluded that in addition to the direct inhibition of HCV targets by DAs, they synergistically interact with OLTs in the infected hepatocytes to influence HCC development. Further experimental investigation on these OLTs is strongly recommended aimed at defining the role(s) of host genetics in the HCV DAA/HCC relationship controversy. Additional wet laboratory analyses are required to identify whether the genetic factor indicates HCC risk or a protective factor. We anticipate that the schema of this study would help in the ongoing revolution of personalized medicine by identifying host genetic factors underlying unexplained ADRs.

Declarations

Author contribution statement

Ahmad M. Zidan, Eman A. Saad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nasser E. Ibrahim, Amal Mahmoud: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Alaa A. Hemeida, Medhat H. Hashem: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at Mendeley (https://doi.org/10.17632/8ws8258hn3.2).

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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