Review of PhD thesis by Anas Khawaja

entitled

A study of the IRES variability: An experimental approach coupled with design of a large-scale mutation database

PhD thesis by Anas Khawaja was carried out at the Department of genetics and microbiology of Faculty of Science, Charles University, in the Laboratory of RNA Biochemistry supervised by RNDr. Martin Pospisek Ph.D. The thesis contributes in understanding the role of hepatitis C virus (HCV)-IRES in modulation of translation efficiency. Thesis pursued important and timely objective of biomedical research.

The thesis is focused on the structural configuration of different HCV-IRES domains and the impact of IRES primary sequence variation on the secondary structure and function. Screening the degree of heterogeneity and variability of IRES accumulated in HCV patients was monitored by denaturating gradient (DGEE) and temperature gradient gel electrophoresis (TGEE). Translation efficiency of transfected IRES constructs was determined by flow cytometry. Anas Kawaja concluded that some of multiple mutations scattered across different domains of HCV-IRES, led to restoration of the HCV-IRES translational activity, although the individual occurrence of these mutations were found to be deleterious. He attributed these observations to long-range inter- and/or intra-domain functional interactions. The stability of the long-range RNA-RNA interaction between the IRES and the core gene was shown to be involved in modulating viral gene expression.

Anas Khawaja contributed to the formation of a large scale HCV-IRES variation database (HCVIVdb), a major scientific project of research group, in which he is integrated. The collation of over 1900 mutations enables systematic analysis of the HCV IRES. By means of HCVIVdb tool, 20 new HCV-IRES mutations were identified and their translational efficiency was determined. Multiple sequence alignment of HCV genome showed the conservation of specific nucleotides and hypervariability of others. Thus, HCVIVdb is a discovery tool to study evolutionary advantage and preservation of specific bases within HCV IRES and relation between sequence, structural conformation and translational efficiency.

The thesis is organized to Abstract, Introduction, Literature review, Material and methods, Results and Discussion, Conclusions, List of publications, References, and Selected publications. List of publications contains papers published in WIREs RNA and BMC Microbiology, where Anas Khawaja is the first author and a manuscript in preparation with Anas Khawaja also as the first author. The thesis is well written and easy to read.

Anas Khawaja should during defense of his thesis clarify or discuss the following points.

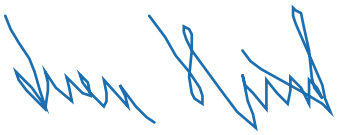
Major points

1. My major concern is that >99.99% of HCV particles in a cell culture or in peripheral blood of infected individuals – the source of RNA for experimental work - is not replication competent. Thus, although the experimental data on translation initiation are valid on biochemical level, their virological relevance will be difficult to evaluate except of construction of full-length HCV replicons mutated in IRES.
2. The author focused his thesis to the effect of variability in the HCV 5´UTR and IRES in initiation of translation. It would be useful to discuss his results from the point of view of other molecular mechanisms dependent on cis-elements present in the HCV 5´UTR or complementary sequence (e.g., binding of miR-122 or of the replication/transcription complex NS5A/B).
3. Studies showing the effect of variability in IRES on HCV replication, cytopathogenicity and sequence development in different clinical settings should be referenced. Clinical significance of sequence variability in IRES should be discussed (see point #1).
4. Please comment from the point of view of your results importance of interaction of benzimidazole inhibitors with subdomain IIa helical bend (p. 20)?
5. Paradoxically, HCV blocks IFN effector function by inducing PKR phosphorylation and HCV translation persists through elF2alpha-independent mechanism (well described on p. 33). Is this mechanism pertinent in your experimental set-up?
6. Origin of HCV analyzed in the study is occult (p. 36). Description of patients´ clinical status is limited to information that patient #7 was IFN therapy non-responder, while the two other (#9, #4) were not treated. Were the patients #9, #4 in acute or chronic phase of infection? What was their ALT/AST levels, virus charge, estimated length of infection, gender and age? No reference is given for method of HCV RNA isolation from plasma or serum. Under which ethical protocol the study was performed? No conclusion (even negative) was made on the basis of data analysis and clinical status of patients.
7. No statistical approach is shown in evaluation of efficiency of the translation activities associated with the HCV IRES of patients #4, 7 and 9 and mutant constructs (Tables 6, 7 and 8). How many times independent measurements were repeated? What was an error? At which level are the differences significant? What statistical method was used?
8. How is defined the consensus sequence in the Figures 4b, 5b, and 6b? Is the consensus sequence experimentally determined on the basis of the clones shown in each figure, or is it the major PCR sequence or a data-base selected sequence? What is the difference if any?

Minor points

1. Definition of HCV genotype should be provided.
2. Numerous errors in lay out and punctuation are present on pp. 42, 43, 49, 50, 53.

In conclusion, in spite of some critical comments, the thesis of Anas Khawaja fulfills requirements demanded for the level of dissertation work. Anas Khawaja is the first author of two papers, one published and another submitted for publication and co-author of further manuscript submitted for publication. I recommend submitted work for defense, and depending on the outcome of defense procedure for approval of doctor degree.



Prague, August 25, 2016 RNDr. Ivan Hirsch, CSc