Abstract

Hepatitis C virus (HCV) has an internal ribosomal binding site (IRES) located near the 5’ end of its genome. The HCV IRES is capable of direct binding to the 40S small ribosomal unit and eukaryotic initiation factor eIF3, and can initiate translation after the assembly of the whole 80S ribosome.

Various molecular types can act as IRES inhibitors. Small molecule compounds seem to be the most promising agent for use in the clinic. The main objective of the thesis was to develop a system for searching for small molecule compound inhibitors of HCV IRES in a library of chemical compounds. Several variants of vector carrying bicistronic cassettes were prepared. After validating their functionality by transient transfection of mammalian cell cultures, mammalian stable cell lines were established. These stable cell lines will allow for automatization of the search for small molecule compound inhibitors of HCV IRES.

Our second objective was to study the variability of HCV IRES sequences in patient samples. The samples were analysed by temperature gradient gel electrophoresis (TGGE). Select specimen were sequenced, cloned into a vector with bicistronic cassette and analysed by flow cytometry. In this was we evaluated the effect of specific mutations in the HCV IRES sequence on the level of IRES dependent translation. By comparing two isolated sets of samples we refuted the possibility of artificial generation of the identified mutations by the experimental setup.

The study has contributed to deepening our knowledge of the HCV IRES structure, dynamics, and their response to inhibitors.

Keywords

hepatitis C virus, HCV, hepatitis C, internal ribosome entry site, IRES, translation, translation initiation, cap-independent translation, HCV IRES inhibitors, small molecule compounds, stable cell line