Nucleic acid sensing in the tumor vasculature

Adrian M. Baris[1], Eugenia Fraile-Bethencourt[1] and Sudarshan Anand[1,2]

[1]Department of Cell, Developmental & Cancer Biology,

[2]Department of Radiation Medicine,

Knight Cancer Institute,

2720, S Moody Avenue, Oregon Health & Science University, Portland, OR 97201

Correspondence to: anands@ohsu.edu

Simple Summary: Our cells can recognize DNA or RNA from pathogens such as viruses. The proteins that recognize these nucleic acids are known as nucleic acid sensors. Upon activation, they trigger immune responses that result in elimination of the infected cells. Recent research has shown how we can mimic this process in cancer and recruit immune cells against the tumor. Among the different cell types in cancer, endothelial cells play a main role here since the blood vessels are highways for the immune cells. In this review, we discuss two different nucleic acid sensors: the Retinoic acid-Inducible Gene 1 (RIG-I) and the Three prime Repair Exonuclease 1 (TREX1); and how they play a role in endothelial cancer cells. We present some approaches to target these pathways within the cancer blood vessels to disrupt the blood supply and attract immune response to cancers.

Abstract: Endothelial cells form a powerful interface between tissues and immune cells. In fact, one of the underappreciated roles of endothelial cells is to orchestrate immune attention to specific sites. Tumor endothelial cells have a unique ability to dampen the immune responses and thereby maintain an immunosuppressive microenvironment. Recent approaches to trigger immune responses in cancers have focused on activating nucleic acid sensors such as cGAS/STING in combination with immunotherapies. In this review, we present a case for targeting nucleic acid sensing pathways within the tumor vasculature to invigorate tumor immune responses. We introduce two specific nucleic acid sensors, the DNA sensor TREX1 and the RNA sensor RIG-I and discuss their functional roles in the vasculature. Finally, we present perspectives on how these nucleic acid sensors in the tumor endothelium can be targeted in an antiangiogenic and immune activation context. We believe understanding the role of nucleic acid sensing in the tumor vasculature can enhance our ability to design more effective therapies targeting the tumor microenvironment.

Keywords: Nucleic Acid sensors, TREX1, cGAS, STING, RIG-I, Tumor angiogenesis, vascular normalization, vascular inflammation, endothelial cells, tumor microenvironment

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What are Nucleic Acid Sensors?

In order to protect itself from outside pathogens and other agents, organisms have developed two interlinked forms of defense systems: an innate and an acquired immunity. The innate immune response reacts rapidly to an infection, which can often exponentially multiply long before the adaptive immune response is able to take effect.[3] These responses should be tightly regulated to prevent dysfunction and damage to the host. The innate immune system responds to Damage Associated Molecular Patterns (DAMPs), which are recognized by Pattern Recognition Receptors (PRRs). Activation of PRRs typically induce a downstream type 1 interferon (IFN-I) and cytokine response. Nucleic acid sensors are a specific type of PRRs that recognize pathogen-derived cytosolic nucleic acids and activate downstream signaling cascades, which produces a proinflammatory response. This response is critical to the ability of nucleic acid sensors stopping pathogens in their tracks.[4] Nucleic acid sensors are unique in that they recognize and differentiate both exogenous and endogenous nucleic acids.[4,5] A number of nucleic acid sensors (NAS) play a prominent role in inflammation.[6] Recent studies have shown that NAS play a large role in endothelial function and dysfunction [2]. Endothelium regulates vascular tone and growth[1], and is critical in pathologies such as viral infections, cardiovascular disease, and cancer. A better understanding of the role NAS play in endothelial function is critical to improving our knowledge of tumor angiogenesis, as well as cardiovascular and infectious disease. Several outstanding reviews discuss the sensors outlined here (Table 1)[7–9]. In this review, we will focus on the DNA sensor Three prime Repair Exonuclease 1 (TREX1) and RNA sensors in the Retinoic acid-Inducible Gene 1 (RIG-I) like receptors (RLR) pathway (summarized in Figure 1).

What is TREX1?

DNase III or Trex1, is an exonuclease that degrades exogenous DNA. TREX1 is a member of the DnaQ family of 3′ → 5′ exonucleases. These exonucleases are known for three conserved

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sequence motifs: Exo I, II and III, which are essential for exonuclease function. The TREX1 protein contains a C-terminal domain of about 75 amino acids and a non-repetitive proline-rich region that is not seen in the other TREX protein, TREX2.[10,11] TREX1 degrades single strand DNA (ssDNA), double strand DNA (dsDNA) and single strand RNA (ssRNA) in the cytosol.[12,13] Cytosolic DNA activates the cGAS-STING-TBK1 pathway and leads to the downstream activation of IRF3 and IRF7, which furthermore induce type I interferons.[14] By degrading cytosolic DNA, TREX1 removes the substrates for cGAS, dampening the nucleic acid sensor response. Therefore, TREX1 is thought to be a negative regulator of interferon signaling and broadly, autoimmune disease. TREX1 interacts with Poly (ADP-ribose) Polymerase (PARP) and is a facilitator of its nuclear translocation and activity[10] suggesting a putative role in DNA damage responses. Indeed, other studies have shown DNA damage can increase TREX1 expression in a dose dependent manner.[16,17] In addition to nucleic acid sensing, TREX1 is also involved in the regulation of an ER resident enzyme oligosaccharyltransferase (OST), which contributes to its role in immune regulation and inflammation.[18,19]

What are the biological roles of TREX1?

DNAse dependent functions

As outlined previously, TREX1 prevents the activation of the cGAS/STING pathway. cGAS is cytosolic cyclic gAMP synthase that is typically in a catalytically inactive autoinhibited state. However, in the presence of DNA, cGAS binds to DNA and undergoes a conformational change which catalyzes the synthesis of cyclic GMP-AMP (cGAMP). cGAMP binds to STING, an endoplasmic-reticulum resident membrane adaptor. This induces a conformational change that activates STING and causes it to traffic to the Golgi, during which TBK1 is activated. The phosphorylation of TBK1 activates its kinase activity, leading to phosphorylation of IRF3 and induces the expression on interferons and inflammatory cytokines.[20] In 2010 it was discovered that TREX1 suppresses the interferon response triggered by HIV. In Trex1 deficient mouse cells

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as well as human immune cells with TREX1 RNAi knockdown, HIV infection produced a type 1 IFN response that inhibited HIV replication and spread.[21] Interestingly, mice deficient in DNAse II, a similar enzyme, die during embryonic development through inflammatory disease. This phenotype is rescued by the loss of STING, as cytosolic DNA was unable to trigger cytokine production through the STING pathway.[22] These findings suggest that the nuclease activity of TREX1 can be an important negative regulator of cGAS STING pathway.

DNAse independent functions

TREX1 has a DNAse independent function that suppresses immune activation through regulation of oligosaccharyltransferase (OST) activity. TREX1 is phosphorylated during mitosis, which disrupts its interactions with the OST complex without affecting its DNAse activity.[19] [11]

Emerging functions of TREX1

TREX1 has a wide range of functions in immunity, DNA damage, and cancer. While it has been widely studied, there are still many new functions still being discovered. For example, TREX1 has been shown to inhibit cGAS activation at micronuclei through the degradation of micronuclear DNA.[23] In addition, cGAS-DNA phase separation sequesters cGAS and prevents access to the negative regulator TREX1. This enhances cGAS DNA sensing activity.[24]

TREX1 knockout mouse

Trex1 deficient mice exhibit reduced survival and develop inflammatory myocarditis. This often leads to cardiomyopathy and circulatory failure.[25] These mice also develop lethal interferon-driven autoimmune disease, however cGAS deficiency is protective in this instance.[26]

TREX1 associated human disease

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Mutations in TREX1 have been linked to several disorders. For example, TREX1 mutations are found in patients with Aicardi-Goutières syndrome (AGS), an encephalopathy that results in severe neurological dysfunction.[16] Patients with AGS because of a TREX1 mutation typically experience neurological defects such as dystonia, seizures, cortical blindness, and progressive microcephaly, and are more likely to be affected at birth.[17] There are 5 unique TREX1 mutations that contribute to AGS. These consist of a G314A transition that results in a nonconservative R to H substitution predicted to be involved in protein dimerization, a missense T602A mutation in the ExoIII motif, and two protein truncating mutations.[27] Type 1 interferon signaling is shown to be upregulated in most TREX1 AGS patients[18]. TREX1 mutations have also been associated with Familial Chilblain Lupus (FCL), and an upregulation of type 1 interferon genes has been associated in this disease.[19]

How does TREX1 function impact the vasculature?

Hereditary vascular retinopathy (HVR) is another rare disorder linked to mutations in TREX1 and eventually leads to blindness. It is a microvascular endotheliopathy without any obvious immunological symptoms, however vascular integrity is compromised.[20,21]TREX1 mutations are also associated with another syndrome Retinal Vasculopathy with Combined Leukodystrophy (RVCL) where capillaries in the retina degenerate leading to vision loss and brain pathology shows DNA damage.[28] We observed similar functional consequences both in a neonatal ocular angiogenesis model and in the tumor vasculature with miR targeting of TREX1.

Our lab has demonstrated that TREX1 silencing exacerbates DNA damage, cell death, inhibits angiogenesis and induces inflammatory cytokines [16]. Similar to our observations, Vanpouille-Box et al showed that TREX1 in tumor cells is induced by high dose radiation and inhibition of TREX1 synergizes with immune checkpoint blockade.[29] Going beyond TREX1, there is emerging evidence for the function of downstream pathways in the vasculature. STING is expressed in both normal and tumor vasculature.[30–32] Additionally there is evidence that STING

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signaling in the high endothelial venule can contribute to endothelial–lymphocyte interaction.[33] Endothelial cell–derived type I IFNs initiate antitumor responses before dendritic cells and $CD8^+$ T cells infiltrate the TME and determine the magnitude of overall immunity.[31] In humans, mutations in the STING-encoding gene *TMEM173*, results in a fatal vasculitis, termed STING-associated vasculopathy with onset in infancy (SAVI).[20,34,35] Both TBK1 and IRF3 were found to be necessary proangiogenic factors in a high-throughput genomic screen of endothelial cell activity.[36] Mice deficient in Tbk1 exhibit immune cell infiltrates and an increase in susceptibility to LPS-induced lethality as well as a decrease in IFN-β and T-cell expression.[37(p1),38] Irf3 deficient mice experience an altered IFN response as well as the ablation of IFNα and IFNβ after influenza infection. They also experience an influx of granulocytes in the lung and a decrease in the activation of the adaptive immune response.[39]

What are the RLR family of intracellular RNA sensors?

In addition to DNA, PPRs can also recognize RNA in the cytoplasm. The RIG-I like receptor family (RLR) contains three RNA sensors: Retinoic Acid Inducible Gene-I (RIG-I), Melanoma Differentiation-Associated protein 5 (MDA5) and Laboratory of Genetics and Physiology gene 2 (LGP2). These three proteins share a C-terminal domain (CTD) and a helicase domain (HD), but only RIG-I and MDA5 bear the effector domain known as caspase activating and recruiting domain (CARD) in the N-terminal. So that, only RIG-I and MDA5 trigger the IFN-I response through interactions with mitochondrial antiviral signaling proteins (MAVS) [40,41,42].

What are the substrates (foreign and self)?

RLRs are activated by cytosolic RNA. RIG-I and MDA5 are similar in structure and function, but they recognize different RNA structures. RIG-I is preferentially activated by blunt-ended 5'ppp short RNAs which bind to the CTD. [43,44] The CTD of RIG-I has a pocket that specifically binds

either a 5'-PPP or a 5'-PP. In normal conditions, CARD domain is bound to the HD in a repressing form. Upon RNA recognition, base-paired region of RNA complexes with the HD of RIG-I, releasing the CARD domain. Thus, stable RNA-RIG-I interaction displaces CARDs, which causes multiple RIG-I proteins to oligomerize and become accessible for MAVS signaling. One main player in this process is the E3 ubiquitin ligase TRIM25. TRIM25 ubiquitination is critical to release RIG-I from autorepression.[42] To interact with MAVS in the mitochondria, the RIG-I complex RIG-I/14-3-3ε/TRIM25 mediates the redistribution or "translocation" of RIG-I from the cytosol to the intracellular membrane compartments. There, RIG-I binds to MAVS through homotypic CARD-CARD interactions.[45] Once activated, MAVS recruits the tumor necrosis factor receptor associated factors (TRAFs), which are essential to activate interferon regulatory factors 3 and 7 (IRF3, IRF7) and NF-kB mediated response.[46] Finally, activation of RIG-I results in the expression of cytokines and IFN-I genes, which recruitments the innate immune cells.[41,47]

RLR are able to recognize self-derived RNAs, leading to either enhance or deplete IFN response in a context dependent manner.[44] A recent study showed that mitochondrial DNA double stranded breaks release mitochondrial RNA into the cytoplasm, triggering the RLR dependent immune response. Moreover, following cellular irradiation, mitochondrial DNA breaks synergize with nuclear DNA to promote the immune response.[48] These emerging studies highlight the potential of RIG-I activation without extrinsic pathogens and could explain the 'sterile inflammation 'in tissues.

What are the biological roles of the RLR family RNA sensors?

While RIG-I and MDA5 undergo very similar signaling pathways, they do differentially induce type 1 IFN response to different pathogens.[40] For example, while RIG-I is activated most potently in response to negative-strand viruses such as the influenza virus[49], MDA5 is activated in response to positive-strand viruses such as hepatitis D virus.[50] In addition, animal models

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show that RIG-I and MDA5 have functional differences *in vivo*, as well as distinct molecular immune functions.[41]

Phenotypes in knockout mice

RIG-I knockout mice show a colitis-like phenotype, reduced Peyer's patches, and show increased effector T cells and decreased naïve T cells.[51] MAVS and MDA5 KO mice lose type 1 interferon production and suffer early mortality in response to infection with Coxsackie B virus (CVB), which has been associated with myocarditis.[52] In a study of RLRs in West Nile Virus, RIG-I x MDA5 double knockout mice lacked the innate immune response against the virus infection. Surprisingly, they did not suffer severe pathological damage in tissues during infection, which was similar to animals lacking MAVS.[53]

Associations with human disease

Singleton-Merten syndrome (SMS) is an autosomal-dominant disorder characterized by aortic calcification, skeletal abnormalities, psoriasis, as well as other conditions. Jang et al performed exome sequencing and found gain-of-function mutations in DDX58, the gene which encodes the RIG-I protein, leads to variable manifestation of SMS, often without the typical dental anomalies.[54] In addition, gain-of-function mutations of MDA5 have been found in SMS patients with upregulated interferon signature genes. The sustained signaling of MDA5 and RIG-I in SMS patients in possibly due to an increase of protein levels, to the recognition of self-RNA or both. It is believed that excess INF-I and other inflammatory cytokines in the endothelial cells in aortic and mitral valves are critical for the SMS development.[55]

Checks and balances on RIG-I signaling

Several intricate pathways have evolved to regulate RIG-I signaling in cells. For instance, lncRNAs ATV and Lsm3b have been shown to directly inhibit the RIG-I CTD.[56,57] Other ncRNAs,

miR-526a indirectly impact RIG-I by downregulating CYLD, an enzyme that inhibits ubiquitination of RIG-I CARD domain.[58] There are other miRNAs, such as miR-485, that directly inhibit RIG-I transcripts.[59]

In addition, several post-translational modifications regulate RIG-I function. For example, TRIM38 mediated sumoylation either in the CARD domain or in the CTD can activate RIG-I.[60] Conversely, acetylation of RIG-I in the CTD at K909 is thought to prevent RIG from binding to viral RNA. Hence, CTD deacetylation by HDACs, especially HDAC6 can enhance RIG-I activation and signaling.[61]

How does RLR function impact the vasculature?

It has been shown that endothelial RIG-I activation leads to endothelial dysfunction. In wild-type mice, endothelial activation of RIG-I leads to endothelial stress, damage, and vessel impairment. After injection with a RIG-I agonist (dsRNA with a triphosphate at the 5 ’end), treated mice experienced vascular oxidative stress and increased circulating endothelial microparticle (EMP) numbers, indicating endothelial dysfunction. In addition, after stimulation with the RIG-I agonist, both human coronary endothelial cells (HCAEC) and endothelial progenitor cells (EPC) shows increased reactive oxygen species formation, and HCAES saw increases in proinflammatory cytokines.[62] Similarly, stimulation of MDA5 led to endothelial apoptosis, formation of reactive oxygen species, and the release of pro-inflammatory cytokines. MDA5 activation in mice endothelium leads to vascular oxidative stress and an increase in circulating endothelial microparticles and endothelial progenitor cells. In addition, chronic MDA5 stimulation exacerbated atherosclerosis.[63] The activation of Toll-Like Receptor 7 also leads to vascular inflammation and impaired vascular growth.[64]

How can viruses trigger vascular dysfunction through the RIG-I pathway?

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Vascular endothelial cells line the inner surface of blood vessels and provide a barrier between organ systems and blood vessels. This makes them critical during viral infections. Viral infection of endothelial cells gives the virus an opportunity to disperse to other organs, as well as a reservoir for long-term persistence. In addition, viral replication and the immune response in the endothelium leads to an increased tissue permeability as well as inflammation. Altogether ends in vascular and pulmonary disease that further exacerbates the viral disease.[65] Endothelial activation and dysfunction has been shown to serve a necessary mechanistic role in the pathology of severe influenza.[66] For example, RLRs and TLR7 signaling have been shown to be necessary for cell survival and for restricting virus growth in mice.[67] However, the role of nucleic acid sensors in viral infection needs to be further investigated. RLRs, as well as other aspects of the innate immune system, were found to induce an antiviral innate and adaptive immune response.[68] Interestingly, in some cases influenza virus has been shown to coopt signaling of TLR7 and RIG-I.[69]

Other viruses rely on endothelial cells for their replication and host response. Particularly relevant of late, endothelial cells have been shown to be essential in the initiation and propagation of SARS-CoV-2, which is responsible of COVID-19 disease.[70] After the initial phase of infection, some patients experience an overactive inflammatory response which leads to lung damage and increased disease severity.[71] It has also been proposed that SARS-CoV-2 may cause endothelial dysfunction and pulmonary vascular changes. Thus, endothelial cell injury and dysfunction caused by SARS-CoV-2 may contribute to COVID-19 life-threatening complications.[72] While there are relevant questions on the role of nucleic acid sensors in the vascular pathologies induced by SARS-CoV-2, they have been well characterized in the vascular inflammatory response to other several infectious pathogens.

How can we target nucleic acid sensors to diminish tumor angiogenesis?

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Tumor angiogenesis involves a number of pro-angiogenic factors in the tumor vasculature.[73] The tumor vasculature is characterized by disorganized and immature vessels[74]. Tumor cells go into to the vasculature to have access to growth factors as well as spread to other areas of the body. Thus, tumor vasculature must be leaky to allow tumor cells access.[75] It has been found that tumor angiogenesis is correlated with more metastatic disease in breast cancer.[76] In this process, ECs were long thought to only provide support for tumors, without playing an active role. However, endothelial cells do regulate a wide variety of cancer cell function. Secretions from quiescent ECs are able to reduce cancer cell proliferation and invasiveness. In fact, altering the secretome of ECs inhibits their ability to suppress cancer progression.[77] For instance, IFN-β is an antiangiogenic cytokine that targets endothelial cells. Endothelial cells are also a major source of type 1 IFNs[79–81] highlighting a potential feedback inhibitory loop where ECs secrete type I INFs that inhibit angiogenesis.

The notion of anti-angiogenic cancer therapy relies on the idea that removing tumor vasculature will prevent nutrients from entering the tumor, leading to an effective therapy. Conventional anti-angiogenic drugs often target agents that promote blood vessel formation and are overexpressed in tumors, such as VEGF-A. However, anti-angiogenesis treatment cannot eradicate the tumor on its own.[74] The use of chemotherapy or immunotherapy in conjunction with anti-angiogenic treatment can provide a more effective strategy.[82] Several studies have shown that combining anti-angiogenic therapy with conventional chemotherapy can lead to improved clinical outcomes.[83–85] Interestingly, STING activation has been shown to normalize the tumor vasculature through increased pericyte coverage and increase in effector T cells across the endothelial barrier, enhancing antitumor immunity. This was most effective when synergized with a VEGF inhibitor, displaying the utility of combining anti-angiogenic drugs with vascular immune therapy.[35]

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We showed that inhibition of endothelial TREX1 through miR-103 and siRNA was shown to decrease angiogenesis and tumor growth.[16] Similarly, our recent efforts in the lab have highlighted a role for RIG-I in the tumor vasculature. The use of a 5'PPP containing siVEGF RNA was shown to provide both antiangiogenic therapy and activate RIG-I, leading to an antitumor effect in a murine model of lung cancer.[86] IRF-1, a known tumor suppressor, has also been shown to inhibit angiogenesis. This has been attributed to a splicing variant involving exon 7 and has implication for anti-angiogenic cancer therapies.[87] Even recently, Myct1 was identified as a transcription factor in the tumor endothelium and shown to play a dual role in tumor angiogenesis and tumor immunity.[88]

Conclusions and future perspectives:

As proangiogenic molecules have been shown to have immunosuppressive effects, anti-angiogenic treatments may also stimulate an immune response. Returning the tumor vasculature to a more normalized state is thought to reverse the immunosuppressive tumor microenvironment and allow the tumor to be treated more effectively.[52] This has been observed with VEGF inhibitors in tumors, however the success is limited due to immune evasion and development of additional strategies.[53,54] Anti-angiogenic drugs can be combined with immunotherapies to produce a more potent anti-tumor response, and have been shown to have clinical potential.[89] We anticipate with more insight into these cytosolic nucleic acid sensing pathways in the tumor vasculature, we will be able to design agents that can both inhibit angiogenesis as well as actively stimulate innate and adaptive immune responses.

Methods:

Please note that this preprint is a review article and does not report any primary data from

human or experimental animals.

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Author Contributions

A.B., E.F-B. and S.A wrote the manuscript. All authors reviewed and edited the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Figure Legend

Figure 1: Nucleic Acid Sensors in Endothelial Cells.

RNA sensors RIG-I/MDA5 respond to exogenous RNA and lead to a type I interferon response via the MAVS pathway in ECs. Conversely, TREX1 degrades ssDNA or dsDNA in the cytosol thereby preventing activation of cGAS/STING pathway that activates type I interferons. Activation of RIG-I pathway or inhibition of TREX1 pathway will inhibit tumor angiogenesis by upregulating interferon responses.

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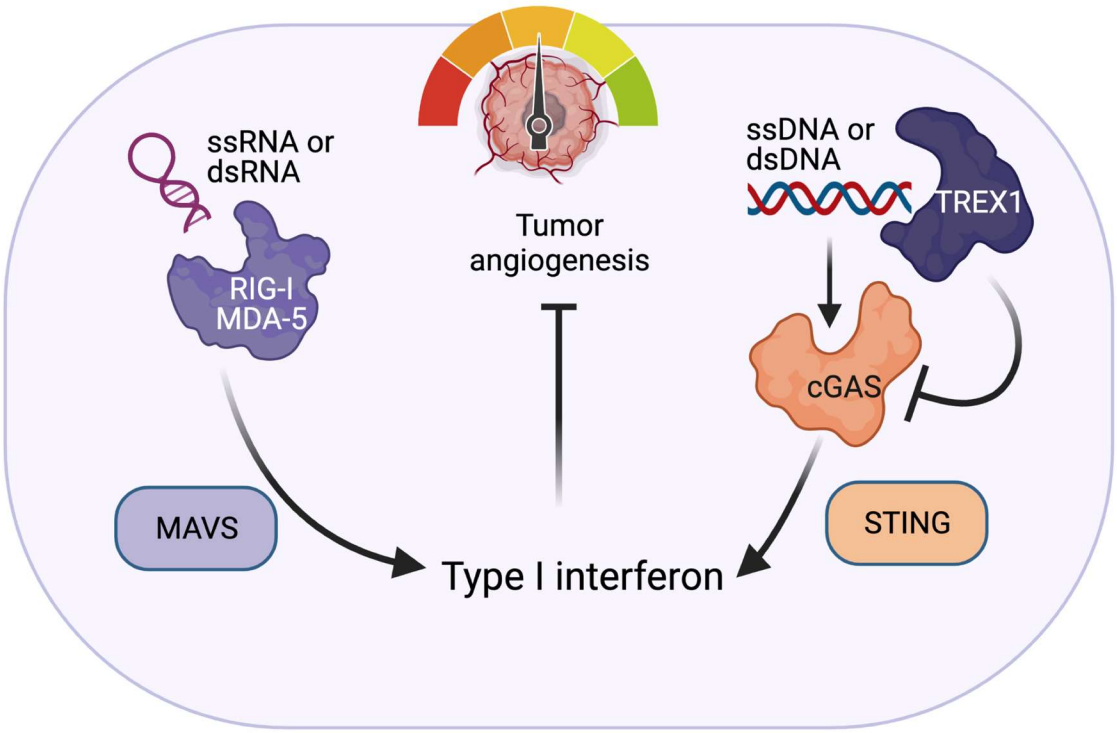

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Gene	Protein	Substrate	Function	Reference
Cgas	cGAS	dsDNA	Apoptosis	Schlee et al, 2016
TLR9	TLR9	RNA-DNA hybrid	Inflammation	Schlee et al, 2016
TREX1	TREX1	dsDNA	Immune suppression	Vanpouille-Box et al, 2019
AIM2	AIM2	dsDNA	Pyroptosis	Schlee et al, 2016
IFI16	IFI16	dsDNA	Pyroptosis	Vanpouille-Box et al, 2019
IFIH1	MDA5	dsRNA	Apoptosis	Vanpouille-Box et al, 2019
DDX58	RIG-I	dsRNA	Inflammation	Vanpouille-Box et al, 2019
TLR3	TLR3	dsRNA	Nectroptosis	Vanpouille-Box et al, 2019
ZBP1	ZBP1	B-DNA, Z-DNA	Necroptosis	Jiao et al, 2020