VIRUS HOST INTERACTIONS IN HEPATITIS B VIRUS INFECTION

Dr. Garaigorta´s short Biosketch

Dr. Garaigorta is a virologist with over 20 years of experience in scientific research. He trained in internationally recognized laboratories including those lead by Prof. Juan Ortín at Centro Nacional de Biotecnología (doctoral training), and Prof. Francis V. Chisari at The Scripps Research Institute in La Jolla (USA) (postdoctoral training). His entire career has been focused on studying viruses that cause disease in humans, with relevant contributions in the fields of influenza, hepatitis C and B virus infections, as well as in the antiviral development field. Dr. Garaigorta is co-director of the CNB-CSIC Antiviral Discovery and Characterization Platform, co-coordinator of the CSIC PTI Global Health work package, focused on antiviral development, and participates in an international NAVIPP consortium project aiming to develop broad-spectrum antivirals for highly pathogenic human viruses. He is author of 24 publications in high impact international scientific journals and has participated as inventor in 15 patent applications. He teaches in three University Master programs and participates in outreaching activities, has directed one Ph.D. and five master students and is currently responsible of training of other three Ph.D. students.

Laboratory Research Overview

Dr. Garaigorta´s laboratory is interested in understanding virus host interactions that regulate the outcome and pathogenesis of virus infections. Our main objective is to identify vulnerabilities that could be exploited to develop new strategies to treat hepatitis B virus (HBV) infections, that are responsible of millions of cases of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide.

Our research is focused on understanding cellular and molecular mechanisms that regulate the infection, including its pathological consequences. We use HBV infection cell culture models to study the cellular requirements for the infection as well as to identify host factors that positively or negatively regulate the infection. In the last years, we have identified several cellular proteins (i.e.: Senataxin, Ku70 and Ku80…) that control HBV gene expression by restricting initial steps that are crucial for the establishment of infection. A main line of research includes understanding the mechanism(s) by which the identified host factors regulate HBV episomal DNA-derived gene expression. To do so, we combine gene silencing and overexpression strategies with the molecular characterization of viral and cellular gene expression analysis, focusing on transcriptomics approaches and chromatin immunoprecipitation analysis. The results from these studies point to a selective regulation of HBV episomal DNA expression, which opens a venue for development of novel therapeutic strategies.

More recently we have extended these observations to other episomal DNA viral models including adeno-associated viral (AAV) vectors that are being used in gene therapy, and integration deficient lentivirus (IDLV) and retrovirus (IDRV) vector systems. Our main objective here is to increase our basic knowledge on how episomal DNA-derived expression is being regulated in order to develop new adjuvants that could be potentially used in gene therapy strategies. In parallel, we have implemented techniques to evaluate the potential undesired risk of vector DNA integration. This is an important safety aspect that should be taken into account when developing viral vector-based gene therapy approaches.

As complement to these studies, we have developed a dual fluorescence cellular reporter screening assay to simultaneously quantify the expression from episomal and chromosomal DNA templates,

which in principle allows the identification of small molecules that selectively inhibit (potential antiviral molecules) or increase (potential adjuvants for gene therapy) episomal DNA-derived gene expression. We have already validated this system with a small library of compounds and by the genetic manipulation of host factors that selectively target episomal DNA.

Finally, our group is also involved in the CNB-CSIC Antiviral Discovery and Characterization Platform, which pursues the development of novel antivirals against viruses with biomedical interest including: hepatic viruses, influenza viruses, viruses transmitted by mosquitos, coronaviruses…

National and International Collaborations

Collaborations in the context of HBV and episomal DNA regulation:

- Dra. Gloria Gonzalez Aseguinolaza and Dr. Carmen Unzu from Centro de Investigación Médica Aplicada (CIMA) in Pamplona. They are experts in liver targeting AAV-based gene therapy approaches. We actively collaborate with them in studies aimed to improve gene therapy efficiency.
- Dra. Barbara Testoni from the Cancer Research Center of Lyon (CRCL) in Lyon, France. She is an expert in the HBV field. She hosted a Ph.D. student from our lab (Enara San Sebastian) and supervised her research during the three months secondment stage she performed in her laboratory.
- Prof. Ivan Marazzi from University of California Irvine (UCI) in USA. He studies epigenetic and chromatin-mediated control of gene expression in the context of viral infections, inflammation and neurodegenerative disorders. Our collaboration aims to explore the function of cellular Senataxin protein on episomal DNA expression.

Collaborations in the context of antiviral, antibody and vaccine development:

- CSIC-Chemical library. We have a very close collaboration with many medicinal and synthetic chemists from different CSIC institutes in the context of the CNB-CSIC Antiviral Discovery and Characterization Platform. They provide us with small molecules that we use to identify antiviral molecules. This collaboration has been proven to be very productive in terms of publications and patent applications.
- Drs. Luis Ángel Fernández, José María Casasnovas and Hugh Reyburn from CNB. We have collaborated in the development and use of neutralization assays to help in the characterization of nanobodies and antibodies directed against the S and M proteins of SARS-CoV-2 virus, leading to two publications and several patent applications.
- Drs. Juan García Arriaza (CNB) and David Sancho (CNIC) with whom we have collaborated in the evaluation of the neutralization capacity of vaccine candidates against SARS-CoV-2 leading to two publications.

Relevant Publications

- Gómez-Moreno A, Coto A, Ho S, Moya J, González-Aseguinolaza G, Unzu C, Marazzi I, **Garaigorta U.** Cellular factor Senataxin restricts extrachromosomal DNA expression at transcriptional level. *(Manuscript in preparation)*.

- Coto A[*], Gómez-Moreno A[*], Contreras D, San Sebastian E, Moya J, **Garaigorta U.** RPA1 protects from non-specific integration of episomal DNAs preventing transgene expression. *(Manuscript in preparation)*.

- Gómez-Moreno A, San Sebastian E, Moya J, Gomollón-Zueco P, Isola S, Vales Á, González-Aseguinolaza G, Unzu C, **Garaigorta U.** Topoisomerase Inhibitors Increase Episomal DNA Expression by Inducing the Integration of Episomal DNA in Hepatic Cells. *Pharmaceutics*. 2023 Oct 13;15(10):2459. doi: 10.3390/pharmaceutics15102459. PMID: 37896219. **(Garaigorta 9/9)**.

- Álvarez EG, Demeulemeester J, Otero P, et al… Tubio JMC. Aberrant integration of Hepatitis B virus DNA promotes major restructuring of human hepatocellular carcinoma genome architecture. *Nat Commun*. 2021 Nov 25;12(1):6910. doi: 10.1038/s41467-021-26805-8. PMID: 34824211. **(Garaigorta 39/43)**.

- Ginex T[#], **Garaigorta U[#]**, Ramírez D, et al… Gil C. Host-Directed FDA- Approved Drugs with Antiviral Activity against SARS-CoV-2 Identified by Hierarchical In Silico/In Vitro Screening Methods. *Pharmaceuticals* (Basel). 2021 Apr 6;14(4):332. doi: 10.3390/ph14040332. PMID: 33917313. **(Garaigorta 2[#]/11; co-first author)**.

- Whitten-Bauer C, Chung J, Gómez-Moreno A, Gomollón-Zueco P, Huber MD, Gerace L, **Garaigorta U**. The Host Factor Erlin-1 is Required for Efficient Hepatitis C Virus Infection. *Cells.* 2019 Dec 2;8(12):1555. doi: 10.3390/cells8121555. PMID: 31810281. **(Garaigorta 7/7)**.

- Kruse RL, Shum T, Tashiro H, et al… Bissig KD. HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. *Cytotherapy*. 2018 May;20(5):697-705. doi: 10.1016/j.jcyt.2018.02.002. Epub 2018 Apr 6. PMID: 29631939. **(Garaigorta 9/11)**.

- Gómez-Moreno A, **Garaigorta U**. Hepatitis B Virus and DNA Damage Response: Interactions and Consequences for the Infection. *Viruses*. 2017 Oct 19;9(10):304. doi: 10.3390/v9100304. PMID: 29048354. **(Garaigorta 2/2)**.

- Billioud G, Kruse RL, Carrillo M, et al… Wieland S. In vivo reduction of hepatitis B virus antigenemia and viremia by antisense oligonucleotides. *J Hepatol*. 2016 Apr;64(4):781-9. doi: 10.1016/j.jhep.2015.11.032. PMID: 26658683. **(Garaigorta 12/15)**.

- Padmanabhan P, **Garaigorta U**, Dixit NM. Emergent properties of the interferon-signalling network may underlie the success of hepatitis C treatment. *Nat Commun*. 2014 May 16;5:3872. doi: 10.1038/ncomms4872. PMID: 24834957. **(Garaigorta 2/3)**.

- Dreux M, **Garaigorta U**, Boyd B, et al… Chisari FV. Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. *Cell Host Microbe*. 2012 Oct 18;12(4):558-70. doi: 10.1016/j.chom.2012.08.010. PMID: 23084922. **(Garaigorta 2/8)**.

- **Garaigorta U[*]**, Heim MH, Boyd B, Wieland S, Chisari FV[*]. Hepatitis C virus (HCV) induces formation of stress granules whose proteins regulate HCV RNA replication and virus assembly and egress. *J Virol*. 2012 Oct;86(20):11043-56. doi: 10.1128/JVI.07101-11. Epub 2012 Aug 1. PMID: 22855484. **(Garaigorta 1/5; co-corresponding author)**.

- **Garaigorta U[*]**, Chisari FV[*]. Hepatitis C virus blocks interferon effector function by inducing protein kinase R phosphorylation. *Cell Host Microbe*. 2009 Dec 17;6(6):513-22. doi: 10.1016/j.chom.2009.11.004. PMID: 20006840. **(Garaigorta 1/2; co-corresponding author)**.