CHAPTER ONE

Introduction

Innate immunity and adaptive immunity

The immune system consists of two major components: innate immunity, which provides early, general reactions to infections, and adaptive immunity, which later recognizes and reacts to microbial and non-microbial substances in specific manner.

Once infection events are detected, the host quickly, usually within minutes to hours, initiate innate immune response as the first lines to defend. Cells involved in these lines include macrophages, natural killer (NK) cells, neutrophils and dendritic cells. These cells are recruited and migrate to the battle field, the infection site where macrophages are activated and produce a large amount of chemokines (such as IL-8, IP-10, and CXCL-10) and cytokines (such as TNF-a, IL-6, IL-12, and type-I interferons) (Aderem 2001). These chemokines and cytokines lead to the effect of inflammation at infection site, including vascular permeability increase, dendritic cells maturation, and neutrophil recruitment. To defend against infection, the innate immunity utilizes generally two methods. One is by the production of cationic anti-microbial peptides, including defensins and cathelicidins. Working together with conventional antibiotics, other peptides, and lysozyme, these peptides can kill bacteria very rapidly and can be effective against antibiotic-resistant infections (Hancock and Scott 2000). The other mechanism to defend infection is to kill by macrophages and neutrophils that have migrated to the site of infection. These cells can intake and eliminate pathogen particles either by phagocytosis or by receptor mediated endocytosis.

Adaptive immune response usually takes place at least one day or several days after the initial contact with a pathogen. Lymphocytes, including B cells and T cells, are the major players in adaptive immunity and they provide host with specific protection with memory. After in contact with antigens that have been processed and presented by dendritic cells and other antigen presenting cells, naïve B cells become activated and expand. Meanwhile, somatic hypermutation and affinity maturation processes increase the affinity of the antibodies produced by mature B cells for specific antigens. Antibodies are secreted and are able to recognize and bind to antigens presented on the surface of pathogens, such as virus and bacteria. The antibody-antigen binding would then initiate pathogen clearance responses. On the other hand, T lymphocytes play central role in cell mediated immunity and are consist of two major subtypes: CD4+ helper T cells (Th) and CD8+ cytotoxic T lymphocytes (CTL). CD4+ T cells produce various types of cytokines to assist other cell type in processes including the activation of macrophages and CTL, maturation of B lymphocytes, and eliminate pathogens or tumors. CD8+ T cells, also called T-killer cells, are responsible to destroy compromised cells, including bacteria or virus infected cells as well as tumor cells.

General model for innate immune response

The innate immunity is a critical immune response that protect the host from infections immediately after they occur. The detection of the threats from viruses or bacteria is through a series of germline encoded receptors, called pattern recognition receptors (PRRs). These receptors usually detect pathogen molecules that are much conserved evolutionarily, termed as pathogen-associated molecular patterns (PAMPs). PAMPs are very specific to pathogens thus provide a mechanism for the immune receptors to differentiate non-self from self. One of the most famous PAMP is lipopolysaccharide (LPS) that is recognized by TLR4 together with an accessory protein MD2 (Kim et al. 2007). Upon ligand binding and activation, the alert signals are usually transmitted from the receptor to its downstream adaptor proteins. TLRs usually dependent on myeloid differentiation primary-response gene 88 (MyD88) (Burns et al. 1998) or TIR domain-containing adaptor inducing IFN-β (TRIF) (O'Neill and Bowie 2007) as adaptor proteins (Akira and Takeda 2004). RIG-I and MDA5 that sense RNA utilize mitochondrial antiviral-signaling protein (MAVS) for downstream signaling transduction (Seth et al. 2005). The newly discovered cytosolic DNA sensor, cGAS, synthesizes a secondary small messenger molecule, cGAMP, to activate its adaptor protein STING (Sun et al. 2013, Ishikawa, Ma, and Barber 2009). The activation of these adaptor proteins serves as a platform for the recruitment and activation of kinases, including TRAF family member-associated NF-kB activator (TANK)-binding kinase 1 (TBK1) (Fitzgerald et al. 2003), IkB kinase (IKK) (Wang et al. 2001), IL-1 receptor (IL-1R)-associated kinase (IRAK) (Burns et al. 1998), Janus kinases (JAKs) (Der et al. 1998), and mitogen-activated protein kinases (MAPKs) (Chang and Karin 2001). These kinases, along with the adaptor proteins, activate transcription factors, including interferon regulatory factor (IRF) (Kawai and Akira 2007), NF-kB, activator protein 1 (AP1) (Kawai and Akira 2007), and signal transducers and activators of transcription (STATs) (Baetz et al. 2004). These transcription factors would translocate into the nucleus and, together with transcriptional co-factors, like p200 and CBP, to promote the production of type-I interferons, inflammatory cytokines and other effector proteins to eliminate infectious threats.

IRF5 signaling and autoimmune disease

The IRF family of transcription factors plays a pivotal role in the development of immune cells and induction of cytokines that are important in immune and inflammatory responses (Honda and Taniguchi 2006, Tamura et al. 2008). The mammalian IRF family consists of nine members, IRF1-9 (Ikushima, Negishi, and Taniguchi 2013). Among these, IRF3 and IRF7 have been extensively studied and shown to be important for the induction of type-I interferons (IFNs) and other cytokines in response to a variety of stimuli, such as virus infection. For example, infection with RNA viruses leads to the activation of RIG-I like receptors (RLRs), which in turn activate the mitochondrial adaptor protein MAVS(Kawai et al. 2005, Meylan et al. 2005, Seth et al. 2005, Xu et al. 2005, Yoneyama et al. 2004). MAVS then activates the kinase TBK1, which phosphorylates IRF3 and IRF7, causing these transcription factors to homodimerize and enter the nucleus to turn on type-I IFNs. MAVS also activates the kinase IKKβ, which activates NF-κB to induce pro-inflammatory cytokines. Stimulation of some Toll-like receptors (TLRs), especially those localized on the endosomal membranes such as TLR3, 4, 7, 8 and 9, also leads to strong activation of IRF3 and IRF7 to induce type-I IFNs(Noppert, Fitzgerald, and Hertzog 2007, Ikushima, Negishi, and Taniguchi 2013).

Compared to IRF3 and IRF7, much less is known about how IRF5 is activated. However, genetic studies have provided compelling evidence for an essential role of IRF5 in the production of inflammatory cytokines, such as TNF-α and IL-6, in response to TLR ligands such as lipopolysaccharides (LPS) (Takaoka et al. 2005). IRF5 also functions together with IRF3 and IRF7 to mediate type-I interferon production in response to viral infections (Lazear et al. 2013). In addition, IRF5 plays important roles in M1 macrophage polarization (Krausgruber et al. 2011) and IgG class switching in B cells(Lien et al. 2010). Polymorphisms in the IRF5 gene have been linked to human autoimmune diseases, including systemic lupus erythematosus (Graham et al. 2006) and Sjogren’s syndrome (Miceli-Richard et al. 2007). Thus, IRF5 is critical for regulating immune and inflammatory responses in health and disease(Lazzari and Jefferies 2014).

Similar to IRF3 and IRF7, IRF5 contains a DNA binding domain (DBD), an IRF association domain (IAD) and a Serine-rich region (SRR) at the C-terminus(Barnes, Moore, and Pitha 2001, Barnes et al. 2002). The SRR is phosphorylated in response to TLR stimulation or virus infection. The crystal structure of a human IRF5 mutant, S430D, which was proposed to mimic IRF5 phosphorylation, showed that IRF5 formed a dimer (Chen et al. 2008). However, the physiological phosphorylation sites of IRF5 had not yet been identified or validated. The kinase that mediates IRF5 phosphorylation was also unknown.

Toll-like receptor-mediated nucleic acid sensing

As the essential genetic information carrier for almost all life forms, nucleic acids are very important pathogen associated molecular pattern (PAMP) molecules for innate immunity recognition. But at the same time, it is very important for the immune system to reduce the risk of recognizing self-nucleic acids, which might lead to autoimmune diseases(Ablasser, Hertrich, et al. 2013).

There are basically two groups of receptors that could sense DNA/RNA, determined by their localization in cells. Toll-like receptors (TLRs) are those localized on the endosome membrane and are expressed in dendritic cells, macrophages and B cells. The other group of receptors stay in the cytoplasm of cells and detect DNA and RNA molecules derived from pathogens that are exposed in the cytosol.

Most of the TLRs that recognize nucleic acids, including TLR3, TLR7, TLR8, TLR9, and TLR13, are exclusively expressed in intracellular membrane organelles, such as endoplasmic reticulum, endosome, and endolysosome. After ligand is taken by cells and processed, TLRs bind with the ligands, dimerize and undergo conformational changes that would recruit adaptor proteins, like MyD88, TRIF, TRAM and TICAM1, to the TIR domain of the TLRs (Akira, Uematsu, and Takeuchi 2006). The adaptor proteins would then activate downstream signaling cascade and elicit the production of type-I interferons and proinflmmatory cytokines.

The ectodomain of TLR3 can bind with double stranded RNA (dsRNA) (Choe, Kelker, and Wilson 2005). The ligand for TLR3 widely used in the laboratory is polyinosinic-polycytidylic acid (poly(I:C)), a dsRNA analog. In physiological conditions, the dsRNA as TLR3 ligands can be derived from the replication process of ssRNA viruses or from symmetrical transcription in DNA viruses (Akira, Uematsu, and Takeuchi 2006). Both TLR7 and TLR8 can recognize ssRNA from RNA viruses. They are phylogenetically close to each other and appear to have different functions in different species (Kawai and Akira 2010). TLR7 is highly expressed in pDCs and can produce a large amount of type-I interferons in response to infection of HIV and influenza viruses (Diebold et al. 2004, Heil et al. 2004). TLR8 is less studied than TLR7. Human TLR8 can also mediate the recognition of viral ssRNA but in mice, TLR8 deficient mice can still normally respond to ssRNA agonists (Kawai and Akira 2010). Different from human genomic DNA, in which CpG dinucleotides are of low frequency and are methylated, DNA from bacteria has large amount of unmethylated CpG dinucleotides thus has very strong stimulatory effect on mammalian immune system (Yamamoto, Yamamoto, and Tokunaga 2000, Sparwasser et al. 1998, Jakob et al. 1998). By specifically binding with non-methylated CpG dinucleotides, TLR9 is used by vertebrate immune systems to distinguish bacterial DNA from self-DNA (Hemmi et al. 2000). More recently, TLR13 has been identified to be the sensor for 23S ribosomal RNA from bacteria (Li and Chen 2012, Oldenburg et al. 2012).

Interestingly, TLRs that recognized nucleic acids are also found to be involved in the pathogenesis of autoimmune diseases. In patients with systemic lupus erythematosus (SLE), a systemic autoimmune disease with production of autoantibodies (Groom et al. 2007), their serum contains self RNAs and DNAs in the form of protein complexes that can activate TLR7, TLR8, and TLR9 (Lafyatis and York 2009). TLR3 has been reported to be activated by RNA released from synovial fluid cells of patients with rheumatoid arthritis (RA) (Brentano et al. 2005). Since the activation of TLRs has been linked with the progression of autoimmune diseases, there are drugs targeting those receptors as treatment for diseases, including IRS 661 that block TLR7 signaling and inhibit the production of IFN-a in human plasmacytoid dendritic cells (pDC) (Barrat and Coffman 2008), and Chloroquine as antagonist for TLR7 and TLR9 (Sun et al. 2007).

Nucleic acid sensing in the cytosol

In addition to the TLRs on the membrane organelles, cells can also be alarmed of invading microbes by cytosolic nucleic acid sensors. One advantage of cytosolic nucleic acid sensors is that unlike TLRs, most of which are specifically expressed in immune cells like macrophages and dendritic cells, cytosolic DNA/RNA receptors are broadly expressed in different types of cells, including epithelial cells and fibroblasts, which are usually the frontlines of infections.

The major group of sensors for cytosolic RNA is the retinoic acid-inducible gene I-like receptor (RIG-I-like receptor, RLR) family, as was first identified in 2004 (Yoneyama et al. 2004). There are three RLRs identified so far, RIG-I, Melanoma Differentiation-Associated protein 5 (MDA5), and Laboratory of Genetics and Physiology 2 (LGP2) (Yoneyama and Fujita 2007). Both RIG-I and MDA5 contains two tandem caspase activation and recruitment domains (CARDs) in the N terminus that are essential for mediating the activation signals to downstream adaptor proteins, mitochondrial antiviral-signaling protein (MAVS) (Jiang et al. 2012, Goubau, Deddouche, and Reis e Sousa 2013). LGP2, however, lacks the card domain(s) and was previously regarded as a negative regulatory factor to virus induced type-I interferon induction (Yoneyama et al. 2005, Komuro and Horvath 2006). But there are also studies indicating that LGP2 also plays a positive role in MDA5-mediated immune response (Satoh et al. 2010). RIG-I can specifically recognize single stranded RNA with 5′-triphosphate, a marker of non-self RNA (Hornung et al. 2006). Though primary transcripts transcribed in the nucleus also have 5′-triphosphate, these RNAs are subjected to various processing and modification processes thus cannot be detected by RIG-I. Besides, Both RIG-I and MDA5 can bind with double stranded RNA, but MDA5 has a preference for longer RNA molecules. According to loss of function researches, RIG-I is essential for the interferon production in response to the infection of Sendai virus, NDV, vesicular stomatitis virus (VSV), influenza A virus, and Japanese encephalitis virus (JEV) while MDA5 is important for detecting cytokine production by encephalomyocarditis virus (EMCV), Thyler's virus and Mengo virus, all Picornaviruses (genus cardiovirus) (Yoneyama and Fujita 2007).

DNA sensing in the cytosol has long been an interest of research and significant progress has been made in recent years. Before the discovery of cGAS as the general cytosolic DNA sensor, a number of proteins had been proposed to have the function of DNA sensing in the cytoplasm. DNA-dependent activator of IRFs (DAI) was first discovered in 2007 as the DNA sensor that elicit type-I interferon production (Takaoka et al. 2007). Though the researchers proved that DAI had DNA binding activity and can associate with TBK1 and IRF3 to promote the transcription of interferon mRNAs, later knockout studies suggest that in cells deficient in DAI has normal interferon response to DNA stimulation (Ishii et al. 2008). Gamma-interferon-inducible protein Ifi-16 (IFI-16) has been identified as a DNA senor that can respond to DNA in both cytosol and nucleus (Orzalli, DeLuca, and Knipe 2012, Unterholzner et al. 2010). And latest publication also shows that IFI-16 works together with cGAS in the activation of STING during DNA sensing in human keratinocytes (Almine et al. 2017). However, there are also studies showing that some cells with IFI-16 knocked down exhibit normal interferon response to cytosolic delivered DNA, suggesting that IFI-16 might not be the general DNA cytosol DNA sensor (Brunette et al. 2012, Abe et al. 2013). Stimulator of interferon genes (STING, also known as transmembrane protein 173, TMEM173), which will be discussed more in the following part, was also proposed to be a direct DNA sensor and can bind with dsDNA through its C terminus domain (Abe et al. 2013). However, the binding affinity of STING with dsDNA is very low and cell lines with STING only (without cGAS) can only respond to cyclic di-nucleotide but not dsDNA (Burdette et al. 2011, Wu et al. 2013), suggesting STING is more of an adaptor protein in DNA sensing pathway. Other less characterized proteins proposed as cytosol DNA sensor are probable ATP-dependent RNA helicase DDX41 (Zhang, Yuan, et al. 2011), LRR Binding FLII Interacting Protein 1 (LRRFIP1) (Yang et al. 2010), DExD/H-box helicase 9 (DHX9) (Kim et al. 2010), and Ku70 (Zhang, Brann, et al. 2011).

An important, well characterized DNA sensor in the cytosol is absent in melanoma 2 (AIM2). Instead of inducing the production of type-I interferons and proinflammatory cytokines, AIM2 activates the inflammasome pathways, represented by the activation of the proteolytic enzyme caspase-1 and the maturation of IL-1β (Muruve et al. 2008, Fernandes-Alnemri et al. 2009, Hornung et al. 2009, Burckstummer et al. 2009, Roberts et al. 2009). AIM2 binds DNA with its HIN200 domain and its pyrin domain can interact with apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) molecule to activate both NF-kB and caspase-1 (Hornung et al. 2009).

Cytosolic DNA sensing by cGAS/STING pathway

Before cyclic GMP-AMP synthase (cGAS) was identified as the essential and general cytosolic DNA sensor, it was known that stimulator of IFN genes (STING, also known as MITA and TMEM173) is an essential adaptor protein for the induction of type-I interferons (Ishikawa and Barber 2008, Zhong et al. 2008, Sun et al. 2009). Later in 2013, the discovery of mammalian cyclic dinucleotides, cGAMP (Wu et al. 2013), and cGAS (Sun et al. 2013) put all pieces in general DNA sensing pathway together.

When cells are transfected with DNA or infected by DNA viruses, cytosolic DNA binds and activate cGAS to form dimeric 2:2 cGAS-DNA complexes (Zhang, Wu, et al. 2014, Li, Shu, et al. 2013), which synthesizes 2′3′-cGAMP from ATP and GTP. cGAMP then acts as a second messenger and binds to the adaptor protein, STING, with very high affinity (Wu et al. 2013). 2′3′-cGAMP binding and activation induce significant conformational changes of STING protein (Gao, Ascano, Zillinger, et al. 2013, Zhang et al. 2013). Specifically, cGAMP binding induced the two STING molecules in the dimeric complex to undergo a ∼20 Å inward rotation, resulting in a deeper pocket for cGAMP binding along with the formation of a β sheet lid that covered the cGAMP binding site (Cai, Chiu, and Chen 2014). The activated STING dimer then translocate from ER to the ER-Golgi intermediate compartments (ERGIC) (Dobbs et al. 2015), during which kinases including TBK1 and probably IKK2 are recruited and activated. Then within the STING-kinase complexes, transcription factors including IRF3 and NF-kB are recruited and activated. After activation, the transcription factors translocate into the nucleus to elicit the production of type-I interferons and proinflammatory cytokines. Meanwhile, STING protein is also phosphorylated and degraded (Konno, Konno, and Barber 2013, Dobbs et al. 2015), probably as a shut-down mechanism for DNA-sensing pathway.

Since the discovery of cGAS/cGAMP pathway, it has been proven that many pathological processes that involves self or non-self-DNA sensing are mediated by cGAS. It has already been established that cGAS is the essential sensor for DNA viruses like Herpes viruses (Sun et al. 2013), Kaposi's sarcoma-associated herpesvirus (KSHV) (Ma et al. 2015), Vaccinia virus (VACV), and Murine Gammaherpesvirus 68 (MHV-68) (Ablasser, Goldeck, et al. 2013, Schoggins et al. 2014). Importantly, cGAS is also the innate immune sensor for human immunodeficiency virus (HIV) and Mycobacterium tuberculosis (MTB). cGAS can be activated by the reverse-transcribed DNA from HIV infection and knockout or knockdown of cGAS in mouse or human cell lines blocked cytokine induction by HIV, murine leukemia virus, and simian immunodeficiency virus. (Gao, Wu, et al. 2013). Activation of the DNA-dependent cGAS/STING pathway in response to MTB infection stimulates autophagy and the production of proinflammatory cytokines thus plays essential roles in host defense against MTB (Collins et al. 2015, Wassermann et al. 2015). So far, it has also been proven that multiple bacteria including Listeria monocytogenes (Hansen et al. 2014), Francisella (Storek et al. 2015), Chlamydia trachomatis (Zhang, Yeruva, et al. 2014), Neisseria gonorrhoeae (Andrade, Agarwal, et al. 2016), and Group B streptococcus (Andrade, Firon, et al. 2016).

Very interestingly, though cGAS/cGAMP and STING are majorly directly involved in cytosolic DNA sensing, it has also been reports indicating that they may also contribute to the immune response to some RNA viruses. cGAS knockout mice show more susceptibility to West Nile virus (WNV) infection, which is a positive sense single-stranded RNA virus (Schoggins et al. 2014). Researchers has also observed that Sting knockout animals infected with VSV are also significantly sensitive to lethal infection compared to controls (Ishikawa, Ma, and Barber 2009). These discoveries suggest that cGAS/STING pathway may also play a role to regulating RNA sensing pathways or contribute to the basal innate immune response.

cGAMP as an endogenous secondary messenger

The cyclic GMP-AMP (cGAMP) synthase cGAS has been defined as the major and key sensor of cytosolic DNA. Upon binding of double-stranded DNA, cGAS catalyzes the production of cGAMP from ATP and GTP. cGAMP in turn functions as an endogenous second messenger to bind and activate STING to induce type I interferons (Sun et al. 2013). Cyclic dinucleotides (CDNs) that contain uniform 3’-5’ linkage, are known to be produced and involved in multiple processes in bacteria and dictyostelium(Romling, Galperin, and Gomelsky 2013). And these CDNs are proposed to activate STING to trigger innate immune response(Burdette et al. 2011). cGAMP is generated by cGAS is the first CDN found in metazoan. Biophysical, structural and cell-based analyses have shown that cGAMP contains two unique phosphodiester bonds: one between the 2’ OH of GMP and the 5’ phosphate of AMP and the other between 3’ OH of AMP and 5’ phosphate of GMP, referred to as 2’3’-cGAMP, which makes it unique from other previously known CDNs (Ablasser, Goldeck, et al. 2013, Diner et al. 2013, Gao, Ascano, Wu, et al. 2013, Zhang et al. 2013).

Upon its discovery, cGAMP, as a key molecule in cGAS DNA sensing pathway, has been proven to be an essential small molecule in many biological processes. Besides its function in sensing microbe DNAs, cGAMP also mediated other important physiological and pathological processes. Aberrant activation of DNA sensing pathway, usually caused by the recognition of self-DNA by cGAS, can cause autoimmune diseases, which also depend on the binding and activation of STING by cGAMP (Gao et al. 2015). It was well known that point mutations in 3′ repair exonuclease 1 (Trex1; also known as DNase III), which is an important 3’ DNA exonuclease, are related to autoimmune diseases like Aicardi-Goutières syndrome (Crow et al. 2006). When the Trex1 deficient mice, which display inflammatory myocarditis (Morita et al. 2004) and accumulation of cytosolic DNA (Yang, Lindahl, and Barnes 2007), were crossed with mice with STING deletion, the double knockout mice were rescued from the autoimmune disease phenotypes (Gall et al. 2012). Similarly, mice deficient in DNase II, a lysosomal endonuclease, are embryonic lethal, due to the large amount of type-I interferon produced by macrophages due to cytosolic DNA accumulation (Yoshida et al. 2005). This phenotype can only be rescued by further knocking out the adaptor protein in the cytosolic DNA sensing pathway, STING (Ahn et al. 2012), but not the endosomal DNA sensor, TLR9 (Okabe et al. 2005), suggesting that cytosolic DNA sensing by cGAS is crucial in the progress of mutation induced autoimmune diseases.

Also, researchers have shown that cGAMP can serve as an effective adjuvant to boost immune response. They showed that 2’3’cGAMP is very effective in boosting the production of antigen-specific antibodies and T cell responses in mice and this effect is dependent on the presence of STING and type-I interferon receptor (Li, Wu, et al. 2013).

Since the rapid division and frequent loss of DNA repair machinery in tumors often cause the accumulation of DNA fragments in the cytoplasm, there is a chance for cGAS to sense the abnormal presence of cytosolic DNA in tumor cells and initiate an immune response to clear these cells. Previous publications have shown that STING is essential for the host immunity to respond to cancer cells (Ho et al. 2016, Woo et al. 2014). The DNA in the cancer cells that is sensed by cGAS could be from micronuclei generated from DNA damage, chromosome missegregation (Santaguida et al. 2017), or cell cycle arrest. Researchers observed rapid cGAS accumulation in the micronuclei (Mackenzie et al. 2017), indicating that cGAS could be activated by DNA present in micronuclei. Recently, it has also been reported that cGAMP is also an effective anti-tumor molecule via STING-TBK1-IRF3 mediated innate immune response (Li et al. 2016, Corrales et al. 2015). The combination treatment of cGAMP, which activates dendritic cells and enhanced cross-presentation of tumor-associated antigens to CD8 T cells, and PD-L1 antibody showed synergistic effect in terms of tumor control (Wang, Hu, et al. 2017), suggesting that cGAMP could be a very promising small molecule for cancer immunotherapy.

Furthermore, as a second messenger, cGAMP can also function non-autonomously by spreading among cells through gap junctions, which represent a novel immune strategy that allow the host to rapidly convey antiviral immune response in a horizontal manner. (Ablasser, Schmid-Burgk, et al. 2013) or by incorporating into newly formed viral particles, which might be utilized to generate cGAMP loaded viral particles for future vaccine development (Bridgeman et al. 2015, Gentili et al. 2015). These current knowledges about cGAMP has set the stage for its potential therapeutic applications.

Host regulation of cGAS/STING pathway

Post-translational modifications (PTMs), including phosphorylation, glutamylation, glycosylation, ubiquitination, and lipidation, are very import ways to regulate protein activity in cells, especially in cell signaling. It has been reported that the protein kinase, Akt, can phosphorylate the catalytic domain on cGAS protein at Serine 305 site (human cGAS, Serine 291 at mouse cGAS) and plays a negative role in cGAS-STING mediated antiviral immune response (Seo et al. 2015), suggesting that Akt might serve as a checkpoint to fine-tune the activity of cGAS. Another inhibitory modification on cGAS are the poly-glutamylation by TTLL6, which abolishes its DNA binding activity, or mono-glutamylation by TTLL4, which blocked it catalytic activity (Xia et al. 2016). Conversely, CCP6 and CCP5 can remove the poly-glutamylation and mono-glutamylation, respectively, on cGAS and thus activate cGAS. When cells are infected by HSV, an interaction between an ER ubiquitin ligase, RNF185, and cGAS was observed (Wang, Huang, et al. 2017). After binding, RNF185 specifically modifies cGAS with K27-linked poly-ubiquitination, which enhanced its catalytic activity in cGAMP production. More interestingly, elevated mRNA level of RNF185 has been found in patient samples with Systemic Lupus Erythematosus, suggesting that unexpected cGAS activity due to hyperactivity of RNF185 could be the direct cause of the disease. cGAS has also been reported to be SUMOylated. One research group showed that sentrin/SUMO-specific protease 7 (SENP7) can reverse the conjugation of SUMO (small ubiquitin-like modifier) to cGAS at lysine 335, 372 and 382, which abolishes the DNA binding activity of cGAS, and thus promote the activation of cGAS (Cui et al. 2017). Interestingly, another group found that the SUMOylation of cGAS by the ubiquitin ligase Trim38 actually prevents cGAS from being ubiquitinated and degraded, thus stabilize cGAS and promote the downstream signaling (Hu et al. 2016). It is interesting but reasonable that SUMOylation by different enzymes on different sites of cGAS lead to different regulation effects. Besides PTM, Polyglutamine binding protein 1 (PQBP1) emerged from a targeted RNAi screening has been proved to bind with HIV -1 derived double stranded DNA as well as bind to cGAS to faciliate the activation of cytosolic DNA sening (Yoh et al. 2015).

As the key adaptor protein that after cGAMP binding, activates downstream kinases including TBK1 and IKK, STING is also intensively regulated by PTMs. After TBK1 recruitment to STING, TBK1 phosphorylate STING at Serine 366, which is essential for STING to further recruit and activate IRF3 (Liu et al. 2015). Moreover, there are also evidence showing that the phosphorylation of STING at Serine 366 is crucial for its subsequent degradation (Konno, Konno, and Barber 2013), which might be the shutdown mechanism for the pathway. After translocating from ER to Golgi, palmitoylation of STING by Golgi factors is required for downstream events, since treatment with palmitoylation inhibitor 2-bromopalmitate (2-BP), which abolishes STING palmitoylation, would also block type-I interferon production (Mukai et al. 2016). Furthermore, multiple ubiquitin E3 ligases have been identified to regulate STING activity. The expression of tripartite motif protein 30α (TRIM30α) is induced by the activation of cytosolic DNA sensing pathway in dendritic cells, which in turn interacts with STING to promote the degradation of STING via K48-linked ubiquitination on its lysine 275 site, acting as a negative regulator of cGAS-STING pathway (Wang et al. 2015). Similarly, the E3 ubiquitin ligase RNF5 also targets STING at K48-linked ubiquitination on its lysine 150 site for degradation in a signal dependent manner, resulting in inhibited IRF3 dimerization and type-I interferon production in response to virial infection (Zhong et al. 2009). On the other hand, TRIM32 ubiquitinates STING with K63-linkage at lysine 20, 150, 224, and 236, which would enhance its interaction with TBK1 and dramatically increase the downstream signaling and interferon production (Zhang et al. 2012). TRIM 56 is also reported to have similar effects on STING. It also targets STING with K63-linked ubiquitination at lysine 150, which is essential for its dimerization and interaction with TBK1 and IRF3 (Tsuchida et al. 2010). Besides, upon cGAMP binding with STING, autocrine Motility Factor Receptor (AMFR) on the ER is recruited to STING dimer in an insulin-induced gene 1 (INSIG1)-dependent manner, which would form a E3 ubiquitin ligase complex and ubiquitinate STING with K27-linkage and this modification is required for TBK1 recruitment as well as STING perinuclear translocation (Wang et al. 2014). So, in conclusion, the ubiquitination modification types and sites on STING determine its function to downstream events and regulate the host immune response to cytosolic DNA. In another study, STING is SUMOylated by TRIM38 during the initial stage of DNA stimulation, when STING stability and activity are enhanced. Later the SUMOylation modification is removed by Sentrin-specific protease 2 (SENP2) so that STING can be degraded to shut down the immune response (Hu et al. 2016).

One unique feature of STING activation is that it will translocate from ER to Golgi after cGAMP binding and in the process of TBK1 recruitment and activation, which can also be regulated by various factors. An small molecule inhibitor of ADP ribosylation factor (ARF) GTPase, Brefeldin A, which can block STING trafficking from ER to Golgi, can abolish the downstream signaling and interferon production, indicating that the trafficking of STING is essential for the pathway (Konno, Konno, and Barber 2013).

Regulation of cGAS/STING pathway by viral proteins

During the evolution process, microbes co-evolve with host immune system to evade the immune response and cGAS-STING pathway is thus target by various proteins from both viruses and bacteria.

ORF52, an abundant gammaherpesvirus-specific tegument protein initially found in Kaposi's sarcoma-associated herpesvirus (KSHV), can bind both cGAS and DNA and inhibit cGAS activity and downstream signaling. And similar mechanism is also found in other gammaherpesviruses, indicating that the inhibitory mechanism is evolutionarily conserved (Wu et al. 2015). NS2B (non-structural protein 2B) encoded by dengue virus (DENV) was reported to specifically target host cGAS for lysosomal degradation and prevent cytosolic DNA sensing pathway to be activated. Interestingly, another protease complex from DENV, NS2B3 was also previously reported to specifically degrade human STING, but not mouse STING to evade host immune response (Aguirre et al. 2012). A tegument protein found in HSV-1, UL41, also known as the virion host shutoff protein, can help the virus to evade the cGAS/STING-mediated cytosolic DNA sensing response by degrade cGAS protein via its RNase activity (Su and Zheng 2017).

IpaJ, an effector protein from Shigella flexneri (a Gram-negative bacterial pathogen), has found to be able to potently inhibit STING signaling by inactivating ARF GTPase and thus blocking STING translocation from ER to the ER-Golgi intermediate compartment (ERGIC) (Dobbs et al. 2015). Viral IRF-1 from KSHV has also been reported to interact with STING at latency stage and this interaction would abrogate the interaction between STING and TBK1, blocking the activation of the pathway (Ma et al. 2015). Oncogenes from DNA tumor virus also target STING to subvert the host immune activity. The Leu-X-Cys-X-Glu (LXCXE) motif in E7 protein from human papillomavirus (HPV) and E1A viral protein from adenovirus can bind with STING and potently inhibit its activation, abolishing the type-I interferon production (Lau et al. 2015).