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Review

Structure and functionality in flavivirus NS-proteins: Perspectives for drug design

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ABSTRACT

Flaviviridae are small enveloped viruses hosting a positive-sense single-stranded RNA genome. Besides yellow fever virus, a landmark case in the history of virology, members of the Flavivirus genus, such as West Nile virus and dengue virus, are increasingly gaining attention due to their re-emergence and incidence in different areas of the world. Additional environmental and demographic considerations suggest that novel or known flaviviruses will continue to emerge in the future. Nevertheless, up to few years ago flaviviruses were considered low interest candidates for drug design. At the start of the European Union VIZIER Project, in 2004, just two crystal structures of protein domains from the flaviviral replication machinery were known. Such pioneering studies, however, indicated the flaviviral replication complex as a promising target for the development of antiviral compounds. Here we review structural and functional aspects emerging from the characterization of two main components (NS3 and NS5 proteins) of the flavivirus replication complex. Most of the reviewed results were achieved within the European Union VIZIER Project, and cover topics that span from viral genomics to structural biology and inhibition mechanisms. The ultimate aim of the reported approaches is to shed light on the design and development of antiviral drug leads.

Contents

Abbreviations: BVDV, bovine viral diarrhea virus; C, capsid protein; CSFV, classical swine fever virus; CCHFV, Crimean-Congo hemorrhagic fever virus; CPE, cytopathogenic effect; dsRNA, double-stranded RNA; ER, endoplasmic reticulum; E, envelope protein; GMP, guanosine monophosphate; GTP, guanosine triphosphate; GTase, guanylyltransferase; NS3Hel, helicase; HIV, Human Immunodeficiency Virus I; HCV, hepatitis C virus; HBS, high affinity binding site; IMP, Inosine 5′-monophosphate; LBS, low-affinity binding site; M, membrane protein; NS5MTase, methyltransferase; N7MTase, (guanine-N7)-methyltransferase; 2′OMTase, (nucleoside-2′-O-)-methyltransferase; NS, non-structural; NLS, nuclear localization sequences; NS3Pro, protease; RC, replication-competent complex; RSV, respiratory syncytial virus; NS5RdRp, RNA-dependent RNA polymerase; NS3RTPase, RNA triphosphatase; AdoMet, S-adenosyl-L-methionine; ssRNA, single-stranded RNA; T-705 RMP, T-705-ribofuranosyl-5′-monophosphate; VIZIER, Viral Enzymes Involved in Replication.

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1. Introduction

The genus Flavivirus, together with Pestivirus and Hepacivirus, belongs to the family of Flaviviradae. Flaviviridae are small enveloped viruses hosting a positive-sense single-stranded RNA genome. The complete genome is 9500–12,500 nucleotides long. It encodes a large polyprotein precursor, which is co- and post-translationally processed by viral and cellular proteases into three structural proteins, building the capsid, and seven non-structural proteins involved in virus replication.

1.1. Emergence and re-emergence of pathogenic flaviviruses

In the Flaviviridae family, the genus Flavivirus occupies a special space within the RNA virus world. The family derives its name from the word flavus (Latin for yellow), with one prominent member being the yellow fever virus (YFV) a landmark reference system in the history of virology. It was introduced in the Americas in the 16th century as a consequence of the African slave trade, recognized by Carlos Finlay as a vector-borne disease as early as 1881, before any virus was isolated. YFV was the first human pathogenic virus isolated in 1927 (Staples and Monath, 2008). Although a safe and efficient vaccine designed in 1937 by Max Theiler shaped our view on the control of viruses, there are still more than 200,000 annual cases in Africa alone, and about 15% of the cases enter a critical phase that only 50% of the patients survive (Ellis and Barrett, 2008). In more recent years, members of the Flavivirus genus gained public visibility due to re-emergence and steadily increasing incidence, such as for West Nile virus (WNV) in the Americas and dengue virus (DENV) in subtropical areas of the world.

WNV, isolated in Uganda in 1937, is endemic in Africa and southern Europe, but its appearance in the Americas in 1999 was followed by a rapid geographic extension from Canada to Argentina by 2008, leaving behind thousands of deaths and disabled patients (Petersen and Hayes, 2008). Likewise, the four DENV serotypes have considerably expanded their geographic distribution in recent years. With billions of people at risk, more than 50 million cases, and about 12,500–25,000 deaths annually, DENV is robustly emerging in a growing number of countries (Vasilakis and Weaver, 2008). The two remaining clinically significant flaviviruses are the Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV), for which existing vaccines should help reduce the current morbidity burden, mostly in Asia and central Europe, respectively. Most flaviviruses are arthropod-borne viruses (arboviruses), transmitted either by ticks (tick-borne viruses, TBV) or mosquitoes (mosquito-borne viruses, MBV), but a number of flaviviruses have no known vectors (NKV) and/or have been isolated from infected animals without a link to any specific disease (Table 1).

1.2. Development of flavivirus treatments

There are a number of environmental, demographic and ecological reasons to believe that either novel or known flaviviruses will continue to emerge. In this respect, the success of vaccination against YFV has been temperated by difficulties encountered when such programs were launched against DENV. In particular, the presence of four DENV serotypes has complicated vaccine design because incomplete protection against one serotype may influence the disease outcome once infection is established by a distinct serotype, through a process referred to as antibody-mediated disease enhancement (Guzman and Kouri, 2008). Therefore, in addition to vaccine design efforts, there has been a growing interest in discovering drugs against DENV and WNV. For instance, a moderate, borderline effect, whose mechanism of action is controversial, was reported for the activity of ribavirin against flaviviruses (Huggins, 1989; Day et al., 2005; Leyssen et al., 2006; Takhampunya et al., 2006). Prior to 2004 there were very few coordinated efforts towards the design of antiflavivirus compounds, flaviviruses being hardly considered interesting candidates for drug design. A notable exception has been the activity at the Novartis Institute for Tropical Disease in Singapore that focused its research efforts on dengue disease since its first opening (in 2003) (Gubler and Clark, 1995; Kroeger et al., 2004). Perhaps even before the launch of the European Union VIZIER Project (Viral Enzymes Involved in Replication) in October 2004, the lack of viral genomics programs was recog-