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Advances in the Host Antiviral CCCH-Zinc Finger Proteins

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Short Commentary

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ABSTRACT

Recognition and degradation of viral RNA are essential for antiviral innate immune responses. Zinc-finger proteins (ZFPs) are gaining intensive concern because they are attractive targets for the control of multiple cellular processes. In this short commentary, we focus on the two popular host antiviral ZFPs, zinc antiviral protein (ZAP) and monocyte chemotactic protein-induced protein 1 (MCPIP1), and discuss their antiviral effects, mechanisms and prospects.

INTRODUCTION

Zinc-finger proteins (ZFPs) are the largest transcription factor family in human genome. They have recently become an area of intense study because of their broad range of biological functions, including development, differentiation, metabolism, autophagy, cancer progression and more recently because they are attractive targets for antiviral therapy [1]. The zinc fingers was first recognized as relatively small protein motifs in Xenopus transcription factor IIIA (TFIIIA), which contain conserved cysteine (Cys) and histidine (His) ligands [1]. Till now, numerous zinc-binding motifs have been identified as zinc fingers which are encoded by 1% of the mammalian genes. To date, 8 different classes of zinc finger motifs have been reported, including Cys2His2 (C2H2) like, Gag knuckle, Treble clef, Zinc ribbon, Zn2/Cys6, TAZ2 domain like, Zinc binding loops and Metallothionein. Different types of zinc finger motifs show great diversity of functions in various cellular processes, such as transcriptional activation, translation regulation, metabolism and cell proliferation and apoptosis [2].

Zinc fingers are structurally diverse and categorized by the nature and spacing of the zinc-chelating residues, most of them are classical CCHH or CCCC zinc finger type [3,4]. The CCCH zinc finger family containing the motif with three Cys and one His residues, which has been well-known to be involved in the RNA stability and metabolism [5,6]. About 60 CCCH zinc finger proteins in mouse and human have been identified and most of those have not been investigated [7]. One of the most early and widely studied members is Tistetraprolin (TTP, also known as Zfp36) family, which contains two tandem CCCH-zinc fingers and could lead mRNA degradation by binding to AU-rich elements (ARE) in the 3'-untranslated region (3'UTR) of mRNA and increasing rates of mRNA deadenylation and destruction [8-10]. TTP knockout mice developed a systemic syndrome of arthritis, skin lesions and autoimmunity, as well as myeloid hyperplasia due to excess tumor necrosis factor-α (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) accumulation [11,12]. Moreover, recent studies revealed that Rc3h1 (also known as Roquin), which contains a ring-finger and a CCCH-zinc finger, repressed autoimmunity by promoting the mRNA degradation of T cell co-stimulator [13]. Thus, the researches about a few known CCCH zinc finger proteins suggest that this family may be critical in the regulation of immune and inflammatory response. In this short commentary, we will emphasize on the two family members- zinc antiviral protein (ZAP) and monocyte chemotactic protein-induced protein 1 (MCPIP1)- and discuss their antiviral effects, mechanisms and prospects.

Zinc-finger antiviral protein (ZAP, also known as zinc finger CCCH-type antiviral 1, ZC3HAV1) prevents the accumulation of viral mRNA in the cytoplasm by targeting viral RNA for degradation. It was originally identified in rat as a host factor that inhibits Moloney murine leukemia virus (MMLV) in cells [14]. Afterwards it was confirmed to suppress the replication of positive-

stranded RNA viruses (Alphavirus and Flavivirus of Togaviridae), negative stranded RNA viruses (*Filoviridae* such as EBOV and MARV) and retroviruses (Retroviridae such as HIV, HBV and MMLV) in human cell lines. Recently, we have demonstrated the antiviral properties of ZAP against coxsackievirus B3 (CVB3), a single-stranded RNA virus of the *Enterovirus* genus within the *Picornaviridae* as a major causative agent of viral myocarditis (VMC), not only in cell line but also in murine model of CVB3-induced acute myocarditis [15].

ZAP is demonstrated to exert its antiviral effect by binding to viral RNA through its CCCH zinc finger motif and recruiting host RNA exosome or cellular decapping complex to degrade viral RNA in the cytoplasm [16,17]. The target virus of ZAP depends on whether it has ZAP-responsive elements (ZRE) in its viral RNA. No obvious common ZRE sequences or structure has been reported. The ZREs in MMLV and XMRV were located in the 3'UTR but ZRE of HIV was mapped to the 5'UTR of spliced Mrna [16,18,19]. Our data reveal that ZAP target non-ARE sequence of 3'UTR and (250- 741) nt fragment of 5'UTR within CVB3 RNA [15]. Currently, there are few reports of antiviral effect of endogenous ZAP in primary cells or *in vivo*. In 2013, Akira group generated ZAP knockout mice and found ZAP deficiency greatly enhanced the replication efficiency of MLV in MEFs which is independent on retinoic acid inducible gene I- like receptor [17]. We used a two-injection (interval of 3 days) of PEI-packaged ZAP plasmids strategy to keep ZAP overexpressing for 7 days without any myocardial pathology and demonstrated that the enhanced antiviral effect of ZAP led to an alleviation of myocarditis. Recently, Gao group constructed ZAP conditional knockout mice and reported its different effect against a neurovirulent Sindbis virus strain (SVNI) [20]. They found that ZAP deficiency led to reduced survival in 10 day-old suckling mice but improved survival in 23 day-old weanling mice, which was further explored and revealed that in the weanling knockout mice SVNI replicated more efficient in lymphoid tissues and induced higher IFN production resulting in the restriction viral spread to the central nervous system [20].

However, expression of ZAP did not induce a broad-spectrum antiviral state. DNA virus like herpes simplex virus 1 (HSV-1), and RNA viruses including vesicular stomatitis virus (VSV), yellow fever virus and poliovirus, could escape from the antiviral activity of ZAP [18-23]. Viral proteins might directly affect the stability of ZAP as revealed by Zheng's group [24] that the HSV-1 tegument protein UL41 abrogated the induction of ZAP expression and antiviral activity of ZAP by targeting its mRNA for degradation after HSV-1 infection. Virus can also escape ZAP-mediated immunity by interfering interaction between ZAP and viral RNAs or ZAP functional domains which is required for its antiviral activity. Murine gammaherpesvirus 68 (MHV-68) infection induced ZAP expression but the MHV-68 transactivator, Replication and transcription activator (RTA), antagonized the antiviral activity of ZAP by indirectly disrupting the N-terminal intermolecular interaction of ZAP in the lytic phase [25]. It indicates that a couple of viruses have evolved strategies to antagonize the antiviral function of ZAP which narrow the antiviral spectrum of ZAP.

Monocyte chemotactic protein-induced protein 1 (MCPIP1, also known as Zc3h12a or regulatory RNase 1), containing a CCCH-zinc finger and a YacP Nuclease domains, has been recently implicated in the exhibiting broad-spectrum antiviral effects through viral RNA binding and degradation [26]. It was originally identified in human peripheral blood monocytes induced by the monocyte chemotactic protein (MCP) as a negative regulator in the macrophage activation [27,28]. Accumulated evidence demonstrate that MCPIP1 could also be induced by IL-1β, TNF-α, LPS or other stimuli and negatively regulate the inflammatory response during innate and adaptive immune responses [29-31]. MCPIP1 was once reported to act as a deubiquitinase to inhibit JNK and NF-κB signaling pathways by removing the ubiquitin moieties of TNF receptor-associated factors (TRAFs) [32]. Other studies suggested that MCPIP1 functioned as an essential PilT N-terminus-like RNase to decay specific mRNAs of IL-12p40 and IL-6 by targeting a conserved non-AU-rich element (ARE) of 3'-untranslated region (UTR) [30,31]. In 2013, Lin group reported that MCPIP1 ribonuclease exhibited broad-spectrum antiviral effects against Japanese encephalitis virus (JEV) and dengue virus (DEN) [25]. Furthermore, infection of other RNA viruses, such as sindbis virus and encephalomyocarditis virus and influenza virus, as well as DNA virus, such as adenovirus, can also be suppressed by MCPIP1 [25]. MCPIP1 was further found to inhibit the replication of human immunodeficiency virus (HIV) and hepatitis virus (HCV) [33,34]. However, MCPIP1 does not exhibit a universal antiviral effect commonly to all viruses and some viruses including *enterovirus* 71 (EV71) and VSV replicate to normal levels in MCPIP1-overexpressed cells [25], yet the underlying molecular mechanism has not been elucidated. The antiviral mechanism of MCPIP1 was dedicated to its degradation of viral RNA directly through its RNA-binding capacity and RNase activity via the CCCH zinc domain and NYN domain [26,33-34], similar mechanism utilized by MCPIP1 to affect the stability of cytokine mRNA [30,31]. MCPIP1 was reported to degrade four different sub-fragments of JEV RNA and later found to cleave the 3'UTR region of HCV genome [26,34]. Our recent study revealed that MCPIP1 suppressed CVB3 replication and virus-mediated inflammatory response by targeting non-ARE region of 3'UTR of CVB3 RNA [35]. All these data suggests that MCPIP1 is also an important host antiviral factor and it's *in vivo* biological relevance needs further investigation.

As members of the CCCH-zinc finger protein family, ZAP and MCPIP1 have their distinct antiviral mechanisms. ZAP binds viral RNA through its zinc finger domain but needs recruitment of exosome component and RNA helicase for the degradation of viral transcripts [17]. MCPIP1 also binds viral RNA through its zinc finger domain but directly degrades viral RNA via its intrinsic RNase activity without additional help of RNA-degradation machinery [26,32,33]. For the RNA recognition motif, no obvious common sequence or structure was recognized by ZAP. The ZAP-targeted ZREs may be located in the 3'UTR of MMLV and XMRV but mapped to the 5'UTR of spliced mRNA of HIV [16,18,19]. We found that ZAP targeted non-ARE sequence of 3'UTR and (250-741nt) fragment of 5'UTR within CVB3 RNA15. The common characteristic of ZAP-targeted ZREs is non AU-rich elements, which is

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different from TTP. MCPIP1, however, targets non-ARE sequence and specific stem-loop structure of the 3'UTR of cytokine mRNA and HCV RNA [26,34]. Not only that, ZAP and MCPIP1 play distinct roles in the regulation of innate and inflammatory responses. ZAP was once reported to modulate innate antiviral immunity by boosting RIG-I signaling in human cell lines [36]. But Akira group reported that ZAP did not regulate RIG-I-dependent type I IFN response in primary mouse cells and inhibited MLV independently of RLR-IRF3/7 signaling axis [17]. Our study also failed to detect any increase of IFN expression after overexpression of ZAP *in vivo* [15]; while MCPIP1 is recognized as a critical negative regulator in innate and adaptive immunity.

ZFPs represent as important innate antiviral effectors and the dominant roles of ZFPs *in vivo* and their physiological relevance await further investigation. Future research using newly developed knockout mice and clinical samples from infected patients should focus on assigning protective or pathogenic functions for different viruses in physiological condition, examining the cell and tissue-type specific mechanisms of action, and exploring strategies for the regulation of ZFPs at different levels in viral infection. Since ZFPs may have a much broader antiviral spectrum than it appears to have at the present, it is necessary to elucidate how ZFPs are activated and regulated upon virus infection, and how their antiviral function is antagonized by viral proteins. New approaches aimed at blocking or inhibiting critical viral restriction factors, such as UL41, would significantly enhance the activity of ZAPs against HSV-1 at innate stage. A better understanding of the molecular mechanisms evolved by viruses to counteract ZFP antiviral activity will hopefully lead to the identification and development of drugs that specifically interfere with viral processes.

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