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Review

Viruses and the nucleolus: The fatal attraction ☆

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ABSTRACT

Viruses are small obligatory parasites and as a consequence, they have developed sophisticated strategies to exploit the host cell's functions to create an environment that favors their own replication. A common feature of most – if not all – families of human and non-human viruses concerns their interaction with the nucleolus. The nucleolus is a multifunctional nuclear domain, which, in addition to its well-known role in ribosome biogenesis, plays several crucial other functions. Viral infection induces important nucleolar alterations. Indeed, during viral infection numerous viral components localize in nucleoli, while various host nucleolar proteins are redistributed in other cell compartments or are modified, and non-nucleolar cellular proteins reach the nucleolus. This review highlights the interactions reported between the nucleolus and some human or animal viral families able to establish a latent or productive infection, selected on the basis of their known interactions with the nucleolus and the nucleolar activities, and their links with virus replication and/or pathogenesis. This article is part of a Special Issue entitled: Role of the Nucleolus in Human Disease.

1. Introduction

Viruses are small obligatory parasites and as a consequence, they have to divert some of the cellular machineries for their own replication. They have developed sophisticated strategies to exploit the host cell's functions and to inhibit its intrinsic and innate defense mechanisms in order to efficiently accomplish their replication cycle. Viral infections are generally associated with specific diseases affecting one or several organs or tissues, some of which can be fatal for the host. Accordingly, studying the interaction between viruses and the cell is extremely informative, not only to understand the virus properties but also to gain a better insight into the cell's functions.

The viral genome is a DNA or RNA molecule that encodes viral components that allow a latent/chronic or lytic infection. Generally, most DNA viruses replicate in the nucleus while most RNA viruses replicate in the cytoplasm. However, exceptions also exist with some DNA viruses and RNA viruses replicating in the cytoplasm and the nucleus, respectively. During latent or chronic infection, only a few viral components are synthesized and the viral genome persists in the infected cell. A typical infectious cycle is usually lytic. It includes attachment of the virus to the cell surface using specific receptor, entry through the plasma membrane to reach the cytoplasm, production of viral RNAs and proteins, genome replication, and at the end of the cycle the newly made viral components are assembled into progeny virus particles that are released from the infected cells and spread to new cells.

The consequences of viral infection on host cell functions are diverse. Surprisingly, despite the important variety of mechanisms, a common feature of most – if not all – viral families is their interaction with the nucleolus, one of the best-known nuclear compartments [1–4]. The interaction of viruses with the nucleolus has been the object of an increasing number of studies since the beginning of the 1990s, some of them establishing a link between their ability to interact with this nuclear compartment and the outcome of virus replication and pathogenesis.

The nucleolus, the most prominent nuclear domain, is a membraneless structure whose existence was established in the 19th century. Until recently, its most well known role was ribosome biogenesis. Indeed, the nucleolus forms around the clusters of genes coding for ribosomal RNAs arranged in a tandem array, and the transcriptional activity of ribosomal genes in the nucleolus gives rise to its characteristic ultrastructural organization: the fibrillar center, surrounded by the dense fibrillar component, which is bordered by the granular component [5]. During mitosis the nucleolus disassembles, then reassembles at the end of mitosis. Subsequently, the nucleolus was discovered to be more than a "ribosome factory" [6]. Studies in the last decades have identified several thousands of different nucleolar components (proteins and RNAs) the roles of which have highlighted that the nucleolus is also involved in other biological functions such as tRNA and mRNA processing, maturation and assembly of ribonucleoprotein complexes, cell cycle regulation and cellular aging, leading to the notion of a plurifunctional nucleolus. In addition, nucleoli are dynamic nuclear domains and their components communicate constantly with other nuclear domains and with the cytoplasm [4,7–12].

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Therefore, due to the multiple functions fulfilled by nucleoli, it is not surprising that in cells infected with various types of viruses, nucleoli are submitted to profound alterations in structure and composition. Indeed, in addition to the numerous viral components that traffic to and from the nucleolus, some nucleolar proteins are delocalized out of the nucleolus, while in other cases non-nucleolar cellular proteins enter the nucleolus to fulfill other function(s) [1,3]. At present, the roles of these virally-induced nucleolar perturbations on viral replication and host cell functions are not fully elucidated for many of them. Even though the infected cells need to support the synthesis of new viral proteins, only a few studies on viral infection focus on mechanisms related with ribosome biogenesis demonstrating that viral proteins interact with rRNAs, inhibit or stimulate rRNA gene transcription, or modulate pre-rRNA maturation [13–17]. By contrast, numerous studies have shown that several of the virally-induced modifications of nucleolar structure and composition rather interfere with other well established fundamental processes in which they are directly or indirectly involved, such as cell cycle regulation, apoptosis and translation. In addition, these studies showed that nucleoli themselves or nucleolar proteins participate directly in specific processes that are crucial for the outcome of infection, like viral DNA replication, virus assembly, and control of intracellular trafficking.

The aim of this review is to highlight the interactions reported between the nucleolus and some viral families, which illustrate the variety of studies in this field and of their potential relevance to the development of treatments against viral infections. To simplify this potentially huge task, we made the choice to focus on a discrete number of viral families chosen for their importance in human or animal disease and their mode of replication. In particular, this review will focus on some single-stranded RNA viruses belonging to the Flaviviridae, Coronaviridae and Togaviridae families, and double-stranded DNA viruses belonging to the Herpesviridae family, which represent viruses replicating in the cytoplasm or the nucleus of the infected cell, respectively. An abundant literature, including several reviews has already been published on the interaction between retro- and lenti-viruses, such the Human Immunodeficiency Virus (HIV), with the nucleolus [18–23]. There is also increasing data showing that plant viruses hijack the nucleolus to promote virus replication [24,25]. The information available on these latter viruses was deliberately omitted and we invite the readers to refer to specific articles for more detailed information on this topic.

2. The nucleolus: a central hub for the replication of pathogenic RNA viruses?

The majority of RNA viruses replicate in the cytoplasm of the infected cell where all the infectious cycle takes places, including transcription, replication of the RNA genome and assembly of newly infectious particles. Not surprisingly however, several studies have additionally described the interaction of a number of these viruses with the nucleus and in particular the nucleolus [26]. This chapter will focus on four different families of RNA viruses that possess a positive (+) strand RNA genome (Table 1). These four families contain viruses that are highly pathogenic in animals and/or primates, including man, and, consequently, most of them have been the focus of recent intensive studies. This is the case, in particular, for members of the Flaviviridae family such as Hepatitis C virus (HCV), a widely spread human virus which causes a chronic infection of the liver which can lead to cirrhosis and hepatocellular carcinoma [27], or the arthropod-transmitted viruses, Dengue virus (DENV), West Nile Encephalitis virus (WNV) or Japanese Encephalitis virus (JEV), which can cause severe hemorrhagic or neurological syndromes in man [28]. Members of the Coronaviridae and Arteriviridae families such as the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), the avian Infectious Bronchitis virus (IBV) and the Porcine Reproductive and Respiratory Syndrome virus (PPRSV) are also considered major pathogens causing severe respiratory diseases in man and animals [29]. Finally, it is of particular interest to also cite the interactions reported for two members of the Togaviridae family, the Semliki Forest Virus (SFV) and the Getah-like alphavirus (GETV) M1 which, even if not considered major pathogens for man, have attracted interest as anti-cancer tools [30] and could also, by extension, predict future interesting interactions for other more pathogenic members of this viral family such as the Chikungunja virus.

2.1. Replicative cycle of positive-strand RNA viruses

Positive-strand RNA viruses are composed of a lipid envelope containing the viral glycoproteins responsible for attachment to the cell membrane and penetration, surrounding a capsid that contains the RNA genome. The size and the shape of the assembled capsid can vary according to the virus but a common feature is that it is composed of multiple copies of a unique protein, called capsid, nucleocapsid (N), or core, which is able to bind and condense RNA and thus constitutes a protective shell for the viral genome. After attachment to the cell surface and delivery of the RNA in the cytoplasm, the viral genome is immediately translated into the enzymes required for its replication, which occurs *via* a negative (−) strand RNA intermediate. Newly replicated viral RNA molecules are used for the synthesis of the viral proteins and as a substrate during particle assembly. All these processes are accomplished by exploiting virus-encoded enzymes and cellular components, in particular cellular membranes which are involved in the formation of particles from intra-cytoplasmic organelles, mainly the endoplasmic reticulum and Golgi apparatus [27].

2.2. Viral factors interacting with the nucleolus

Despite the diversity of proteins encoded by the genomes of (+) strand RNA viruses, it is striking to observe that most of the reported interactions with the nucleolus concern the same structural protein, namely the capsid, which under different names has several common properties among all viral families including its small size (generally <50 kDa), clusters of basic amino acids (aa), and its ability to bind viral and sometimes cellular RNA. It is unclear if this finding reflects a true predilection of the nucleolus for this structural component or if it simply results from the fact that this is one of the most abundant viral proteins which is, therefore, easier to detect in particular in a compartment such as the nucleolus where the proteins rapidly shuttle in and out. Interestingly, some studies have reported the presence of non-structural viral proteins in the nucleolus. This is the case for the accessory protein 3b from the SARS-CoV, which was found to predominantly localize in the nucleolus [31,32]. Further studies indicated that this protein, which inhibits type I interferon (IFN I) production, could shuttle from the nucleus and mitochondria but, surprisingly, there was no further investigation as to its nucleolar localization [33]. Another example is provided by the nsP2 protein of SFV, which is a multifunctional protein essential for viral replication and maturation which was found localized mostly in the nucleus and nucleoli [34,35]. Surprisingly, again, this latter property was not re-investigated in further studies, which focused exclusively on its nuclear localization [33]. Lastly, deletion of the membrane-anchoring domain of the RNA-dependent RNA polymerase (RdRP) NS5B of HCV induced the delocalization of the protein in the nucleolus also suggesting that this viral enzyme contained a cryptic nucleolar localization signal (NoLS), allowing its transient traffic through the nucleolus [36].

2.3. Mechanisms of nucleolar import and export of viral proteins

Localization of viral proteins in the nucleolus is frequently not exclusive and sometimes hard to visualize. In some cases, nucleolar localization was revealed or enhanced by introducing deletions into domains of the protein suggesting that the signals involved in nucleolar targeting were masked by other domains or that this subcellular localization is restricted to some cleaved forms. This was particularly evident for the