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Viruses and the human DEAD-box helicase DDX3: inhibition or exploitation?

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Abstract

Human DDX3 is a DEAD (Asp-Glu-Ala-Asp)-box RNA helicase that appears to be a prime target for viral manipulation. While two viruses that manifest major global health threats, HIV and HCV (hepatitis C virus), utilize DDX3 for their replication, other viruses inhibit DDX3's newly identified function in innate antiviral signalling. This review discusses the role of DDX3 in antiviral immunity and its inhibition or exploitation by different viruses.

Introduction

DDX3(X), or DBX, is a member of the DEAD (Asp-Glu-Ala-Asp)-box helicase family and was first identified in 1997 [1]. DEAD-box helicases are involved in a large variety of cellular processes involving RNA, such as splicing, mRNA export, transcriptional and translational regulation, RNA decay and ribosome biogenesis [2]. DDX3 itself has been implicated in nearly all of these processes; however, the extent of its involvement and its exact mechanism of action is unclear for most of them [3]. Therefore it is fair to say that we currently lack a solid understanding of the functions of DDX3 in healthy cells. However, we and others have recently demonstrated that DDX3 contributes to antiviral innate immunity [4,5]. This is particularly interesting because, conversely, DDX3 is required for the replication of two viruses that pose major global health threats, namely HIV and HCV (hepatitis C virus) [6–8]. Owing to these findings, the inhibition of DDX3 has been suggested as a strategy for the development of novel therapeutics against these two viruses [9]. However, DDX3 also has reported roles in the regulation of protein translation, cell cycle control and apoptosis [3], in addition to its aforementioned novel function in antiviral immune signalling. Therefore it seems absolutely crucial to understand how exactly DDX3 contributes to these processes in order to design informed strategies for its therapeutic inhibition or manipulation. In the past, studying a viral protein and its host targets has often provided novel insights, not only about the virus, but also about the function of its host target. In our case, studying the immunoregulatory function of the VACV (vaccinia virus) protein K7 revealed a novel role for its host target, DDX3, in antiviral immunity [4].

Key words: DEAD-box helicase, innate immune signalling, type I interferon, viral host factor, viral immune evasion.

Abbreviations used: CRM1, chromosome region maintenance 1; ds, double-stranded; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; IKKε, IκB (inhibitor of nuclear factor κB) kinase ε; IPS-1, IFNβ promoter stimulator-1; IRF, IFN regulatory factor; mda-5, melanoma differentiation associated gene; NF-κB, nuclear factor κB; pol, polymerase; PRR, pattern recognition receptor; RIG, retinoic-acid inducible gene; RLH, RIG-like helicase; TBK1, TANK [TRAF (tumour-necrosis-factor-receptor-associated factor)-associated NF-κB activator]-binding kinase 1; TIR, Toll/interleukin-1 receptor; TLR, Toll-like receptor; VACV, vaccinia virus; ZBP, Z-DNA-binding protein.

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The role of DDX3 in innate immune signalling

Viral detection by innate immune cells is mediated by different sets of PRRs (pattern recognition receptors), mainly endosomal TLRs (Toll-like receptors) and cytoplasmic RLHs [RIG (retinoic-acid inducible gene)-like helicases], both of which recognize different classes of viral nucleic acids. Recently, several cytoplasmic DNA receptors were also identified that contribute to the immune response against DNA viruses. The engagement of these different antiviral PRRs triggers signalling cascades, which result in the activation of the transcription factors NF-κB (nuclear factor κB) and IRF3/7 [IFN (interferon) regulatory factor 3/7]. While NF-κB activation is a general feature of PRR signalling resulting in the expression of pro-inflammatory cytokines, IRF3/7 activation is a hallmark of the antiviral subsets of PRRs and leads to the induction of type I IFNs, which are potent antiviral mediators. The kinases TBK1 {TANK [TRAF (tumour-necrosis-factor-receptor-associated factor)-associated nuclear factor-κB activator]-binding kinase 1} and IKKε [IκB (inhibitor of NF-κB) kinase ε] phosphorylate and activate IRF3/7 downstream of TLR3, TLR4, the RLHs and (most likely) the cytoplasmic DNA receptors (Figure 1). Many viruses have evolved mechanisms to evade this immune response, either by avoiding recognition or by actively interfering with IFN induction or signalling. The VACV protein K7 potently inhibits IRF3/7 activation and IFNβ promoter activation induced by a range of different antiviral PRRs [4]. The identification of DDX3 as a molecular host target of K7 allowed us to implicate DDX3 in the antiviral signalling pathway leading to type I IFN induction [4]. We showed that DDX3 transiently associates with IKKε after virus stimulation. Knockdown of endogenous DDX3 using siRNA (small interfering RNA) led to decreased induction of the IFNβ promoter and IRF3/7-mediated

transactivation. This, together with the finding that K7 blocks Sendai virus-induced IRF3 phosphorylation, pointed towards an involvement of DDX3 in IKKε-induced IRF3/7 activation and, consequently, IFNβ promoter activation [4]. Concurrently, Soulat et al. [5] identified DDX3 as a TBK1 phosphorylation target. Their study also confirmed a role for DDX3 in PRR-induced IFNβ promoter activation and its function downstream of TBK1/IKKε [5]. Interestingly, they showed that DDX3 associates with the IFNβ promoter after PRR stimulation [5], suggesting that it acts as a transcriptional cofactor after activation by TBK1/IKKε (Figure 1). There is other evidence suggesting that DDX3 can indeed act as a transcriptional co-activator at the promoter level, e.g. it has been shown to be recruited to the promoter of the tumour suppressor p21/waf in an Sp1 (specificity protein 1)-dependent manner [10] and to bind and repress the E-cadherin promoter [11]. Therefore this could be a conceivable mechanism of action for DDX3. On the other hand, it has also recently been suggested that DDX3 acts in fact upstream of IKKε/TBK1 at the level of RIG-I and IPS-1 (IFNβ promoter stimulator-1) [12]. In a yeast two-hybrid screen, Oshiumi et al. [12] identified DDX3 as a protein that interacts with the CARD (caspase recruitment domain) domain of IPS-1, the mitochondrial adaptor molecule that mediates the initial signalling step after RLH triggering. Like all DEAD-box helicases and DExD/H-box helicases, DDX3 binds to RNA and potentially also DNA [13]. Oshiumi et al. [12] postulated that DDX3 acts as a sensor of viral RNAs and interacts with the RLHs, RIG-I and mda-5 (melanoma differentiation associated gene), and their adaptor molecule IPS-1 to initiate signalling. While RIG-I levels in uninfected cells are low and get up-regulated by IFNs during the course of viral infection, DDX3 is constitutively expressed in most cells at relatively high levels. Oshiumi et al. [12] therefore suggest that DDX3 senses viral RNA and facilitates signalling through IPS-1 at the initial stages of the infection when RIG-I levels are low (Figure 1).

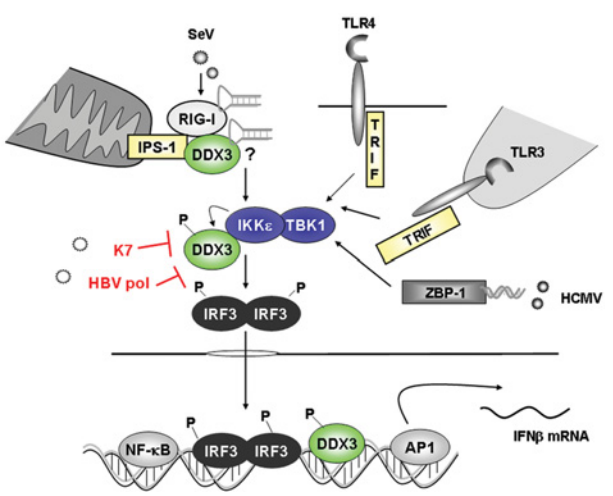
It is not inconceivable that DDX3 and other DEAD-box helicases can act as sensors for viral nucleic acids in the cytosol of infected cells, much in the same way the related DExD/H helicases RIG-I and mda-5 do. Two more DExD/H helicases, DHX36 and DHX9, have recently been shown to sense viral DNA (CpG-A and CpG-B respectively) in the cytosol and to couple to the TIR (Toll/interleukin-1 receptor)-adaptor molecule MyD88 (myeloid differentiation factor 88) for signalling [14]. It is therefore quite likely that additional DExD/H helicases will be discovered to play a role in RNA/DNA sensing.

However, the role of DDX3 does not appear to be limited to the sensing of viral RNA and IPS-1-dependent signalling pathways. DDX3 has also been shown to play a role in the TRIF (TIR domain-containing adaptor inducing IFNβ)-dependent TLR4 and TLR3 signalling pathways [4,5] and ZBP1 (Z-DNA-binding protein 1)-mediated induction of IFNβ by HCMV (human cytomegalovirus) [15]. In addition, several of the aforementioned studies support an involvement of DDX3 downstream of TBK1/IKKε, a point of convergence for the TLR3/4, RLH and ZBP1 signalling pathways [4,5,15–17].

So, while the Oshiumi et al. [12] study further supports a role for DDX3 in antiviral signalling and the induction of type I IFNs, it also opens up more questions about its exact function in this pathway: Does it act as a sensor, a signalling intermediate or a transcriptional regulator? Is it possible that DDX3 can be involved at three different levels? It is imaginable that DDX3 senses viral RNA, then orchestrates the assembly of signalling intermediates at the mitochondria and finally translocates into the nucleus to exert a direct effect

Figure 1 | The role of DDX3 in antiviral innate immune signalling

DDX3 contributes to IRF3/7 activation and IFNβ promoter induction downstream of different antiviral PRRs. Its exact function and placement in this signalling pathway remains to be determined, as DDX3 was suggested to act as RNA sensor, signalling intermediate and transcriptional co-activator. Recently, DDX3 was suggested to detect viral RNA and interact with IPS-1 to induce signalling in the early stages of infection [12]. Previously, DDX3 was shown to interact with, and be phosphorylated by, TBK1 and IKKε, the key kinases that phosphorylate IRF3/7 in the depicted antiviral signalling pathways [4,5]. The interaction between DDX3 and IKKε was transiently induced during Sendai virus (SeV) infection, linking DDX3 into the signalling pathway from RIG-I to IRF3 activation and IFNβ promoter activation [4]. IKKε and TBK1 also mediate IRF3 activation downstream of other pattern recognition receptors, such as TLR3 and TLR4 and cytoplasmic DNA receptors such as ZBP1. Consequently, DDX3 also contributes to IFNβ promoter induction downstream of these receptors [4,5,15–17]. DDX3 was also found to associate directly with the IFNβ promoter, suggesting that it can act as a transcriptional co-activator in this context [5]. The VACV protein K7 binds to the N-terminus of DDX3, which is required for IFNβ promoter induction. K7 is a potent inhibitor of IRF3 activation and IFNβ promoter induction. This suggests that K7 binding to DDX3 prevents it from exerting its effect on IFN induction in a typical viral immune evasion strategy [4]. The HBV pol was also recently shown to inhibit the function of DDX3 in this pathway in a manner similar to K7 [16,17]. AP1, activator protein 1; HCMV, human cytomegalovirus; TRIF, TIR domain-containing adaptor protein inducing IFNβ. Adapted from [3] with permission.



on the IFNβ promoter (after being activated by TBK1/IKKε-mediated phosphorylation). For example, it is an intriguing possibility that DDX3 could recruit IKKε to IPS-1, possibly by means of its N- and C-terminal regions respectively both of which have been suggested by different studies to be involved in its effect on IFNβ induction [4,12]. Further investigation of the temporal and spatial orchestration of these signalling events will clearly be necessary to clarify the role of DDX3 in this process.

Inhibition of DDX3 by viral proteins

The VACV protein K7

As mentioned above, we have recently identified DDX3 as a molecular host target for the VACV protein K7, a viral protein that potently inhibits TLR- and RLH-dependent IRF3/7 activation and IFNβ promoter induction [4]. This led us to identify the novel role for DDX3 in this innate immune signalling pathway as described above [9]. Interestingly, K7 binds to the N-terminal region of DDX3 (amino acids 1–139) that we found to be required for the effect on the IFNβ promoter [4]. In a more recent study, we have narrowed down the K7-DDX3 binding site to the region between amino acids 61 and 90 of DDX3 and shown that two phenylalanine residues in this region of DDX3 are required for its effect on IRF activation [18]. K7 therefore seems to target this region of DDX3 in a typical immune evasion strategy that the virus has evolved to suppress production of type I IFNs (Figure 1).

VACV is a ds (double-stranded) DNA virus that replicates in the cytoplasm, and mda-5 [19] and the newly described cytoplasmic DNA sensor Ifi16 (IFN-inducible 16) contribute to IFNβ induction in response to this virus [20]. By targeting DDX3, K7 is likely to interfere with both of these signalling pathways, as well as TLR3 and TLR4 signalling, assuming DDX3 indeed acts downstream of IKKε/TBK1.

The HBV pol

Another viral protein that interacts with DDX3 is the HBV (hepatitis B virus) pol (polymerase) [21]. HBV is a hepadnavirus and replication of its genome occurs by reverse transcription of a pregenomic RNA template. This occurs entirely within nucleocapsids and is mediated by HBV pol. The interaction between DDX3 and HBV pol appeared to be independent of their interaction with RNA. DDX3 was incorporated into nucleocapsids together with HBV pol and inhibited the initial step of reverse transcription in a manner that was dependent on the ATPase activity of DDX3 [21]. This suggested an antiviral role of DDX3 in HBV infection; however, it remained unclear how DDX3 interfered with viral replication. Interestingly, it has been shown that DDX3 levels are decreased in a fraction of HBV-induced HCC (hepatocellular carcinoma) cases [22]. It is possible that the virus down-regulates DDX3 levels to relieve the inhibitory effect of DDX3 on its replication. As DDX3 is a potential tumour suppressor, this might contribute to the development of HBV-induced HCC [22]. More recently, it was shown by two independent studies that HBV pol acts in a similar fashion to VACV K7 and inhibits IRF3 activation and IFNβ promoter induction by targeting DDX3. These studies demonstrated that HBV pol disrupts the interaction between IKKε and DDX3 and support a role for DDX3 downstream of TBK1/IKKε and upstream of IRF3 activation (Figure 1) [16,17].

It is currently unclear how and whether this finding is linked with the effect of DDX3 on HBV genome replication. It is unlikely that DDX3 exerts its inhibitory effect on HBV genome replication through IFNs, as ATPase activity of DDX3 was required for this effect but not for IFN induction. While all three studies support an antiviral role of DDX3 in HBV infection, the two more recent studies indicate that HBV pol inhibits DDX3 function (in IFN induction), while the earlier study suggested that DDX3 inhibits HBV pol function (in the replication of the viral genome). Perhaps, the DDX3–HBV pol interaction represents a stalemate in the co-evolution between host and virus, but further studies are required to ascertain the role of DDX3 in HBV infection. At present, it is unclear whether disrupting the HBV pol–DDX3 interaction would benefit the virus (by relieving the inhibitory effect of DDX3 on genome replication) or the host (by restoring DDX3 function in the IFN induction pathway).

Exploitation of DDX3 by viruses

HCV and DDX3

HCV core protein was the first viral protein to be described as a DDX3-interacting protein [23–25]. However, up to now, the functional relevance of this interaction is still unclear. HCV core protein is a structural protein, which forms the viral nucleocapsid, but there is evidence that the core protein has other functions, e.g. in the modulation of viral and cellular gene expression. The three original studies suggested that HCV core protein targets DDX3 to manipulate splicing [24], transcriptional [25] or translational regulation [23] respectively, but their studies were hampered by the scarcity of data being available on the cellular function of DDX3 at the time of discovery. HCV core binds to the C-terminus of DDX3 (amino acids 553–611), and the interaction is mediated by the N-terminus of HCV core (amino acids 1–40) [24,25].

It has recently been demonstrated that DDX3 is required for HCV RNA replication [6,7]. This suggested that HCV utilizes DDX3 for the replication of its RNA (possibly by unwinding dsRNA species); however, the exact contribution of DDX3 remained unclear. It is also unclear whether the DDX3 interaction with HCV core mediates the effect of DDX3 on HCV replication. It was recently shown that a JFH1 strain virus (HCV genotype 2a) carrying a mutation (Y35A) in the core protein that disrupts DDX3 binding has no replication defect. The mutated virus also continued to depend on DDX3 for its replication [26]. This would suggest that the virus can recruit DDX3 for its replication process in a core protein-independent manner. However, another recent

study observed an inhibitory effect of peptides derived from the DDX3-binding site of HCV core protein in a HCV replication system based on genotype 1b. The inhibitory effect of the core-derived peptides was reversed by DDX3 overexpression, suggesting that they were indeed acting by targeting DDX3 [27]. We will have to await further studies to clarify the requirement for the DDX3–core interaction in HCV replication.

HIV rev and DDX3

Interestingly, DDX3 is also an essential host factor for HIV replication [8]. HIV Rev protein interacts with DDX3 and the cellular nuclear export protein CRM1 (chromosome region maintenance 1), and thereby tags unspliced and partially spliced viral RNAs to this complex for export from the nucleus. Knockdown of DDX3 inhibited the export of these HIV RNAs from the nucleus and, consequently, HIV replication [8]. DDX3's helicase activity was required for its role in HIV RNA export, therefore its function in this process might be to unwind secondary structures within the HIV RNA or to remove RNA-bound proteins. It is currently unclear whether DDX3 is also required for the nuclear export of selected cellular CRM1 cargos, but it does not appear to have a general function in protein or RNA export [3]. It is an exciting possibility that DDX3 could be targeted for the development of novel anti-HIV therapeutics. Cellular and viral helicases have been successfully targeted therapeutically in the past [9], and knockdown of DDX3 in cells can inhibit HIV replication without affecting cell viability [28]. Two studies successfully used small-molecule inhibitors directed at the ATPase activity of DDX3 to inhibit HIV replication [29,30], which holds some promise for the therapeutic targeting of its helicase function. However, we will need to understand the cellular functions of DDX3 much better, in particular the role of its helicase activity and the physiological role of its interaction with CRM1, to design therapeutic strategies that safely target DDX3 in the context of HIV infection.

Concluding remarks

It is fascinating that DDX3 appears to be a prime target for viral manipulation. So far, four very different viruses have been shown to interact with DDX3 and to manipulate its function in very different ways. Two of the viruses that pose the biggest global health threats, HIV and HCV, recruit and require DDX3 for their replication. This opens exciting possibilities for the therapeutic targeting of DDX3. HIV/HCV co-infections are not uncommon, and it is an intriguing idea that a drug targeting DDX3 could combat both viruses simultaneously. On the other hand, DDX3 has an antiviral role in the induction of type I IFNs. Two viruses, HBV and poxvirus, have already been shown to target this function of DDX3 in a typical immune evasion strategy, and it is not unlikely that additional viruses target DDX3 in this way. It is also a possibility that viruses recruiting DDX3 for their replication simultaneously sequester it from its function in antiviral immunity. For DDX3 to become a viable therapeutic target in virus infections and/or autoimmunity, we have to develop a much better understanding of its multiple functions in order to target them selectively and with minimal disruption to cell homoeostasis. Studying the ways viruses target DDX3 may well provide us with useful insights for developing our own tools to manipulate DDX3 function.

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