STING Agonists/Antagonists

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The cGAS STING pathway has received much attention in recent years, and it has been recognized as an important component of the innate immune response. Since the discovery of STING and that of cGAS, many observations based on preclinical models suggest that the faulty regulation of this pathway is involved in many type I IFN autoinflammatory disorders.

cGAS STING cancer

1. The cGAS/STING, a Nucleic Acid Sensing Pathway, Plays a Role in Many Diseases

Nucleic acids are an important component of the cell. They store genetic information and provide guidance to the cell on how to execute it. Nevertheless, when nucleic acids are found outside the cell or when large amounts of them are misplaced in the cytosol, which occurs because of damage to the cell (intrinsic cell death, viral infection, mitochondria damage), nucleic acids are recognized as harmful agents (as pathogen-associated molecular patterns or “PAMPs”) and trigger a strong immunological response. Such a response is observed in many autoinflammatory and autoimmune diseases, where the activation of nucleic acid sensors has been suggested as a major molecular determinant driving the pathology [1].

Two novel gene products (cGAS and STING) have been recently identified as the key players in the recognition of excess cytosolic dsDNA [2][3][4]. Upon binding to dsDNA, cGAS (a cyclic GMP/AMP synthase) converts GTP and ATP to the cyclic dinucleotide called cGAMP [3]. STING (Stimulator of Interferon Genes) [5] binds cGAMP, undergoing a conformational change, thereby facilitating the phosphorylation of the transcription factor IRF3, finally leading to a large increase in the expression of type I IFN genes [6]. cGAMP is a cyclic dinucleotide composed of one molecule of GMP and one of AMP, with a very unusual 2′,5′ linkage and a classical 3′,5′ linkage [7], and it represents a novel “2nd” messenger. Activation of this pathway leads to a strong type I interferon response which is generally paralleled by an increase of transcription of many ISG (interferon-stimulated genes). Diseases showing a strong type I IFN signature are defined as interferonopathies [8].

A well-characterized genetic-linked interferonopathy is the so-called Aicardi-Goutières-Syndrome (AGS). In around 25% of AGS patients, uncontrolled type I IFN response is linked to mutations of the cytosolic DNase Trex1, which results in an increase of cytosolic dsDNA that activates cGAS. A similar mechanism is common to AGS patients, who have mutations in other DNA processing enzymes (RNASEH2A, RNASEH2B, RNASEH2C, and SAMHD1) [9]. The clinical manifestations in AGS patients are very similar to those observed in lupus patients. A milder form of the

disease is found in Familial Chilblain Lupus patients, who are carrying a heterozygous mutation in Trex1 [10]. Among the Mendelian diseases related to TREX1 loss-of-function mutation, a less severe form leads to RVCL (autosomal dominant retinal vasculopathy with cerebral leukodystrophy), which is characterized by an adult-onset of vasculopathy, leading to retinopathy and juvenile ischemic stroke [11]. STING-associated vasculopathy with onset in infancy (SAVI) is another lupus-like disease with a link to the cGAS/STING pathway that is the consequence of the uncontrolled activation of the pathway. Identified as one of the interferonopathies observed prevalently in young persons, this disease was shown to be the consequence of mutations hyperactivating STING, resulting in a chronic type I IFN response. Manifestations of this pathology are evidenced by skin rashes, lung inflammation, and inflammation in the extremities, leading in extreme cases to amputation [12]. Diseases with a defect in the DNA-processing enzymes (as for the Trex AGS patients) are expected to respond well to cGAS inhibition: preclinical work has documented that the increase of cytosolic dsDNA leads predominantly to the activation of cGAS, while the contribution of other DNA sensors such as AIM2 seems to be minor [13]. In contrast, for SAVI patients, STING antagonists are the therapeutics of choice, although it is not yet known if one compound might be capable of blocking, to the same extent, all (hyperactivated) STING mutants that have been identified so far.

Learning from AGS and SAVI patients might teach us where, in man, the cGAS/STING pathway plays an important role: for example, prominent damage of blood vessels has been observed in SAVI patients [12], suggesting that activation of STING might play a role in some of the non-genetically linked vasculitis disorders, although evidence for the latter is still fragmentary.

Besides the rare genetic diseases, there is evidence suggesting that the cGAS/STING pathway might play a role in chronic diseases, where programmed cell death is not efficiently clearing cellular debris [14]. In lupus patients, the chronic damage of different organs leads to the appearance of antiDNA antibodies, which suggest a contribution of the cGAS/STING pathway in this disease [15].

Diseases such as subtypes of systemic lupus erythematosus (SLE), lupus nephritis (LN), and dermatomyositis, which have been suggested to be triggered by DNA viruses such as EBV, cytosolic dsDNA, or mitochondrial dsDNA, are also expected to be driven (at least in part) by the aberrant activation of cGAS. A prominent role of cGAS in the development of Sjogren's Syndrome (SS) would not be unexpected since this disease shares many similarities with SLE; this has been confirmed by a recent publication showing hypersensitivity to cGAS/STING activators in SS patients [16].

Low molecular weight inhibitors of cGAS might also be effective in treating the skin rashes/reddening associated with SLE, a pathology that is often observed when SLE patients are exposed to UV light [17]. There has also been some evidence that deposition of dsDNA in joints might be responsible for the inflammation observed in rheumatoid arthritis patients [18], although it is unlikely that cGAS/STING inhibitors will be superior to the TNF blockers that are used in the clinics: evidence from preclinical models suggests that TNF also controls, among others, the cGAS activation in joint inflammation [19].

Some reports have suggested cGAS/STING modulation as a potential treatment of ulcerative colitis and inflammatory bowel disease (IBD) [20][21][22][23]. At the same time, other reports showed that blocking the cGAS/STING pathway worsened the outcome of colitis [24]. In the case of these diseases affecting the gastrointestinal tract, researchers need to better understand the role of the microbiome in modulating the cGAS/STING pathway since evidence has accumulated that the bacteria producing cyclic dinucleotides are capable of activating STING [25]. Based on recent observations, it was proposed that blocking cGAS/STING might show some efficacy in inflammation driven by sepsis [26], although to achieve a robust clinical remission in this disease, it might require combining cGAS/STING inhibitors with drugs targeted to TLRs and other DNA sensing pathways.

A large body of evidence has indicated that both cGAS and STING are involved in lung inflammation. Damage to lung epithelial (by different agents) causes the release of DNA, appearing in bronchoalveolar lavage (BAL), which seems to be sufficient to activate the cGAS/STING pathway. Intratracheal application of DNAse strongly reduced the type I IFN response in a model of silicosis-driven lung inflammation, suggesting that the released DNA has an inflammatory effect [27]. Different publications using genetically modified animals confirmed the role of STING in models of lung inflammation [27][28][29]. Although therapeutic intervention in the cGAS/STING pathway might lead to some improvements in diseases such as cirrhosis and endomyocardial fibrosis [30][31][32][33][34][35], more data would be needed to confirm a strong general effect in fibrosis. Aberrant cGAS/STING activation might also play a role in diseases such as nonalcoholic steatohepatitis (NASH) and chronic obstructive pulmonary disease (COPD); nevertheless, this still requires further evaluation. In a mouse model of SAVI patients, conditions were identified under which bacterial-derived cyclic nucleotides were driving lung inflammation. However, the same mice, which were made germ free, uncovered an unexpected protective function of the cGAS/STING pathway [36]. This suggests that contributions by the cGAS/STING pathway could, at the same time, worsen or protect disease pathology: it will be critical to figure out which of the two components is having the strongest effect before moving to a clinical setting.

cGAS and STING have been shown to play a role in cellular senescence [37][38], and there is some evidence that such findings will have an impact beyond the cellular phenotype [39]. To the protective effect, it needs to be considered that enhancing the survival of cells might lead to an increased risk of tumorgenicity. There is accumulating evidence that cGAS activation is involved in neuroinflammatory diseases such as Parkinson's disease (or at least a subtype of them) [40], Alzheimer's disease [41], and amyotrophic lateral sclerosis (ALS) [42]. In these latter diseases, controlling the cellular senescence component might have a therapeutic advantage.

2. STING Agonists in Cancer

5,6-dimethylxanthenone-4-acetic acid (DMXAA) had been, for a long time, considered a promising tumor drug candidate; it belongs to a class of compounds with strong antivascular activity. In view of the very promising rodent preclinical l data, this compound was moved in human clinical trials [43]. DMXAA and related compounds, despite their acceptable pharmacokinetic properties, failed to show any effect in human patients. It was demonstrated that DMXAA and other flavonoids were highly specific mouse STING agonists [44], which provided a first glimpse at the

mode of action of this class of drugs. At the same time, data showed that DMXAA was completely inactive at human STING, explaining the lack of efficacy of this drug in human clinical trials [44]. Great effort was made to chemically modify DMXAA to gain activity at human STING. Despite very elegant early crystallography studies [45], which gave insight into the DMXAA-STING structure, it was not possible to find a derivative with good activity at human STING. Activities in this direction have now been paused since interesting novel STING agonists have been identified.

The lessons learned from mouse biology and the realization that the activation of STING leads to a strong killing of tumor cells, mediated by type 1 IFN response, have, in between, gained the interest of the oncology community. After the identification of cGAMP as the product of the dsDNA-mediated activation of cGAS, it was shown that this molecule had tumor suppressive activity in different preclinical tumor models. Many pharmaceutical companies and academic institutions launched chemical optimization efforts to transform cGAMP into a “drug-like” molecule. The investments seemed to pay off, as shown by the good efficacy of ADU-S100 in a mouse tumor model [46]. ADU-S100 had comparable activity toward mouse and human STING, making it the first candidate to move to early clinical studies. One of the challenges encountered with the cGAMP derivatives was how to circumvent their strong systemic effect upon oral/intravenous application. Therefore, early clinical trials were started with the local application of ADU-S100 at the tumor site. These trials did not progress as quickly as hoped, and some were terminated ahead of time [47]. The final reports on the outcome of these studies have not been fully published, but it is fair to assume that the efficacy of this STING agonist, as a single therapeutic agent, was not sufficiently convincing to progress it further in the clinics. Combinations of STING agonists with other cancer drugs is still an appealing strategy, although it has been difficult identifying the ideal pathway(s)/drug for such combination trials. These studies also underline how difficult it is to move from very successful animals model studies to human patients.

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