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Research paper

Phylogenetic analysis, structure modeling and docking study of HCV NS3 protease for the identification of potent inhibitors

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ABSTRACT

The nonstructural protein 3 (NS3) helicase of HCV is believed to be a plausible target for the identification and designing of potent antiviral drugs. NS3 protein is involved in a positive sense single-stranded viral replication as well as it also cleaves viral poly protein into diverse mature proteins at different time spans. Structural exploration of NS3 revealed that HCV helicase could also act as translocase. In order to identify potential inhibitors for HCV-3a, the current study has been designed. Serum samples from the Pakistani HCV positive patients were collected, sequenced and after purification included in the present study. Phylogenetic analysis on the samples clustered around it in the same group with those from India. Using homology modeling technique, we determined 3D structure of NS3 gene of HCV-3a and employed further in docking studies to discover potent inhibitor against it. As a result of docking Compound 1, with IC50 value of 0.015 and −14.4 kcal/mol energy, ranked as a most pungent inhibitor among all the studied inhibitors. Compound 1 also exhibited good hydrogen bond interactions with the modeled protein. The finding of present study could be used as a lead in future to design an effective dual inhibitor against HCV-3a.

1. Introduction

Hepatitis C virus (HCV) belongs to Flaviviridae, is a blood-borne pathogen which infects about 180 million individuals throughout the world; most of the cases lead to end stage liver diseases, fibrosis, cirrhosis and hepatocellular carcinoma (Choo et al., 1989; Major and Feinstone, 1997; Wasley and Alter, 2000). About 10 million populations of Pakistan are infected with HCV, with rare cases of spontaneous clearness; most of them progress to chronic cases (Ali et al., 2016). The most prevalent genotype among eleven HCV genotypes in Pakistan is 3a (Waheed et al., 2009). For a decade pegylated interferon a (PEG-IFN-a) plus ribavirin (RBV) remained treatment of the choice, is expensive, associated with severe side effects and effective for certain genotypes (Dillon, 2004; Farci et al., 2006; Ferenci, 2006; Ni and Wagman, 2004; Reichard et al., 1998). Different treatment response pattern to HCV genotypes is due to the genetic heterogeneity of virus. Considerable variability is shown by the virus which directly interferes with the disease treatment. The response to treatment varies according to HCV genotype and subtype (Bastos et al., 2016). The sustained virological response rate (SVR) in Pakistani population infected with 3a genotype to IFN-α and RBV combination therapy is 87.5%, approximately 2.45% of the patients discontinued treatment due to adverse side effects (Ali et al., 2016). Lacking of efficient treatment regimens and increased incidence rate of HCV infection has created a pressure for the therapeutic compounds that can efficiently target the HCV (López-Labrador, 2008). Nonstructural protein NS3/NS4A serine protease and helicase are considered as potential drug targets for the development of effective anti-HCV compounds (Ashfaq et al., 2011). The main role of NS3/NS4A is to cleave viral poly protein into different mature proteins at various time intervals as well as involved in viral replication; HCV helicase affects the viral life cycle at two steps for unwinding of double strand RNA intermediate required for the movement of HCV NS5B polymerase (Piccininni et al., 2002). NS3 structural analysis revealed a new function of HCV helicase as translocase and considered as a potential specific inhibitor to block NS3 helicase (Gu and Rice, 2010). For predication and comparison of molecular and physicochemical properties and mechanisms of reactions of different therapeutic compounds, molecular modeling techniques are widely used (Elfiky et al., 2013; Saleh et al., 2014). For HIV-1 and HCV proteases effective inhibitors are designed through these techniques (Elfiky et al., 2013; Ibrahim et al., 2012a; Ibrahim et al., 2012b). Computational studies not only motivated researchers for the identification of novel therapeutic targets but also

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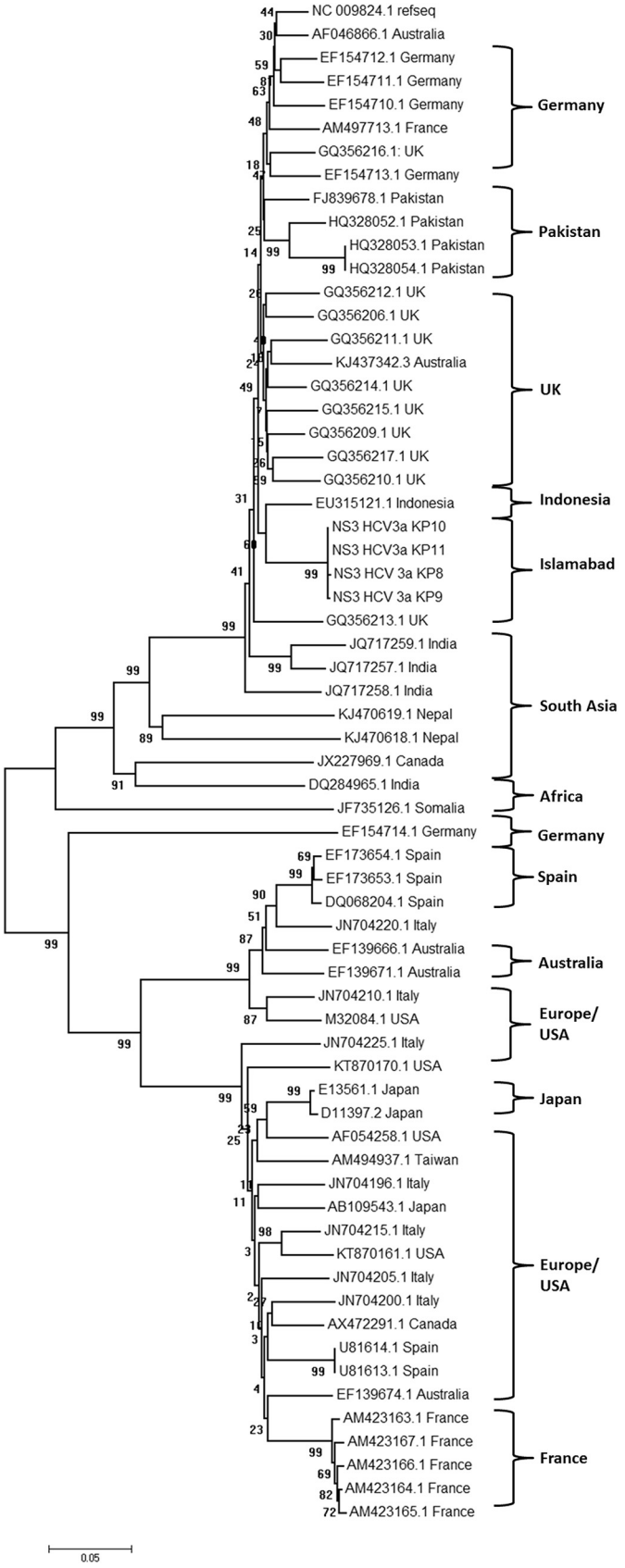

Fig. 1. Phylogenetic analysis of the HCV isolates from the underprivileged part of Islamabad representing the regional distribution of these isolates.

fortify the drug designing/development process. The field of computational biology and its latest uses has offered reliable methods for the enhanced understanding of biological systems of interest (Azam et al., 2009a; Azam et al., 2009b, 2009c; Azam et al., 2010; Azam et al., 2012; Barreca et al., 1999; Cheng et al., 2002). In the current study, the potent NS3-3a protease inhibitors, previously designed for NS3, have been identified by employing different computational techniques such as homology modeling, and molecular docking.

2. Methods

2.1. Complementary DNA synthesis and amplification of NS3 gene

HCV positive patients with genotype 3a from the underprivileged part of Islamabad were collected and included in the present study. RNA was extracted from the serum samples using Virus RNA isolation Kit (INSTANT, AJ Roboscreen, GmbH Germany). The RNA was reverse transcribed in to complimentary DNA (cDNA) using 100 units of M-MLV reversed transcriptase (Invitrogen), with 5 pM of outer antisense primer 5-GGCGACACTCCACCATAGAT-3. Amplification of NS3 gene using sense primer 5-GGCCGTGAGGTGTTGTTGG-3 and anti-sense primer 5-TGGTTACTTCCAGATCGGCTG-3 was carried out according to Sabri and colleagues (Sabri et al., 2014). After amplification and purification of the PCR product sequencing was carried out by Sanger method.

2.2. Phylogenetic analysis

The sequences obtained from Sanger method were subjected to a phylogenetic analysis for determination of regional distribution of NS3. HCV NS3 sequences from the regions of UK, Germany, France, Australia, India, Nepal, Canada and Somalia were extracted from NCBI database. Alignment and phylogenetic tree construction has been carried out using MEGA 7.0. For multiple sequence alignment clustalw algorithm was used (Sievers et al., 2011). The tree was constructed using neighbor joining algorithm (Fig. 1). Neighbor-Joining method was used in order to infer the evolutionary history (Saitou and Nei, 1987). The optimal tree along with branch length sum equal to 1.88376007 is presented. The phylogenetic tree was drawn to scale in the same branch length units as of evolutionary distance. Maximum Composite (Tamura et al., 2004) Likelihood method was employed to compute evolutionary distances in the number of base substitution per site units. Total sequences involved in the analysis amounted to 32. Missing data and all the positions presenting gaps we eliminated. Final dataset comprised of a total of 1850 positions. Further, MEGA7 was used to conduct evolutionary analysis (Kumar et al., 2016). Fig. 1.

2.3. Homology modeling

The crystallographic three-dimensional structure of HCV NS3 genotype 3a, the genotype most prevalent in Pakistani community, has not been reported yet. The sample used in the present study was collected from the underprivileged part of Islamabad, after sequencing the sample was designated KP8/NS3 sequence, in FASTA format. In order to identify suitable templates from RCSB PDB, NCBI BLASTp search of a target sequence was accomplished (http://www.pdb.org/pdb/) (Altschul et al., 1997). BLAST searching resulted in the most appropriate template (PBDID 4B6E) with a resolution of 2.64 Å from Hepatitis C virus (Saalau-Bethell et al., 2012). To generate a 3D model by alignment by means of Clustal Omega, coordinates of 4B6E were used as a template (http://www.ebi.ac.uk/Tools/clustalw2/index.html) (Sievers et al., 2011). To characterize the areas of similarity, pair-wise sequence alignment was performed. This characterization might emphasize evolutionary, functional, structural relationships among target and template two.

In total, 5 homology models of NS3 for genotype 3a, with differing geometric conformations, were generated by using MODELLER 9v12

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