Abstract

Emerging and re-emerging RNA viruses have been the cause of epidemics within the past few years, raising public health concerns throughout the world. The 2014-2016 outbreak of Ebola Virus (EBOV) resulted in over 28000 infections with more than 11000 deaths. In 2015, a widespread epidemic of an emerging mosquito-borne flavivirus, Zika Virus (ZIKV), was associated with the increased incidence of neurological complications in South and Central America.

As an early response to viral infection, type I interferon (IFN-α/β) produced by mammalian cells exerts antiviral activity. The RIG-I-like receptors are critical sensors for RNA viruses, including EBOV and ZIKV. The IFN response begins with recognition of pathogen-associated molecular pattern by pattern recognition receptors, whose activation leads to a signaling cascade resulting in the production of IFN-α/β. Once secreted, type I IFN binds to the specific receptor activating the IFN signaling cascade leading to the induction of hundreds of IFN-stimulated genes (ISGs), which establish the antiviral state of the cells, targeting specific aspects of the viral life cycle. The majority of viruses have evolved strategies to disarm the IFN-I response: (i) blocking IFN-I production, (ii) IFN-I signaling, (iii) ISGs action. The antagonism of the IFN system has significant implications in both EBOV and ZIKV immune pathogenesis and virulence. EBOV multifunctional protein VP24 is one of the main determinants of EBOV virulence by virtue of its inhibition of the IFN signaling cascade. ZIKV antagonism of IFN response is also correlated to virulence, in particular mutations in residues of NS1 and NS4B have been associated with increased viral replication and neurovirulence in mice.

Today no approved drugs are available to treat EBOV and ZIKV infections. Therefore, the knowledge gained by characterizing the mechanisms through which both viruses evade the IFN response is at the base for the attenuation of the pathogenesis, contributing to the development of countermeasures directed against novel potential pharmacological targets.

This PhD thesis, on one hand, focuses on the study of EBOV VP24 protein and its inhibition of the IFN signaling cascade. Being a key factor for EBOV virulence, with the aim of identifying novel therapeutic agents against VP24, a new cellular drug screening assay has been developed. The assay is based on the transfection of cells with a luciferase reporter under the control of the promoter of ISGs (pISRE-luc) and an expression plasmid for VP24 that results in the inhibition of the IFN-mediated ISRE transcription. Addition of compounds leads to the partial restoration of the IFN signaling cascade. We optimized the assay to achieve excellent signal, robust performance and high reproducibility.

Among plants components, flavonoids have been reported to be active against many viruses, including EBOV, and to potentially bind VP24 *in silico* studies. Hence, using our drug screening assay, we screened a number of flavonoids among which Quercetin was identified to be the most active derivative against VP24, allowing to restore the IFN response with an IC_{50} value of 7.4 μM. Quercetin is the first compound reported to target specifically the EBOV VP24 IFN inhibitory function, thus, being potentially able to block EBOV infection at the early steps.

On the other hand, driven by the need for a comprehensive understanding of the molecular pathogenesis of the newly emergent ZIKV, the present PhD thesis aims to identify the ZIKV proteins with IFN signaling inhibitory functions. For the first time, we describe new functions for ZIKV NS2A and NS4B as IFN signaling inhibitors, being able to block ISRE expression and ISGs transcripts through the suppression of STAT1/STAT2 phosphorylation. Given their impact on IFN response, they may represent novel and attractive targets for the further development of antiviral therapies against ZIKV.