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Inflammatory mitochondrial signalling and viral mimicry in cancer

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Abstract

Endogenous transposable elements (TEs) are receiving increasing attention as potential targets to develop novel immunostimulatory strategies against cancer. Indeed, the defective epigenetic suppression of TEs in malignant cells offers a therapeutic window to enable their re-activation with at least some degree of selectivity. In line with this notion, multiple clinically employed epigenetic modifiers such as DNA-demethylating agents have been shown to promote the re-expression of TEs in preclinical tumour models, hence driving powerful inflammatory responses that enables increased sensitivity of immunitary immune cells to immunotherapy with immune checkpoint inhibitors (ICIs). This phenomenon is commonly referred to as "*viral mimicry*" as (at least in part) it impinges on the activation of immunological pathways commonly driven by viral infection, notably the detection of cytosolic nucleic acids by pattern recognition receptors. Here, we critically discuss the molecular mechanisms through which the mitochondria-dependent cGAS-STING and MAVS pathways enable viral mimicry as elicited by the re-activation of TEs in neoplastic cells, as we comment on the therapeutic potential of using epigenetic modifiers to harness these mechanisms in support of restored ICIs sensitivity across cancer types.

Keywords Viral mimicry, Cancer immunotherapy, Pattern recognition receptors, Mitochondria, Endogenous transposable elements, Inflammatory responses

Introduction

The success of immunotherapy is limited to few indications due to the overpowering immune tolerance regulating the multicellular networks of the tumour microenvironment (**TME**) that counterbalance immune effector mechanisms [1]. A better mechanistic explanation for the phenomenology that determines with innate and adaptive immune systems the immune-mediated cancer rejection and affects the balance between tolerance and rejection may lead to novel strategies expanding the effectiveness of immunotherapy to broader applications. To this end, recent attention has been directed to epigenetic mechanisms that lead to the aberrant activation of repetitive elements (**RE**) scattered throughout the human genome that in turn leads to the enhancement of cancer-intrinsic immunogenicity. This concept is supported by the observation that DNA-demethylating drugs and other epigenetic modifiers can drive cancer cell immunogenicity past the tolerance threshold by causing activation of interferon signalling within the targeted cancer cells [2, 3]. Thus, cancer-specific ways of modulating RE may offer new therapeutic promises [4–7].

Mammalian cells contain dormant viral remnants, known as endogenous retroviruses (**ERVs**) that are silenced in normal cells but can be reactivated by cellular

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stress or epigenetic changes resulting in the production of viral-like genetic material [2, 8]. The activation generates double-stranded RNA (**dsRNA**) and/or DNA (**dsDNA**), a signal that the immune system interprets as a product of viral infection and triggers the expression of type I and III interferons (**IFN**). This phenomenon is particularly common in cancer and draws immune effector cells to the TME to initiate the anticancer immune response [1], whereas regulatory T cells, tumour-associated macrophages, and myeloid-derived suppressor cells dampen the immune response against tumours by suppressing the activity of effector T cells and other immune cells. Thus, therapeutically disrupting the dormancy of ERVs can initiate an immune response that could be leveraged for cancer therapy [9]. However, while stimulating anti-tumour immunity is beneficial, prolonged inflammation can also induce a compensatory immune suppression that fuels cancer growth [10–12]. Therefore, carefully controlling the balance between anti-cancer versus pro-cancer immune responses is essential [5].

Epigenetic modifying drugs have been known to regulate ERV activation. However, recent findings suggest that multiple mechanisms beyond DNA methylation or histone modifications can also play a role. The "*fire alarm hypothesis*" proposes that defects in cellular homeostasis cause a buildup of immunogenic ERV products, which act as an alarm system by triggering cancer cell-intrinsic activation of inflammatory processes and secondarily result in the recruitment of innate immune responses within the multicellular network of the TME [1, 9].

This review explores the intricate relationship between transposable elements (**TEs**) that are REs resulting from genetic rearrangements that duplicate ERV sequences across the genome, and cancer immunogenicity, beginning with the classification and function of TEs, followed by their role in oncogenesis and immune activation. Particular attention is given to the cyclic GMP-AMP (**cGAMP**) synthase (**cGAS**)- stimulator of IFN genes (**STING**) pathway, which senses cytosolic nucleic acids (**CNAs**) and mitochondria triggering inflammatory signalling. Additionally, we examine the therapeutic potential of viral mimicry in cancer treatment, focusing on epigenetic therapies that induce TE reactivation. Lastly, we discuss how combination therapies targeting these pathways could open new avenues for cancer immunotherapy.

Table 1 Summary of the two major taxonomic groups of type I and type II TEs

	Type I TEs (Retrotransposons)	Type II TEs (DNA Transposons)
Transposition Mechanism	Copy-and-paste (via RNA intermediate)	Cut-and-paste (excision-reinsertion)
Enzymes Involved	Encode reverse transcriptase and integrase	Typically encode transposase
Intermediate	RNA	None (DNA directly)
Subgroups	LTR Retrotransposons: Have Long Terminal Repeats (e.g., Human Endogenous Retroviruses) Non-LTR Retrotransposons: Lack LTRs (e.g., LINEs, SINEs)	
Human Relevance	LINEs and SINEs are major retrotransposons in the human "mobilome"	Also present in humans, but different mechanism
Activity in Humans	Human Endogenous Retroviruses likely no longer transpose; LINEs and SINEs are significant active elements	Can be active, but different mechanisms from retrotransposons

From repetitive elements to immunity and beyond

Up to two-thirds of the human genome are composed of repetitive DNA sequences or REs that originate from segmental duplication, simple sequence repeats, processed pseudogenes, and tandem repeats/satellite DNA sequences. The most represented category is TEs [13, 14], which are interspersed repetitive DNAs composing most of the telomeric and centromeric regions of human chromosomes. TEs with their ability to mobilize portions of DNA can significantly influence the organization and function of the genomic architecture of mammals.

Two major taxonomic groups distinguish type I or type II TEs [15]. The first includes "*retrotransposons*" that transpose across the genome following a copy-and-paste replication strategy dependent upon reverse transcription. Type II TEs are DNA transposons that use a cut-and-paste excision-reinsertion mechanism [14]. Since type I TEs exploit reverse transcription, they encode proprietary reverse transcriptase and integrase enzymes that produce an RNA intermediate. TEs retrotransposons are differentiated according to the presence or absence of long terminal repeats (**LTR**) at their ends. All human endogenous retroviruses that likely no longer transpose in humans contain LTRS [16], whereas non-LTR TE include long interspersed nuclear elements (**LINEs**) and short interspersed nuclear elements (**SINEs**) that represent the main retrotransposons of the human "*mobilome*" [17–19] (Table 1).

TEs work as natural mutagens and can be the cause or consequence of cancer progression. Malignancy due to TE activity is dependent upon the oncogenic potential of retrotransposition that modifies the structure of chromosomes and gene expression regulation or directly disrupts gene structures. Moreover, resulting architectural changes in cryptic *cis*-regulatory elements in which enhancers and promoters play a key role in regulating gene expression without modifying the primary DNA sequence, can stimulate cancer development [20].

Moreover, TEs activation in cancer cells results in the expression of their bio-products expression at both the

nucleic acid and peptide level. This depends mostly upon DNA hypomethylation observable to various degrees in most cancers [21]. Indeed, the retrotransposition process produces nucleic acids that are recognized by endosomal toll-like receptor 3 (**TLR3**), cytosolic sensors such as melanoma differentiation-associated gene 5 (**MDA5**), and retinoic acid inducible gene-I (**RIG-I**) both belonging to the RIG-I-like receptors (**RLRs**) [22], and cGAS. In particular, RLRs are RNA-sensing cytosolic surveillance systems whereas cGAS is a DNA-sensor [23–25]. Endogenous dsRNAs are often derived from all type I TEs, i.e., LTR, LINEs, and SINEs [2, 26, 27], , whereas LINEs reverse transcribe complementary DNA (**cDNA**) [28] (Fig. 1).

CNAs are detected by CNA pattern recognition (**CPR**) elements promoting IFN signalling [29]. dsRNA or RNA molecules with a triphosphate group at the 5' end known as 5'pppRNA are recognized by RLRs first followed downstream by interactions with the mitochondrial antiviral-signalling protein (**MAVS**) that contains an RLRs adaptor in its amino-terminal caspase recruitment domain (**CARD**) [30, 31]. The homotypic interaction between similar domains as the CARD of RIG-I or MDA5 with MAVS CARD promotes the recruitment of other MAVS on the outer mitochondrial membrane. MAVS CARDs aggregation stimulates the prion-like conversion of MAVS into larger functional assembles [32] responsible for the amplification of RLRs signalling,

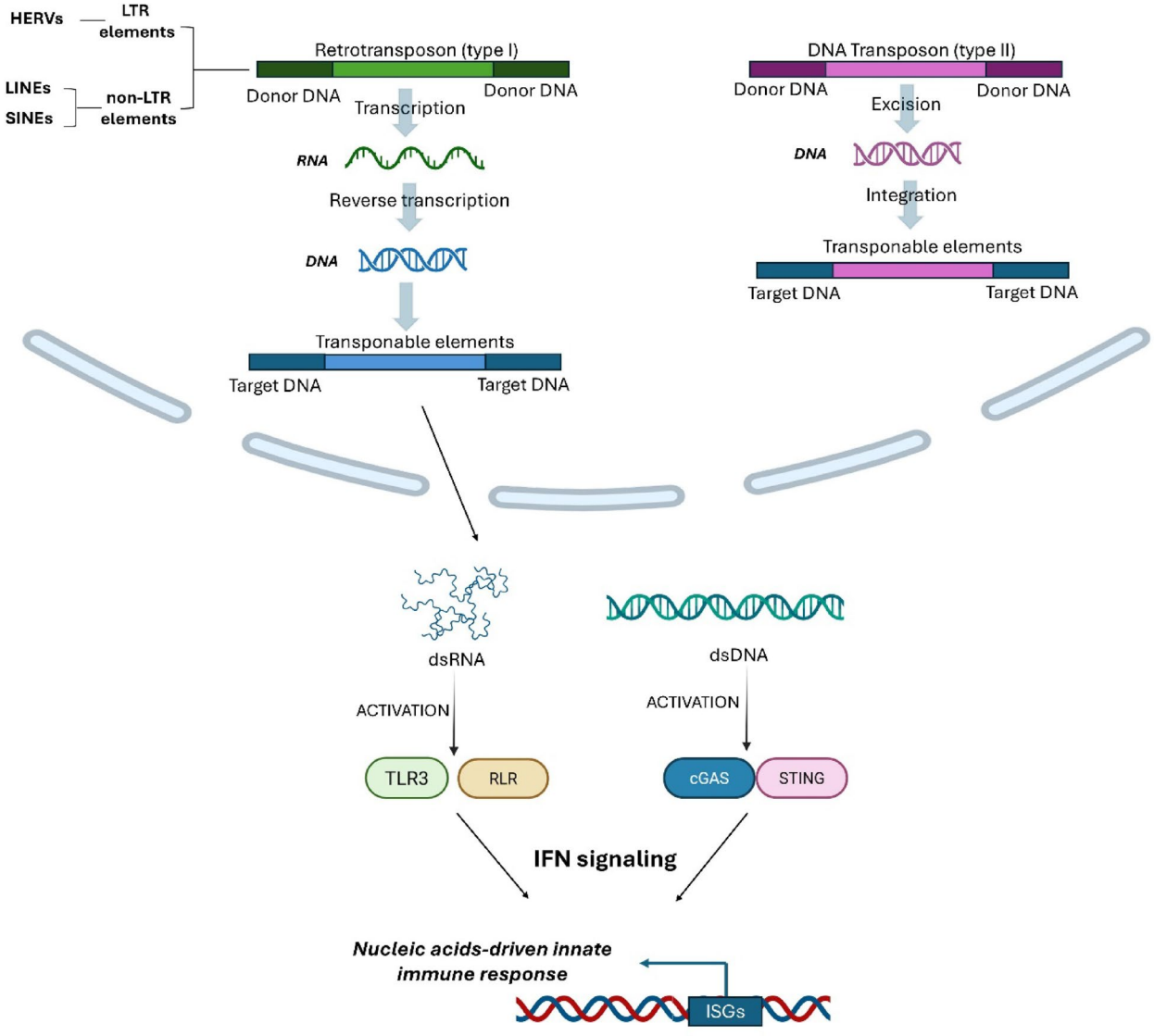

Fig. 1 TE-derived viral mimicry strategies. dsRNA and dsDNA produced by type I TEs can be detected by the cytosolic sensors RLR (specific for dsRNA) and cGAS (specific for dsDNA). This detection induces type I and III IFN signalling, which results in the transcription of interferon-stimulated genes (ISGs) and the induction of an inflammatory response, a process called "viral mimicry" that ultimately leads to the stimulation of the innate immune response by endogenous nucleic acids. Figure created with BioRender (BioRender.com)

downstream IFN signalling and cytokines production through the interferon regulatory factor (**IRF**) 3 and IRF7, and NF-κB [33]. Likewise, the cytosolic detection of DNA (nuclear, mitochondrial, or viral) activates cGAS-STING that, together with MAVS, forms a hub for innate immunity activation through production of type I and type III interferons (**IFN**) [30, 34].

IFN released by cells functions through autocrine and paracrine signalling by binding to IFN receptors triggering Janus kinase (**JAK**) signal transducer and activator of transduction (**STAT**) [35] leading to the induction of IFN-stimulated genes (**ISGs**). Since the components of IFN signalling are present at baseline state, synthesis of new proteins is not required and the molecular process of transduction in the cytosol occurs rapidly [36]. Indeed, each IFN receptor chain binds JAK in an inactive conformation inducing its transphosphorylation that sustains its activation [37]. IFN receptor chains phosphorylated by JAKs are established by the binging of STAT via Src homology 2 (**SH2**) domain interactions. Consequently, conserved Tyr/Ser residues of STATs are phosphorylated, and STATs are released in the cytosol. SH2 domains in STATs are also involved in the promotion of the subsequent homo- hetero-dimerization of phosphorylated STATs [35, 38]. In the cytosol, the complex interacts with IRF9 forming a new complex named IFN-stimulated gene factor 3 (**ISGF3**). ISGF3 translocates into the nucleus binding the IFN-stimulated regulatory elements (**ISREs**) leading to the increased transcription of ISGs [35, 39] (Fig. 2). The resulting presence of proinflammatory cytokines and chemokines facilitates macrophage activation and recruitment of neutrophils enhancing activation of the immune system [1].

Mitochondrial antiviral signalling protein and cellular homeostasis

MAVS is an outer mitochondrial membrane adaptor central to innate antiviral immunity [40]. Upon activation by cytosolic RLRs (RIG-I or MDA5), MAVS initiates downstream signalling cascades, culminating in type I IFN and pro-inflammatory cytokine production [30]. Furthermore, MAVS modulates mitochondrial dynamics, apoptotic signalling, and metabolic pathways maintaining cellular homeostasis under stress. This dual function underscores the significance of MAVS in both host defence and overall cellular health, with implications for pathological conditions such as cancer and maintenance of immune homeostasis [41].

The detection of short or long-dsRNA by RIG-I or MDA5 stimulates the conformational change in RLRs leading to exposure of their CARD motifs necessary for MAVS interaction/activation. CARD-CARD bond between exposed CARD domains of RIG-I or MDA5 and the CARD domain of MAVS triggers MAVS oligomerization. The polymerization of MAVS by CARD domains forms the prion-like aggregates that are responsible for amplifying signalling by recruitment of the adaptor proteins, such as the TNF Receptor–Associated Factor (**TRAF**) family members [42]. In particular, TRAF3 engages TANK-binding kinase 1 (**TBK1**) that phosphorylates IRF3 and IRF7. In parallel, another TRAF protein (i.e., TRAF6) activates the IkappaB kinase (**IKK**) complex of the NF-κB pathway. IKK composed of the catalytic subunits IKKα and IKKβ, and a regulatory subunit NEMO, is central to activating the NF-κB pathway [43]. IκB binds to NF-κB in the cytoplasm, keeping it inactive by masking its nuclear localization signals. Once the IKK complex is activated, IKKβ phosphorylates IκB on specific serine residues. Therefore, the phosphorylation on IkB creates a recognition site for the E3 ubiquitin ligase complex. IκB in the phosphorylated state dissociates from NF-κB undergoing a polyubiquitination responsible for IκB degradation by the proteasome. NF-κB released from IκB inhibition is translocated into the nucleus where it binds to specific κB sites in the DNA. Similarly, the phosphorylated forms of IRF3 and IRF7 can dimerize and translocate into the nucleus promoting the expression of type I IFN genes (IFN-α and IFN-β) establishing an antiviral status that recruits other immune cells to the TME (Fig. 3).

MAVS can "*spontaneously*" combine in the absence of viral infection triggering autoimmune diseases. The two most prevalent post-translational modifications (**PTMs**) that regulate MAVS activation are phosphorylation [44, 45] and ubiquitination [46]. Therefore, the cell must deploy stringent regulation of PTM to prevent spontaneous MAVS prion-like aggregation maintaining its monomeric form in the resting state. In addition to these PTMs, succinylation has the ability to influence viral replication. Succinyl-CoA is the precursor to PTM by succinylation under viral stimulation. The desuccynilation of MAVS is responsible for reduced MAVS oligomerization and is controlled by Sirtuin 5 as a new regulatory mechanism of immune homeostasis to viral mimicry events [47]. This ensures the "*closed*" state of the RIG-I/MDA5-MAVS signalling pathway triggered by MAVS oligomerization [48]. Moreover, mitophagy and mitochondrial degradation can clear spontaneously aggregated MAVS, through the mitochondrial protein Nix that binds to autophagy-related proteins to mediate PINK-PARKIN-independent mitophagy [49]. However, these homeostatic mechanisms are often altered in cancer which can also contribute to cancer immunogenicity.

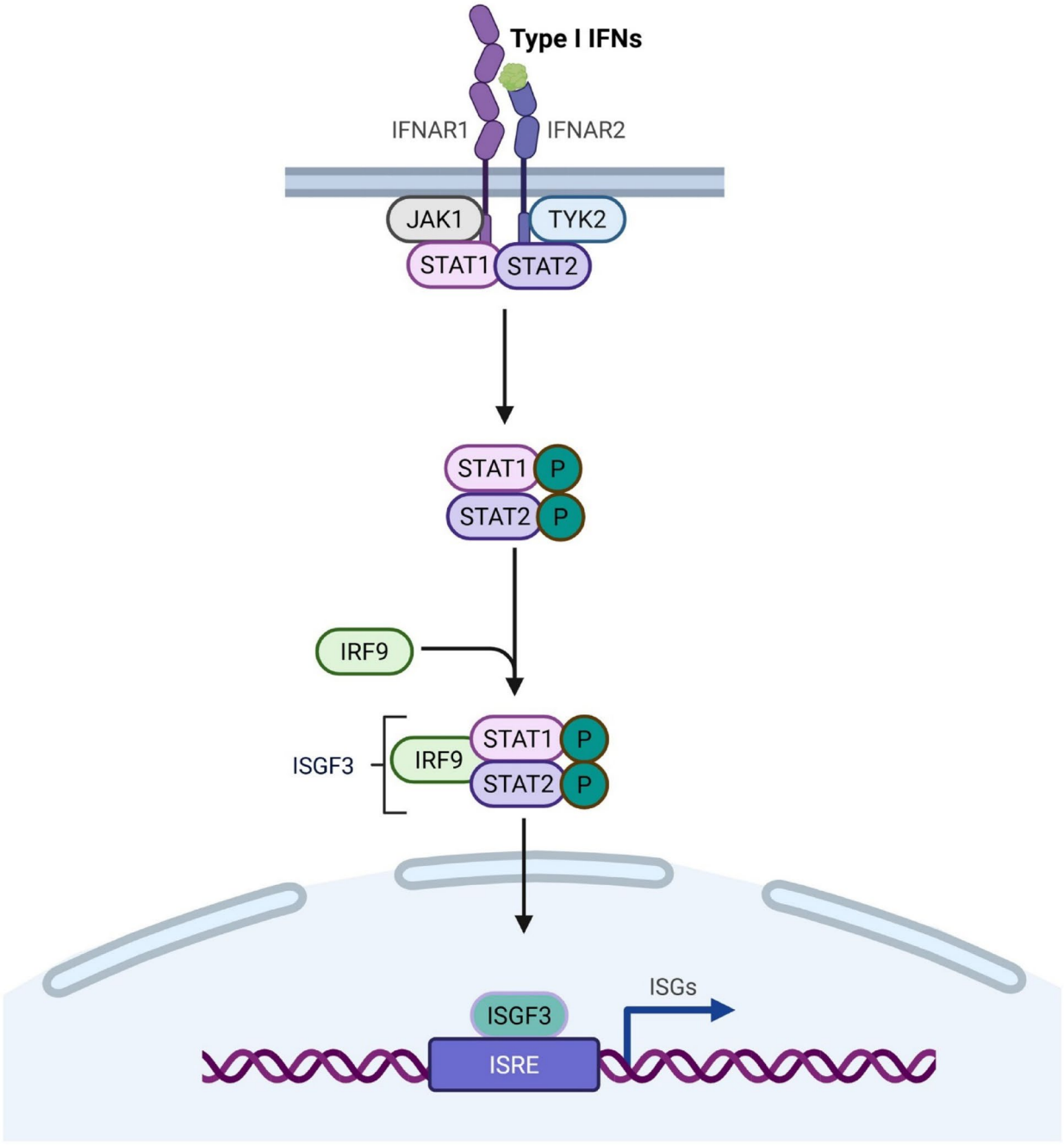

Fig. 2 ISGF3-mediated transcription of ISGs activated by type I IFNs signalling. The binding of IFNs to their receptors causes receptor dimerization, leading to the recruitment of JAKs. JAKs are activated via a process of transphosphorylation and in turn phosphorylate STAT proteins. Hetero- or homo-dimers of STAT recruit IRF9 to create a trimolecular complex, called ISGF3, which translocates to the nucleus to induce the expression of ISGs. Figure created with BioRender (BioRender.com)

cGAS-STING signalling in the inflammatory process by viral mimicry and mtDNA: two possible choices

The cGAS-STING pathway is the major dsDNA sensor. cGAS is a cytoplasmic protein that can also be found in the nucleus and the plasma membrane, a localization that facilitates DNA sensing [50]. Dimerized cGAS, binds along the entire length of dsDNA to generate a ladder network, a mechanism that allows it to be activated only when it senses short stretches of dsDNA. This interaction requires a minimum of 18 base pairs of dsDNA for binding, and more than 40 base pairs to stabilize the "*ladder network*" and increase its catalytic activity [51]. Cytosolic dsDNA binding is sequence-independent, but cGAS only binds the canonical structure of DNA known as B-form,

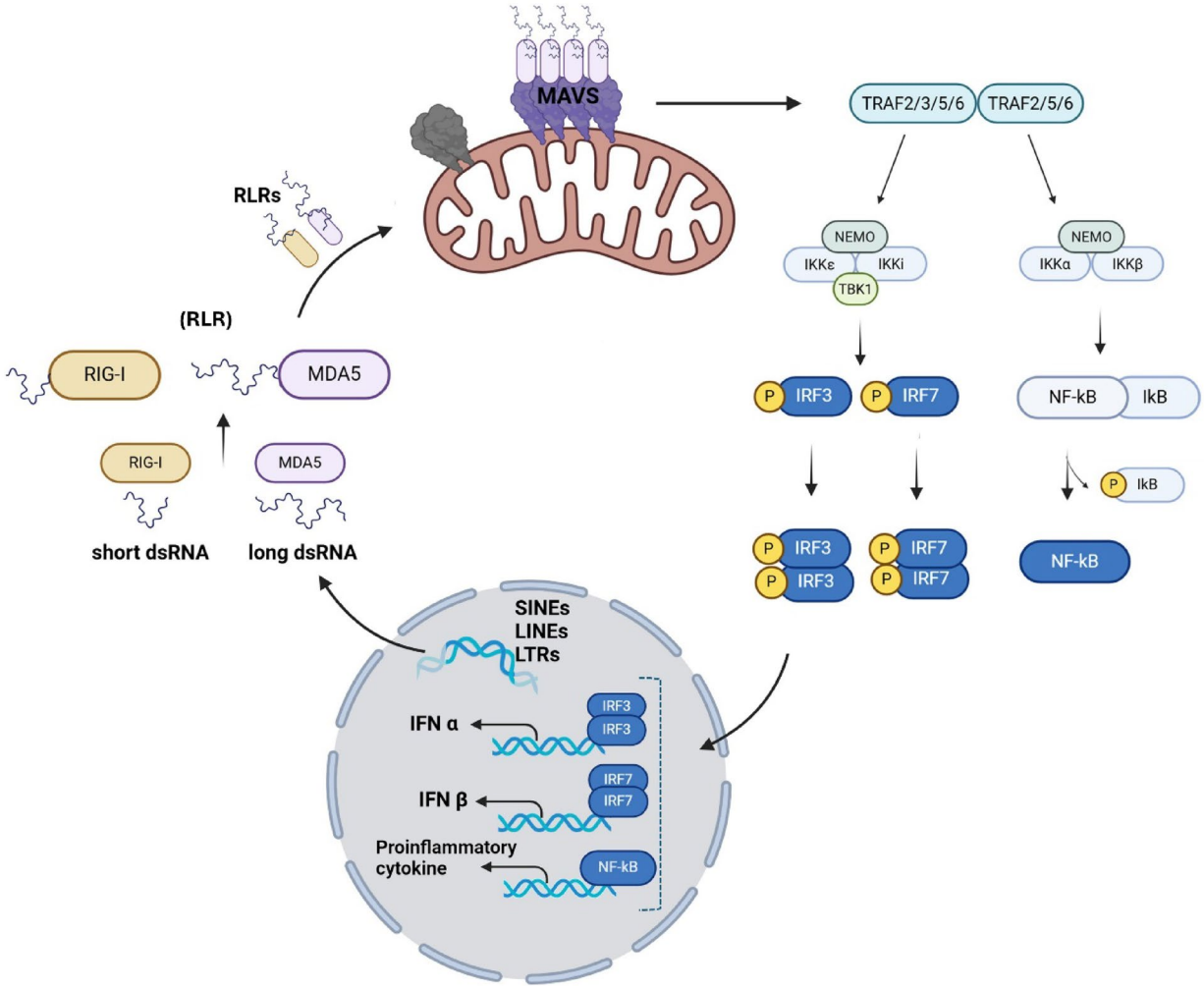

Fig. 3 The RLR signalling pathway is shown. RIG-I and MDA5 recognize and bind dsRNAs generated by TEs (LTRs, LINEs, and SINEs). In this form, RIG-I and MDA5 recruit and activate the downstream adaptor protein MAVS. Interaction of exposed CARD domains of RIG-I or MDA5 and the CARD domain of MAVS triggers the MAVS oligomerization and initiates the production of interferon signalling. Through a phosphorylation process, the activation of IRF3/IRF7 and NF-κB is triggered, which translocate to the nucleus where they promote the transcription of IFN-I and inflammatory cytokines, respectively. Figure created with BioRender (BioRender.com)

which is the right-handed double helix structure [52]. At the molecular level, cGAS-STING shows a bilobed configuration at the C-terminal catalytic domain to house the nucleotidyltransferase core activity and a conserved zinc-ion-binding module, which mediates DNA binding and cGAS dimerization. The large cleft between the bi-lobal structure binds the ATP and GTP substrates. Then, the nucleotidyltransferase activity of cGAS synthetizes cGAMP [53, 54]. cGAMP can be exported into the extracellular space through transmembrane transporters, exploiting channels or extracellular vesicles, and imported by other cells, or through intracellular connecting proteins that form gap junctions [55]. In the absence of cGAMP, STING is a domain-swapped homodimer that consists of a complex of two identical STING proteins. Upon binding to cGAMP, STING undergoes conformational changes, including a 180° rotation of the ligand binding domain (**LBD**). STING bound to cGAMP forms an ordered β-sheet that covers the LBD [56]. In this conformation, STING oligomerizes. To perform the phosphorylation STING-dependent by promoters TBK1, STING needs to translocate from the endoplasmic reticulum (ER) to the ER-Golgi intermediate compartment (**ERGIC**) or the Golgi apparatus to obtain the active form (Fig. 4).

The translocation is driven by the ER-Golgi transport machinery composed of coatomer protein complex II (**COPII**) vesicles, the component SEC24C, the ARF-GTPase ARF1, and the GTPase SAR1A. However, a mechanism is known by which the adaptor protein SURF4 interacts with STING in the Golgi to facilitate the encapsidation of STING in COPII vesicles and activate the retrograde transport of STING to the ER [57, 58]. In the Golgi, STING recruits TBK1, to a highly conserved

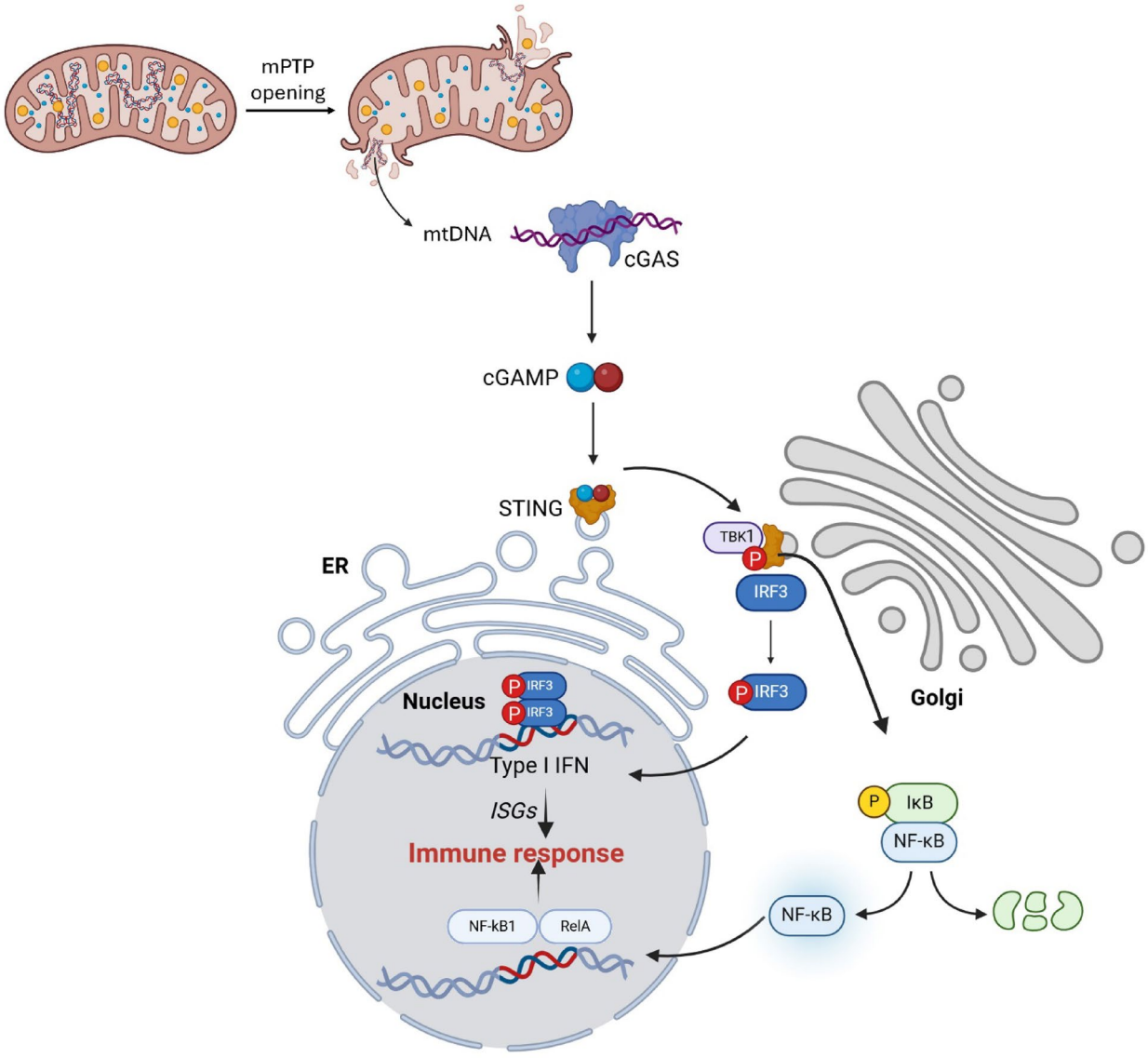

Fig. 4 Activation of the cGAS–STING pathway. dsDNA from intracellular sources is sensed by cGAS. cGAS binds dsDNA and transforms the substrates, GTP and ATP, to form the product cGAMP. The second messenger cGAMP is sensed by STING located in the ER. This induces STING to translocate from the ER to the Golgi, recruiting TBK1. TBK1 phosphorylates and activates IRF3 and NF-kB, which translocate to the nucleus, culminating in the transcription of type I IFNs and inflammatory cytokines. Figure created with BioRender (BioRender.com)

motif in the CTT of STING leading to trans-phosphorylation of TBK1. This leads to phosphorylation of CTT, generating a docking site for IRF3 [59]. TBK1 then phosphorylates IRF3, which dimerizes and translocates to the nucleus, where it induces the expression of many target genes such as inflammatory cytokines and type I IFNs (Fig. 4). IFNs exported outside of the cell bind and activate heterodimeric receptor complexes consisting of IFNα receptor 1 and 2 [60].

The cGAS-STING pathway may be regulated by PTMs that occur on cGAS and STING. Phosphorylation processes such as protein kinase B, which phosphorylates cGAS on the human Ser305 residue near the catalytic site, suppress cGAMP synthesis [61], or aurora kinase B hyper-phosphorylates the N-terminus, preventing cGAS from sensing chromatin. Polyglutamylation of cGAS on Glu272 by the enzyme tubulin tyrosine ligase-like 6 also prevents the binding of cytosolic dsDNA [62]. SUMOylation and ubiquitination also modulate cGAS activity in a target amino acid residue-dependent manner [63, 64]. Ubiquitination is the modulatory process of STING activity and is also dependent on the ubiquitinated amino acid residue, such as TRIM56, an interferon-inducible E3 ubiquitin ligase that promotes STING ubiquitination on K150 77, causing STING dimerization and TBK1 recruitment [65], while, an E3 ubiquitin ligase,

RNF5, and RNF90 catalyse K48-linked ubiquitination of STING, causing STING degradation through the proteasome-dependent pathway [66]. cGAS-STING signalling can be considered an excellent target for improving existing immunotherapies by activating the antitumor immune response in TME. STING antagonists could lead to surprising results. 5,6-dimethylxanthenone-4-acetic acid, as the first STING-targeted therapy, has been evaluated in oncology clinical trials and is the only agent to have reached Phase III. Conversely, Cyclic dinucleotide analogues as ADU-S100, MK-1454, and BI-1,387,446, directly activate STING, inducing a strong immune response.

As another contributor to viral mimicry, mitochondrial DNA (mtDNA) is recognized by the cell as a damage-associated molecular pattern (DAMP) when released into the cytosol. Mitochondrial oxidative stress or apoptotic cell death [67] cause the release of mtDNA through the mitochondrial permeability transition pore (mPTP) or through BAK/BAX macropores, respectively. However, although mtDNA is recognized by cytoplasmic cGAS in both cases, activating the cGAS-STING pathway, the apoptotic pathway does not involve a pathway-mediated inflammatory response due to the degradation of cGAS and IRF3 by caspase 3, which mediates proteolytic cleavage in apoptosis [68]. Conversely, release of mtDNA from the mPTP [69] activates the cGAS-STING pathway acting as an immunostimulant [55]. Inactivation of mPTP in cancer cells might prevent the activation of the cGAS-STING system. Conversely, employing mPTP opening with a poorly defined mechanism [70], mtDNA can pass through the inner mitochondrial membrane and transported in the cytosol to trigger the cGAS-STING signalling. mtDNA following the effect of viral mimicry can boost immune responses by increasing type I IFN production [71, 72]. The disruption of mtDNA homeostasis, leading to mPTP-dependent release and subsequent cGAS-STING activation, could serve as a cell-intrinsic indicator of how mitochondria contribute to stimulating innate immunity in the pathogenesis of cancer cells [72].

Viral mimicry–inducing cancer-specific therapeutic vulnerability

Ancient endogenous retroviruses and retrotransposons normally repressed in the mammalian genome may be reactivated in most cancers upon therapy. When the resulting antiviral pathways are triggered an immune response is induced [73]. The reactivation occurs mostly through global DNA hypomethylation characteristic of the cancer cell genome. In particular, intergenic regions, including REs and oncogenes are primarily affected. Associated with the global reduction in DNA methylation, is a "compensatory" hypermethylation of promoters of tumour suppressor genes that leads to transcriptional-silencing [73–75] and suppression of gene expression [76]. The epigenetic profile of cancer cells is, therefore, useful in pharmaco-epigenomics to guide towards new therapeutic strategies [77].

For instance, DNA demethylating agents, histone deacetylases or lysine-specific demethylase 1 inhibitors, can induce viral mimicry responses that enhance the effectiveness of immunotherapy and radiotherapy [78–81] although due to their poor cancer specificity, these therapeutics may target normal cells, causing significant side effects [82]. An example of a drug stimulating a viral mimicry response is 5-aza-2-deoxycytidine (**5-A-dC**) [3]. As a cytidine analogue, 5-A-dC integrated into DNA causes global demethylation through a nonspecific mechanism of DNA (cytosine-5-) methyltransferase sequestration [75, 83]. Administration of 5-A-dC to cancer cells at low doses induces the production dsRNA from TEs and the resulting viral mimicry causes transcription and production of type I IFN involving MDA5/MAVS-dependent IRF7 activation [30].

Another promising approach is 5'→3' exoribonuclease 1 (XRN1) that regulates nucleic acid metabolism. XRN1 knockout leads to increases in the level of dsRNA and consequent ISG expression. On the contrary, XRN1-dependent degradation of RNA prevents the accumulation of dsRNA below the threshold required for activation of viral mimicry [84]. Moreover, XRN1-resistant cell lines with low baseline dsRNA levels become sensitive to the induction of viral mimicry by the use of dsRNA-elevating drugs such as palbociclib or 5-A-Dc [85, 86].

The identification of REs silencing mechanisms specific to cancer cells be the basis for developing a new generation of cancer Immunotherapeutics. For instance, FBXO44 expression is inversely correlated to IFN signalling, and cytotoxic T-cell infiltration in human tumours [87]. FBXO44 has been shown to play an important role in keeping REs silent in cancer cells by binding H3K9me3-modified nucleosomes together to protein complexes formed of SUV39H1, CRL4, and Mi-2/NuRD. Conversely, FBXO44 inhibition induces the reactivation of REs and viral mimicry with production of IFN through stimulation of cGAS-STING or the MAVS pathways [82].

Epigenetic therapies combined with other modalities

Cytosolic double strands of nucleic acids induced by viral mimicry-enhancing therapies can increase the efficacy of conventional cancer-specific therapeutics [5]. For instance, treatment of acute myelogenous leukaemia cells by azacytidine induces type I IFN response enhancing T cell-mediated anti-leukemic activity making leukemic cells more susceptible to T cells effector activity [88]. Moreover, conventional chemotherapeutics developed according to their ability to preferentially kill malignant

cells may exploit immunological mechanisms inducing viral mimicry that in turn increase responsiveness to immune therapy modalities such as immune checkpoint inhibitors (**ICI**) [79, 81, 89, 90]. These combinations may be particularly useful for the treatment of immune-excluded or immune-desert cancers, which are less responsive to ICI [91]. Other drugs targeting proteins or metabolic pathways not involved in the oncogenesis of cancer but needed for cancer cell survival and proliferation can induce viral mimicry-related pro-inflammatory changes in the TME, converting resistant tumours into ones more responsive to ICI [92].

Radiotherapy not only exerts direct cytotoxic effects but also has immunomodulatory properties that influence both tumour cells and surrounding tissues. Radiotherapy-induced chromosome breakage and/or genome fragmentation, producing membrane-bound DNA, can sustain inflammatory signals exploiting the cGAS-STING pathway [78]. These immune effects go beyond the irradiated site and contribute to abscopal responses, whereby radiotherapy induces systemic antitumour immunity [93, 94].

As an example of future mechanisms that could be exploited to enhance cancer cell-intrinsic immunogenicity is ADAR1, which physiologically dampens viral mimicry caused by the accumulation of cytoplasmic double-stranded nucleic acids. Indeed, genetic variations that disrupt ADAR1 ability to edit dsRNA can lead to inappropriate immune activation and inflammation [95] due to the misidentification of self-RNA as foreign [96]. Exploiting this phenomenon by inhibition of ADAR1 could enhance cancer cell-intrinsic immunogenicity [26] and support a novel therapeutic strategy, particularly in combination with ICI to expand their effectiveness to less susceptible cancers.

In conclusion, targeting mitochondria-dependent signalling that leads to viral mimicry may identify alternative therapies that could overcome the shortcomings of immunotherapy, including ICI. Reactivation of TEs holds promise as a novel therapeutic strategy exploiting epigenetic modifiers that may result in the death of cancer cells associated with enhanced anti-tumour immunity.

Abbreviations

5-A-dC	5-aza-2-deoxycytidine
CARD	Caspase Recruitment Domain
cDNA	Complementary DNA
cGAMP	cyclic GMP-AMP
CNAs	Cytosolic Nucleic Acids
COPII	coatomer protein complex II
CPR	CNA Pattern Recognition
DAMP	Damage-Associated Molecular Pattern
dsDNA	Double Stranded DNA
dsRNA	Double Stranded RNA
ERGIC	ER-Golgi intermediate compartment
ERV	Endogenous Retrovirus
ICI	Immune Checkpoint Inhibitors
cGAS	cGAMP synthetase
IFN	Interferon
IKK	IkappaB kinase
IRF	Interferon Regulatory Factor
ISG	Interferon-Stimulated Gene
ISGF3	IFN-stimulated gene factor 3
ISRE	IFN-stimulated regulatory elements
JAK	Janus Kinase
LBD	Ligand Binding Domain
LINE	Long Interspersed Nuclear Elements
LTR	Long Terminal Repeat
MAVS	Mitochondrial Antiviral-Signalling Protein
MDA5	Melanoma Differentiation Associated Antigen 5
mPTP	Mitochondrial Permeability Transition Pore
mtDNA	Mitochondrial DNA
PMT	Post-Translational Modification
RE	Repetitive Element
RIG-I	Retinoic acid Inducible Gene-I
RLR	RIG-I-Like Receptors
SH2	Src homology 2
SINE	Short Interspersed Nuclear Elements
STAT	Signal Transducer and Activator of Transcription
STING	Stimulator of Interferon Genes
TBK1	TANK-binding kinase 1
TE	Transposable Elements, TLR, Toll-Like Receptor
TME	Tumour Micro-Environment
TRAF	TNF Receptor–Associated Factor
XRN1	5′→3′ exoribonuclease 1

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Authors' contributions
Salvatore Nesci contributed to the conception, design and drafted the manuscript; Saverio Marchi contributed to revising the manuscript; Joyce Hu contributed to revising the manuscript; Francesco M. Marincola contributed to the conception and substantively revised the manuscript; Cristina Algieri contributed to drafted and revised the manuscript.

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Not applicable.

Consent for publication
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