DNA sensor C GAS- CANSOR DETECTION

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ABSTRACT

The host takes use of pattern recognition receptors (PRRs) to defend against pathogen invasion or cellular damage. Among microorganism-associated molecular patterns detected by host PRRs, nucleic acids derived from bacteria or viruses are tightly supervised, providing a fundamental mechanism of host defense. Pathogenic DNAs are supposed to be detected by DNA sensors that induce the activation of NFκB or TBK1-IRF3 pathway. DNA sensor cGAS is widely expressed in innate immune cells and is a key sensor of invading DNAs in several cell types. cGAS binds to DNA, followed by a conformational change that allows the synthesis of cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) from adenosine triphosphate and guanosine triphosphate. cGAMP is a strong activator of STING that can activate IRF3 and subsequent type I interferon production. Here we describe recent progresses in DNA sensors especially cGAS in the innate immune responses against pathogenic DNAs.

KEYWORDS *cGAS ,cGAMP ,innateimmunity, cytosolic DNAs, DNA sensors*

INTRODUCTION

Innate immunity provides the first defense line of host to invading microbes (Wu and Chen, 2014). The host has Innate immunity provides the first defense line of host to invading microbes (Wu and Chen, 2014). The host has developed a series of pattern-recognition receptors (PRRs) to recognize and fight against pathogen-associated molecular patterns (PAMPs) that are present on microbes, such as peptidoglycans (Schwandner et al., 1999), lipopolysaccharides (LPS) (Brightbill et al., 1999; Zhang et al., 1999) and flagellin (Mizel et al., 2003). PRRs can also detect damage associated molecular pattern molecules (DAMPs) that are derived from the host itself under stresses, including heatshock proteins (Wick et al., 2014), HMGB1 (Bangert et al., 2016), ATP (Kouzaki et al., 2011), uric acid (Andrews, 2005), heparin sulfate (Tsunekawa et al., 2016) and DNA (Chan and Gack, 2016). The most known PRRs are the Toll-like receptor (TLR) family that are expressed on innate immune cells such as dendritic cells (DCs), macrophages and neutrophils (Yin et al., 2015). Most TLRs detect extracellular PAMPs (Gay et al., 2014). For example, TLR1 and TLR2 recognize triacylated lipoproteins from bacteria and GPIanchored proteins from parasites (Kirschning et al., 1998). TLR2 and TLR6 detect diacylated lipoproteins from Gram positive bacteria and zymosan from fungi (McCurdy et al., 2003; Ozinsky et al., 2000). TLR4 is a receptor for LPS from Gram negative bacteria (Poltorak et al., 1998) and TLR5 recognizes flagellin from motile bacteria (Hayashi et al., 2001). Finally, TLR11 can be activated by uropathogenic E. coli and T. gondii (Lauw et al., 2005). Collectively, the above TLRs render the host to distinguish self from non-self PAMPs that are derived from invading pathogens.

Besides above extracellular PAMPs, microbes can also deliver PAMPs to the cytosol of host cells through bacterial secretion system or engulfment by cells (Saito and Gale, 2007; Wilkins and Gale, 2010). These cytosolic PAMPs are surveyed by intracellular PRRs (Beachboard and Horner, 2016). For instance, intracellular LPS can be recognized by inflammatory caspases (Shi et al., 2014; Yang et al., 2015), and intracellular flagellin is detected by NAIP5 (Gong and

Shao, 2012; Zhao et al., 2011). Engulfed CpG rich DNAs are sensed by TLR9 in the endosomal compartment (Hemmi et al., 2000; Krieg, 2003). In general, all microbes rely on DNA or RNA for their basic life activities such as protein encoding, movement and proliferation (Hornung et al., 2014).

When encountered microbial DNAs, the situation is a little complicated. Microbial DNAs do not have tri-phosphorylated groups in their 5' ends and they are constituted by the same elements as are host DNAs (Abdullah and Knolle, 2014; Holm et al., 2013). However, eukaryotic genomic DNAs are surrounded by nuclear walls that separate DNAs from the cytosol (Beachboard and Horner, 2016). Meanwhile, invading pathogens enter the host cytosol at first and some pathogens live in the cytosol, leaving the cytosolic compartment to be contaminated by microbial DNAs (Holm et al., 2013; Hornung, 2014; Hornung et al., 2014). The cellular localization of invading DNAs provides the possibility that the host utilizes cytosolic DNA sensors to respond to these stimuli. Up to now, many cytosolic DNA sensors have been reported to recognize intracellular pathogenic DNAs (Fig. 1). For example, DDX41 (Zhang et al., 2011b), IFI16 (Orzalli et al., 2012; Unterholzner et al., 2010) and DAI (Takaoka et al., 2007) detect double stranded DNAs (dsDNAs) and activate the STING-TBK1-IRF3 pathway. LRRFIP1 binds dsDNA and triggers IRF3 activation through β-catenin (Yang et al., 2010). DHX9 and DHX36 associate with dsDNA and lead to NFκB activation through MyD88 (Kim et al., 2010). Ku70 is reported to bind dsDNA and promote production of type I interferon (IFN) through activation of IRF1 and IRF7 (Zhang et al., 2011a). AIM2 interacts with dsDNA and activates inflammasomes by recruiting ASC and pro-caspase-1 (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009). Of note, Sox2 is expressed in the cytosol of neutrophils and activates the Tab2/TAK1 complex upon binding to dsDNA in a sequence-dependent manner (Xia et al., 2015)

cGAS (encoded by MB21D1 gene) has been recently defined a well-known DNA sensor that recognizes cytoplasmic DNA (Ablasser et al., 2013a; Ablasser et al., 2013b; Gao et al., 2013a; Li et al., 2013b; Schoggins et al., 2014; Sun et al., 2013; Wu et al., 2013). Double stranded DNAs (dsDNAs) longer than 36 bp are optimal for cGAS activation (Gao et al., 2013b). Once detect DNA, cGAS undergoes a conformational change that allows ATP and GTP to come into the catalytic pocket, leading to the synthesis of cGAMP a strong activator of the STING-TBK1 axis (Civril et al., 2013; Gao et al., 2013b; Kranzusch et al., 2013; Wu et al., 2013; Zhang et al., 2014). cGAS is involved in immune recognition of various DNA viruses, certain retroviruses, and even intracellular bacteria (Collins et al., 2015; Li et al., 2013b; Wassermann et al., 2015; Watson et al., 2015; Yoh et al., 2015). cGAS can be activated by dsDNAs and DNA-RNA hybrids (Mankan et al., 2014). Besides sensing exogenous pathogenic DNAs, cGAS is also responsible for monitoring self-originated DNA (Rongvaux et al., 2014; White et al., 2014). During HSV infection, mitochondrial integrity is destroyed, causing the release of mitochondrial DNA (mtDNA) that can be detected by cytosolic cGAS (West et al., 2015). Moreover, in caspase deficient cells, mtDNA is delivered to the cytoplasm, causing cGAS activation during the process of apoptosis (Rongvaux et al., 2014; White et al., 2014). In this review, we will introduce the discovery of cGAS and describe the properties of cGAS as a DNA sensor. Mysteries prior to cGAS discovery

As mentioned above, many DNA sensors have been defined to mediate recognition of exogenous DNAs. However, most of them rely on STING to initiate the production of type I IFNs. STING plays a central role in innate immune responses against foreign DNAs. Structural analysis shows that STING does not have the cavity to bind dsDNAs. Interestingly, STING has a high affinity for cyclic dinucleotides such as c-di-GMP or c-di-AMP. Cyclic dinucleotides are important secondary messenger molecules involved in signaling of bacteria. They are secreted into the cytosol of host during the invasion or replication of bacteria by multidrug efflux pumps (Whiteley et al., 2015). c-di-AMP secreted by L. monocytogenes activates the STING-IRF3 pathway, leading to elevated type I interferon levels that hinder the clearance of intracellular bacteria (Auerbuch et al., 2004; O'Connell et al., 2004). It is curious that STING has such a high affinity for cyclic dinucleotides which seems irrelevant to exogenous DNA recognition. Many genes involved in innate immune responses are interferon-stimulated genes (ISGs) that are upregulated post infection. Through large-scale ISG screening, six important ISGs were identified as below: IRF1, RIG-I, C6orf150 (also known as cGAS, encoded by MB21D1 gene), IFITM3, HPSE and MDA5 (Schoggins and Rice, 2011; Schoggins et al., 2011). Among these ISGs,

C6orf150 and HPSE were less investigated with unknown functions in the antiviral process. Meanwhile, whether these ISGs play an essential role in foreign DNA recognition remained elusive.

cGAS and cGAMP

Based on activity assays using cell extracts, cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) was identified to be an activator of STING (Wu et al., 2013). Mammalian cytoplasmic extracts catalyze the synthesis of cGAMP from adenosine triphosphate (ATP) and guanosine triphosphate (GTP) in the presence of dsDNAs. Both transfected dsDNA and viral DNA initiate the synthesis of cGAMP that binds to STING and initiates IRF3 activation and interferon production. Through fractionation of cytosolic extracts, cGAMP synthase (cGAS) was identified as the missing link between foreign dsDNA and cGAMP (Sun et al., 2013). cGAS, a member of the nucleotidyltransferase family, binds to dsDNA directly in the cytosol and catalyzes cGAMP synthesis from ATP and GTP (Fig. 1). cGAS overexpression promotes IRF3 activation and subsequent type I IFN production. In contrast, cGAS knockdown suppresses the IRF3 activation and type I IFN generation in a STING-dependent manner. In addition to transfected dsDNAs and viral DNAs, cGAS also mediates immune responses against retroviruses such as HIV (Gao et al., 2013a).

As a nucleotidyltransferase, cGAS catalyzes the synthesis of a product that is totally different from cyclic dinucleotides characterized previously. cGAS catalyzes the synthesis of cGAMP that has a 2′-5′ and a 3′-5′ phosphodiester linkage ([Gp(2′-5′)Ap(3′-5′)]) (Ablasser et al., 2013a; Gao et al., 2013b). cGAS promotes the synthesis of cGAMP from ATP and GTP through two steps (Ablasser et al., 2013a). cGAS first catalyzes the synthesis of a 2′-5′-linked dinucleotide from ATP and GTP, followed by another catalysis of a 3′-5′ phosphodiester linkage that finally cyclizes ATP and GTP. During the first step of the synthesis, the attacking nucleotide determines the phosphodiester bond type generated from ATP and GTP. If 5′-GTP is the attacking nucleotide, a 2′-5′-linkage will be formed. If 5′-ATP is the attacking one, a 3′-5′-linkage will be generated. However, cGAS prefers GTP as the attacking nucleotide, generating only linear pppGp(2’-5’)A. This feature of cGAS results in almost absolute synthesis of [Gp(2′-5′)Ap(3′-5′)] in the second step of cGAMP production. This 2′-5′-linked dinucleotide is a strong agonist of STING and binding of cGAMP to STING leads to STING conformational change and subsequent IRF3 activation (Gao et al., 2013b). STING forms a “closed” conformation from a “open” conformation once binds to cGAMP (Zhang et al., 2013). Structural analyses show that STING exists as a symmetrical dimer with or without binding to its ligands. The STING dimer forms a V-shaped conformation with a pocket between two STING molecules. Ligand binding causes STING conformation change with an inward-shift of the symmetrically-related α2- hilices on STING. For human STING protein, positions 230 and 232 are essential for best responses to 2’-5’-linked cGAMP. Substitution of amino acid 232 impairs the immune responses triggered by c-di-GMP but not cGAMP.

Structural basis of cGAS activity

Structural analyses provide a better understanding of how cGAS works in the process of DNA recognition. Mouse cGAS has an NTase domain from amino acid 148 (aa148) to aa370 and a Mab21 domain from aa199 to aa498 (Sun et al., 2013). The Mab21 domain is composed of two lobes divided by a deep cleft (Civril et al., 2013). One lobe has the NTase domain with a twisted β-sheet flanked by two α-helices (Fig. 2). Along the cleft, two β-sheets harbor the catalytic residues. Opposite of the catalytic site is a fairly flat surface that compromises the nucleotide-binding loop (Fig. 2). A protrusion is located on the edge of the surface, comprising conserved histidine and cysteines. This is a Zn2+ ion binding loop denoted as “Zn thumb” (Fig. 2). Functional analyses with cGAS mutations show that the active site residues. cGAS dimerization is also an important prerequisite for cGAS activation (Li et al., 2013a; Zhang et al., 2014). cGAS exhibits an autoinhibited conformation that the activation loop directs to the outside, hindering the synthesis of cGAMP from ATP and GTP (Fig. 2). However, when cGAS binds to dsDNA, the positively charged residues on the nucleotidebinding loop associate with negatively charged nucleotide residues, leading to conformational changes that allow cGAMP catalyzation. Of note, cGAS is a monomer in the absence of dsDNAs. When cGAS binds to dsDNA, a 2:2 dimer is formed between cGAS and dsDNA (Li et al., 2013a; Zhang et al., 2014). Residues N389 to E398 in the Zn thumb are essential for cGAS dimmer formation. Moreover, residues A346 and

K347 also favor dimer formation through forming two hydrogen bonds with residue E398.

cGAS mediates immune responses

cGAS is essential for immune recognition of foreign DNAs from various sources, such as DNA viruses, retroviruses and intracellular bacteria (Fig. 3) (Collins et al., 2015; Gao et al., 2013a; Wassermann et al., 2015; Watson et al., 2015; Yoh et al., 2015). The role of cGAS in HIV infection has been intensely investigated post cGAS discovery. There are two types of HIVs in humans: HIV-1 and HIV-2. Compared with HIV-2, HIV-1 is more common and pathogenic. Besides infected T cells, HIV-2 also replicates in DCs accompanied by activated innate immune responses (Manel et al., 2010). Further analysis shows that cellular factor SAMHD1 restricts HIV-1 infection in DCs and macrophages through negative regulation of interferon responses (Hrecka et al., 2011; Laguette et al., 2011). However, HIV-2 encodes Vpx that mediates SAMHD1 degradation through the CRL4-DCAF1 E3 ubiquitin ligase, resulting in HIV-2 replication in DCs and macrophages (Hrecka et al., 2011; Laguette et al., 2011). DCs sense reverse-transcribed viral cDNA of HIV-2 by cGAS, leading to type I IFN production and surrounding T cell activation. HIV-1 encoded capsid protects its viral cDNA from cGAS-mediated sensing in DCs (Lahaye et al., 2013). DCs play an essential role in immune responses against HIVs. Co-infection of HIV-1 and HIV-2 leads to a delayed lling HIV infection (Esbjornsson et al., 2012). During the reverse transcription of HIV viral RNAs, only single-stranded DNA is generated. However, based on previous knowledge on cGAS, only double-stranded DNA can associate with and activate cGAS (Wu and Chen, 2014). Then how HIV-derived DNA is recognized by cGAS? Actually, HIVderived single-stranded DNA forms short base-paired DNA stretches that are strong activators of cGAS (Herzner et al., 2015). These short dsDNAs (also called Y-form DNA) formed from single-stranded DNAs are the predominant viral DNAs in the cytosol during the early infection of host cells. Y-form DNAs are characterized by unpaired guanosines flanking the stem region. They are flexible in respect of the numbers of unpaired guanosines and represent a newly discovered robust PAMP.

CONCLUDING REMARKS

Although cGAS plays an important role of cytosolic DNA sensing in cell types such as macrophages, DCs and fibroblasts, there are also some cell types do not express cGAS but have normal cytosolic DNA sensing potential. For instance, in CD4 T cells, IFI16 is the main cytoplasmic DNA sensor for HIV reverse-transcribed DNAs (Monroe et al., 2014). In neutrophils, Sox2 acts as a DNA sensor for invading cytosolic bacterial DNA (Xia et al., 2015). Sox2 localizes in the cytoplasm of neutrophils and recognizes microbial DNA through its HMG domain. Moreover, Sox2 associates with TAB2 upon bacterial DNA stimulation and activates the TAB2-TAK1 kinase complex through dimerization. Interestingly, Sox2-mediated DNA recognition is sequence-dependent. Sequence-specific recognition of exogenous DNAs has long been used by low forms of life such as eubacteria and archea. Restriction endonucleases are DNA sequence-specific enzymes that are utilized by bacteria to cleave invading viral DNAs (Jinek et al., 2012). These enzymes provide the innate defense against phage DNAs. In addition, the recently developed CRISPR/Cas9 technology is also originated from bacteria. Bacteria synthesize guiding RNAs from previous integrated virus DNAs to target and degrade incoming virus DNAs (Sampson et al., 2013). This sequence reliable system is a kind of acquired immunity which mimics adoptive immunity in higher organisms. Therefore, it is plausible that higher organisms might reserve these sequence-specific defense systems during evolution, just like the DNA sensing by Sox2 in human and mouse neutrophils.

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