Type-I-interferons in infection and cancer: Unanticipated dynamics with therapeutic implications

Martina Musella, Gwenola Manic, Ruggero De Maria, Ilio Vitale, and Antonella Sistigu

ABSTRACT

If there is a great new hope in the treatment of cancer, the immune system is it. Innate and adaptive immunity either promote or attenuate tumorigenesis and so can have opposing effects on the therapeutic outcome. Originally described as potent antivirals, Type-I interferons (IFNs) were quickly recognized as central coordinators of tumor-immune system interactions. Type-I-IFNs are produced by, and act on, both tumor and immune cells being either host-protecting or tumor-promoting. Here, we discuss Type-I-IFNs in infectious and cancer diseases highlighting their dichotomous role and raising the importance to deeply understand the underlying mechanisms so to reshape the way we can exploit Type-I-IFNs therapeutically.

Abbreviations: AIM2, absent in melanoma 2; AP-1, activated protein-1; ATM, ataxia-telangiectasia mutated; CARD, caspase activation and recruitment domain; CARDIF, CARD adaptor-inducing IFN-β; CDKN1A, cyclin-dependent kinase inhibitor 1A; cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; cGAS, cyclic GMP-AMP synthase; CSC, cancer stem cell; CSF1, colony stimulating factor 1; CTL, cytotoxic T lymphocyte; CXCL10, C-X-C motif chemokine ligand 10; DAI, DNA-dependent activator of IFNs; DAMP, damage-associated molecular pattern; DC, dendritic cell; DDX, DExD/H-box helicase; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; FDA, Food and Drug Administration; FASLG, FAS ligand; HER2, human EGFR 2; HLA, human leucocyte antigen; HSPC, haematopoietic stem/progenitor cell; ICD, immunogenic cell death; IFI16, IFN-β; IFNGR, IFN-γ; IFNAR, IFN-α/β receptor; IPS-1, IFN-stimulated gene; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISGF3, IFN-stimulated gene factor 3; JAK, Janus kinase; LGP2, laboratory of genetics and physiology 2; LPS, lipopolysaccharide; Mal, MyD88 adaptor-like; MAPK14, mitogen-activated protein kinase 14; MAVS, mitochondrial antiviral signaling adaptor; MCA, 3′-methylcholanthrene; MDAS, melanoma differentiation-associated protein 5; MDSC, myeloid-derived suppressor cell; MHC-I, major histocompatibility complex-I; MyD88, myeloid differentiation primary response gene 88; MX1, MX dynamin-like GTPase 1; NF-κb, nuclear factor-kB; NK, natural killer; NLR, NOD-like receptor; NOD2, NOD-containing protein 2; OAS, 2′-5′-oligoadenylate synthetase; PAMP, pathogen-associated molecular pattern; pDC, plasmacytoid DC; PD-L1, programmed death-ligand 1; PKR, protein kinase R; POLR3, RNA polymerase-III; PRR, pathogen recognition receptor; ps3/TTP3, tumor suppressor protein ps3; RANK, receptor activator of NF-κb ligand; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor; ROS, reactive oxygen species; SARM, sterile armadillo-motif-containing protein; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; STING, stimulator of IFN genes; TAA, tumor-associated antigen; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; TME, tumor microenvironment; TNEM173, transmembrane protein 173; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TRAM, TRIF-related adaptor molecule; Treg, regulatory T cell; TREX1, 3′ prime repair exonuclease 1; TRIF, TIR-domain containing adaptor protein-inducing IFN-β; TYK2, tyrosine kinase-2; VEGF, vascular endothelial growth factor; VISA, virus-induced signaling adaptor.

Introduction

The sensing of altered-self, such as changes in tissue/organ homeostasis or integrity, and hence the need to detect and protect against potential danger (e.g., cellular stress, damage, or abnormal death), is upsetting the traditional view of immunity as a response to solely alien microbes and molecules.1 In particular, it is now clear that cancer cells, either transformed by foreign pathogens (e.g., human papillomavirus, hepatitis-B virus, Epstein-Barr virus, human T-lymphotropic virus-I, hepatitis-C virus, Kaposi’s sarcoma herpervirus or Helicobacter pylori) or totally aseptic, differ antigenically from their normal counterparts and, similar to virus-infected cells, emit danger signals to license the immune system. Such signals,
best known as damage-associated molecular patterns (DAMPs), de facto favor the establishment of a productive and long-lasting immune response allowing to clear virus-infected cells (because they express virus-encoded proteins) and tumor cells (because they express tumor-associated antigens, TAAs). Intriguingly, antiviral and antitumor immune responses share common DAMPs, among which Type-I-interferons (IFNs) emerge as the primum movens for the sequential events bridging innate and cognate immunity.\(^7\)

IFNs and their receptors are a subset of the class-2 \(\alpha\)-helical cytokines that have been found in all vertebrates, although a systemic phylogenetic knowledge is lagging behind. Based on criteria such as their cellular source, their general biologic properties, their gene structure and the receptor through which they signal, IFNs have been categorized into three distinct families: Type-I, Type-II and Type-III. In humans, Type-I-IFNs consist of 13 partially homologous IFN-\(\alpha\) cytokines, a single IFN-\(\beta\) and several not yet well characterized single gene products (IFN-\(\gamma\), IFN-\(\tau\), IFN-\(\omega\), IFN-\(\delta\) and IFN-\(\chi\)) all of which are mostly non-glycosylated proteins of 165–200 aminoacids.\(^8\) The reason for the existence of multiple subtypes may be ascribed to differences in tissue-specific expression, the kinetic of production and the spectrum of biologic activities.\(^4\) Almost all cells in the body can produce Type-I-IFNs following the recognition of molecules, such as foreign and self-nucleic-acids and a minority of other non-nucleic-acids (collectively known as pathogen-associated molecular patterns, PAMPs), by the so-called pathogen recognition receptors (PRRs) located in the plasma membrane, cytosol or endosomal compartments.\(^5\) In the canonical Type-I-IFN signaling, Type-I-IFNs bind to a heterodimeric transmembrane receptor termed IFN-\(\alpha\)\(\beta\) receptor (IFNAR), in turn activating the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. This cascade induces the transcription of few hundreds of IFN-stimulated genes (ISGs), which steer the multiple facets of the cellular response.\(^6\) The Type-II-IFN family consists of a single IFN-\(\gamma\) glycosylated protein of 140 aminoacids, which is produced exclusively by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells under immune and inflammatory stimuli. IFN-\(\gamma\) signals through the heterodimeric IFN-\(\gamma\) receptor (IFNGR), composed of IFNGR1 and IFNGR2 and characterized by a JAK1 binding domain and a STAT1 docking site.\(^7\) The Type-III-IFN family consists of the subtypes IFN-\(\lambda1\), IFN-\(\lambda2\), IFN-\(\lambda3\) [also known as interleukin (IL)-29, IL-28A and IL-28B, respectively] and the newly identified IFN-\(\lambda4\).\(^8,9\) Type-III-IFNs are structurally similar to IFN-\(\gamma\) but functionally identical to IFN-\(\alpha\)\(\beta\). Only epithelial-like cells and, to a lesser extent, some immune cells respond to IFN-\(\lambda\)s. Type-III-IFNs engage a receptor complex composed of the IFN-\(\lambda1\)R (or IL-28AR) and IL-10R2 chains to induce signaling pathways similar to those of Type-I-IFNs.\(^8\)

This Review focuses on Type-I-IFNs and how pathogens and danger signals cross-regulate IFNAR signaling to mount immune defenses against virus-related and -unrelated diseases such as cancer. We conclude with open questions, future perspectives and implications for new clinical uses of Type-I-IFNs in oncology.

**Pathways triggering production of Type-I-IFNs**

As reported in the introduction, Type-I-IFNs can be produced by all nucleated cells in the body. The production of Type-I-IFNs is transient and occurs upon stimulation of an array of transmembrane and cytosolic PRRs with viral or other xenogeneic or autologous nucleic acids (Fig. 1). Currently identified PRRs include Toll-like receptors (TLRs), RIG-I-like receptor (RLRs), NOD-like receptors (NLRs) and DNA sensors.\(^10\) Although viral nucleic acids are the predominant ligands, other molecules, including viral proteins, bacterial lipopolysaccharide

![Figure 1. Major intracellular pathways leading to Type-I-IFN production. Families of sensors, known as PRRs, are available in the cells to detect viral and danger products, and induce the expression of Type-I-IFNs. One set of PRRs is localized in endosomal vesicles, while another set senses components in the cytoplasm. The endosome-associated TLR3 and the cytosolic MDA5, RIG-I and NOD2 sense double-stranded and single-stranded RNAs through the activation of adaptor molecules such as TRIF and MAVS, respectively. TRIF and MAVS in turn converge to activate the TBK1-IKK\(e\) kinase complex. This culminates in the activation of several transcription factors IRF3 and IRF7, which translocate to the nucleus and participate in the induction of a first wave of IFN-\(\beta\) production (1). IFN-\(\beta\) in turn acts in an autocrine/paracrine manner binding to the heterodimeric receptor IFNAR1-IFNAR2. This is followed by the activation of a JAK-STAT signaling pathway leading to a second wave of IFN-\(\alpha\) production as well as to the transcription of other antiviral genes (2). Other PRRs sensing DNA are DAI and cGAS, with this last catalyzing the formation of ligands for STING upstream of the TBK1-IKK\(e\) complex, which finally drives the expression of IFNA and IFNB. cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; cGAS: cyclic GMP-AMP synthase; DAI: DNA-dependent activator of IRFs; IFNs: interferons; IFNAR: IFN-\(\alpha\)\(\beta\) receptor; IKK\(e\): IkB kinase e; IRF: IFN regulatory factor; ISGs: IFN-stimulated genes; JAK: Janus kinase; MAVS: mitochondriald antiviral signaling adapter; MDA5: melanoma differentiation-associated protein 5; NOD2: nucleotide oligomerization domain 2; PRRs: pathogen recognition receptors; RIG-I: retinoic acid-inducible gene I; STAT: signal transducer and activator of transcription; STING: stimulator of IFN genes; TBK1: TANK-binding kinase 1; TLR3: Toll-like receptor 3; TRIF: TIR-domain containing adaptor protein-inducing IFN-\(\beta\); TYK2, tyrosine kinase-2.
(LPS), lipoproteins, or endogenous ectopic proteins, can bind PRRs ultimately leading to Type-I-IFN production and innate immune responses.10

TLRs, the first PRRs identified, are transmembrane receptors either expressed on the cell surface or associated with intracellular vesicles.11 To date, 10 functional TLRs have been identified in humans, each of them detecting specific PAMPs. Briefly, lipoproteins are recognized by TLR1, TLR2 and TLR6; double-stranded and single-stranded RNAs by TLR3, TLR7 and TLR8; LPS by TLR4; flagellin by TLR5; and DNA by TLR9.12 Although recent evidence suggests that TLR10 could have either immune-stimulatory13 or immune-suppressive14 properties, its exact activating ligand(s) and function are not yet known. TLRs signal through five different adaptor molecules: myeloid differentiation primary response gene 88 (MyD88), MyD88 adapter-like (Mal), TIR-domain containing adaptor protein-inducing IFN-β (TRIF), TRIF-related adaptor molecule (TRAM) and sterile armadillo-motif-containing protein (SARM).15 The association with these proteins recruits and activates the IkK kinase ε (IKKe)/TANK-binding kinase 1 (TBK1) complex (Fig. 1). This, in turn, is responsible for the phosphorylation and activation of the IFN regulatory factor (IRF)3, nuclear factor (NF)-κB, and activated protein (AP)1, all of them leading to the first-wave of IFN-β production. IFN-β then triggers the autocrine and paracrine expression of a related factor, IRF-7, which is responsible for a positive feedback loop initiating the synthesis of several IFN-α subtypes as the second wave of Type-I-IFNs.16

Among the cytosolic PRRs, RLRs are a family of DExD/H box RNA helicases (DDXs) sensing PAMPs within viral RNA. To date, three RLR members have been identified: (1) retinoic acid-inducible gene (RIG)-I, (2) melanoma differentiation-associated protein (MDA)5 and (3) laboratory of genetics and physiology (LGP)2. RIG-I and MDA5 detect a variety of viruses and share several structural similarities, including their organization into three domains: a tandem caspase activation and recruitment domain (CARD) to the N-terminal, a central DDX helicase region, and a repressor domain to the C-terminal that, in the case of RIG-I, is involved in autoregulation.16 Although presenting a similar organization, LGP2 lacks the N-terminal CARD and is currently thought to be a regulator of RIG-I and MDA5 rather than a bona fide PRR.17 Upon binding to double-stranded-RNAs, RLRs directly interact with a downstream molecule named independently by four different groups as mitochon- drial antiviral signaling adaptor (MAVS),18 IFN-β promoter stimulator (IPS)-1,19 virus-induced signaling adaptor (VISA),20 and CARD adaptor-inducing IFN-β (CARDIF).21 As for TLRs, the association with this mitochondrial-resident protein via CARD induces Type-I-IFN production by the IKKe/TBK1 complex (Fig. 1).

NLRs are cytoplasmic PRRs with a tripartite structure consisting of a variable N-terminal effector domain, a middle nucleotide-binding domain and a C-terminal leucine-rich repeat domain.22 Among the more than 20 NLRs identified in humans so far,22 only the cytosolic molecular sensor NOD-containing protein 2 (NOD2) was clearly shown to recognize single-stranded RNAs leading to Type-I-IFN production through a mechanism dependent on MAVS and IRF3 activation (Fig. 1).23 Other NLRs are mainly described as regulators of the major histocompatibility complex-I (MHC-I),24 the inflammasome multiprotein complex assembly25 and some regulated cell death pathways (apoptosis, pyroptosis and pyro-necrosis).25 All these functions go beyond their sensing of DAMPs and PAMPs, which instead remains largely unknown.

The first described PRR for DNA, and still the only known endosomal-based DNA sensor, was TLR9.26 TLR9 is expressed preferentially in plasmacytoid dendritic cells (pDCs) and acts as a potent inducer of IFN-α via a signaling network dependent on Myd88 and IRF7.26 Moreover, DNA can end-up in the cytosol through several routes (e.g., intracellular pathogenes, lysosome-internalized exogenous DNA from dead cells, or endogenous DNA replication debris) where it can be recognized by more than 10 cytosolic receptors.27 The search for cytosolic DNA sensors first led to the identification of the DNA-dependent activator of IRFs (DAI) (Fig. 1).28 When exogenously expressed in L929 murine fibroblasts, DAI increased Type-I-IFN production in a dose-dependent manner following stimulation by both B- and Z-form DNA.28 Similarly, knockdown of DAI with specific siRNAs impaired Type-I-IFN production in response to cyto- solic DNA.28 RNA polymerase-III (POLR3), the second cytosolic DNA sensor discovered, was reported to use AT-rich and herpesvirus DNA as a template to produce 5′-triphosphate RNAs, which then induce Type-I-IFNs by activating RIG-I.29 However, POLR3 could not account for DAI-independent sensing of non-AT-rich DNA suggesting the existence of additional cytosolic DNA sensors. Remarkably, an adaptor molecule referred to as stimulator of IFN genes (STING) was identified as being crucial for recognizing cytoplasmic DNA and inducing innate immune responses to a variety of DNA pathogens even including certain RNA viruses.30 Nonetheless, despite the wealth of recent information on the mechanisms whereby STING contributes to signal Type-I-IFN induction, the upstream DNA-sensing events remain largely unknown. Recent evidence suggests that cyto- solic DNA is perceived by the cyclic GMP-AMP synthase (cGAS), which then becomes catalytically active and generates the second messenger cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) (Fig. 1). cGAMP in turn binds to STING stimulating its transit from the endo- plasmic-reticulum to perinuclear endosomes where it triggers IRF3 activation via TBK1.30,31 Of note, STING-dependent Type-I-IFN production can also be activated by single-stranded DNA resulting from DNA damage or replication stress,32 by mitochondrial DNA released following apoptotic mitochon- drional outer membrane permeabilization33 and possibly by retroelements not properly metabolized by the three prime repair exonuclease (TREX).34

Two essential mediators of distinct DNA-activated innate responses seem to be the PYHIN proteins absent in melanoma (AIM)2 and IFN-γ-inducible (IFI16).35,36 Moreover, the heli- cases DDX3, DDX41, DHX9, DDX60, DDX1 and DHX36 were recently involved in DNA immune sensing through a pathway dependent on STING and TBK1.37 In particular, Liu and cow- workers found that, in mouse splenic myeloid DCs with limited basal IFI16 expression, DDX41 was the initial sensor of cyto- plasmic DNA inducing Type-I-IFNs and the subsequent IFI16
expression, with this latter operating as an amplifier of innate responses.37

Along with PAMPs and DAMPs, Type-I-IFNs can also be produced in response to rare physiologic stimuli such as colony stimulating factor (CSF),38 receptor activator of NF-κB ligand (RANK)39 and estrogens.40 More recently, an intriguing correlation between Type-I-IFNs and tumor protein p53 (TP53/p53) was reported.41 In sum, the absence of p53 was associated with extensive DNA hypomethylation that resulted in a massive transcription of normally silent retroelements and satellite DNA. The subsequent accumulation of these newly generated double-stranded RNA species eventually triggered a “suicidal” Type-I-IFN response.41

Overall, Type-I-IFN production is tightly regulated by major families of heterologous receptors engaged by diverse ligands during infectious and cancerous diseases. Each of the Type-I-IFN subtypes then induces a unique and partially overlapping set of ISGs able to act at different steps of virus and cancer life cycle.

**ISGs: A complex net of host defenses**

Type-I-IFN-mediated innate immune response is hardwired within genomes to provide a robust first-line of host defense and preserve homeostasis. Once secreted by cells, Type-I-IFNs bind to the same ubiquitous heterodimeric IFNAR1-IFNAR2 receptor.42 The assembly of IFNAR1, Type-I-IFN and IFNAR2 in a 1:1:1 stoichiometry seems to occur via a two-step process whereby Type-I-IFN first binds to one IFNAR and then promotes the recruitment of the second IFNAR without identified interactions between the two IFNARs.42 Once assembled, this ternary complex promotes the phosphorylation and activation of IFNAR1-associated tyrosine kinase (TYK)2 and IFNAR2-associated JAK1, which, in turn, phosphorylate cytosolic STAT1 and STAT2 (Fig. 1). This results in the formation of STAT1-STAT2 heterodimers that dissociate from receptors and migrate into the nucleus where they bind IRF9 to form the heterotrimeric transcriptional complex IFN-stimulated gene factor (ISGF)3. In the final step, ISGF3 binds to specific DNA response elements transactivating hundreds of ISGs.6 The nature and precise mechanisms through which ISGs prime cells for enhanced pathogen/danger detection and clearance, and then allow them to recover to normal function are not entirely elucidated. Recent evidence, reviewed in ref. 4, showed that Type-I-IFNs lead to cell-type and context-dependent patterns of ISG expression through a complex modulation of all seven STAT family members and other kinases (e.g., PI3K, p38, ERK and JNK) in addition to JAK. This may explain the complexity to regulate the pattern and magnitude of so many different biological functions in so many different cells during infection, cancer and inflammation.4 For more insights in these issues refer to databases on signaling pathways and immune cell types such as Interferome (Interferome.org), Innate DB (http://www.innatedb.com) and the NIAIDs Systems Biology (http://www.niaid.nih.gov/labresources/laboratories/about-laboratories/lsb/Pages/).

Similar to most cytokines, Type-I-IFN cascade is tightly regulated by positive and negative feedforward and feedback loops, which collectively ensure that the strength and duration of the response are effective yet limited, thereby preventing the toxic consequences of excessive/prolonged signaling.43 This balance is finely tuned by host factors operating at multiple levels, including signaling, transcription and translation. To give an example, many components of upstream PRR pathways (including receptors and IRFs) are ISGs.44 Type-I-IFNs are also reported to induce a network of inhibitors of their own signaling, such as members of the suppressor of cytokine signaling (SOCS) protein family.45 Overall, a complex net of signaling pathways makes proper use of the Type-I-IFN-ISG system to induce host protection while limiting tissue damage and preventing responses to self. Accumulating evidence indeed suggests that an aberrant activation of immunity by high levels of Type-I-IFNs contributes to the development of autoimmune diseases, such as systemic lupus erythematosus.46 This observation highlights the importance of understanding the mechanisms maintaining strict control over Type-I-IFN signaling to support the development of smart therapies for eradicating the danger and alleviate autoimmune diseases.

**Type-I-IFNs in cancer**

Type-I-IFNs are back in the oncological spotlight due to a greater understanding of their role in tumor generation, pathogenesis and treatment. Regardless of their source in the tumor microenvironment (TME), Type-I-IFNs have the potential to exert their opposed anti- and pro-tumorigenic effects acting directly on tumor cells and indirectly on immune infiltrating cells (Fig. 2).

**Cancer-intrinsic effects of Type-I-IFNs**

The cancer cell-intrinsic effectiveness of Type-I-IFNs is well documented in experimental animal systems and is reported to depend on specific cellular effects such as growth inhibition,47 modulation of apoptosis,48 differentiation,49 migration,50 alteration of cell surface expression of TAA50 and promotion of the epithelial-to-mesenchymal transition (EMT).51 Type-I-IFNs are known to affect different phases of the mitotic cell-cycle (panel 1, Fig. 2) with the most common perturbation being the G1 arrest.51 In a seminal work, Balkwill et al. showed that in vitro treatment of human breast cancer cell lines with exogenous crude preparations of Type-I-IFNs had a direct anti-proliferative effect that was attributed to the prolongation of the cell cycle.52 Accordingly, observations from two independent studies showed that IFN-α inhibited the growth of human prostatic cancer cells and murine macrophages stalling the G1-S transition through the increased expression of the cyclin-dependent kinase inhibitor (CDKN)1A, best known as p21.53,54 Type-I-IFNs are also reported to induce other CDK inhibitors, including CDKN1B and CDKN2B (best known as p27 and p15, respectively), whose upregulation leads to cell-cycle blockade at the G1 phase.55 More recently, Katayama and colleagues provided evidence that, in human colon cancer cells, the anti-proliferative action of Type-I-IFNs relied on a p21-dependent prolongation of the S phase rather than a G1 block.56 Yet other nets involved in Type-I-IFN-induced cell-cycle arrest are believed to include the downregulation of the transcription factor MYC and the activation of mitogen-activated protein kinase (MAPK)14 or the adapter molecule CRK.57,58 Contrasting
Experimental findings indicate that Type-I-IFNs can either induce tumour cell death or protect cancer cells from chemically induced apoptosis (panels 2 and 5, Fig. 2). This discrepancy may be ascribed to the degree of cellular differentiation, tumour-related factors and differences in the TME. Indeed, the administration of Type-I-IFNs was reported to modulate the two major apoptotic responses: the extrinsic or death receptor-mediated pathway and the intrinsic or mitochondrial pathway. Briefly, the former cascade requires ligation of cell-surface death receptors, such as the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), to activate the initiator caspase-8, whereas the latter requires the release of apoptotic factors such as cytochrome c1 from the mitochondria to activate other cytoplasmic initiator caspases. The ISGs involved in apoptosis include (but are not limited to) FAS, FAS ligand (FASLG), protein kinase R (PKR) and oligoadenylate synthetase (OAS), particularly the 9–2 isozyme (extensively reviewed in ref. 61).

The in vitro modulation of cultured tumor cells by Type-I-IFNs has also been documented. Some early reports showed that IFN-β has the ability to boost human leucocyte antigen (HLA)-class-I expression (panel 3, Fig. 2) and modulate the antigenic landscape of cultured melanoma cells (panel 4, Fig. 2). More recently, these discoveries were characterized by Dunn et al., who showed that IFN-α simultaneously augments TAAs (e.g., Melan-A/MART-1, gp100 and MAGE-A1) and HLA-class-I thus increasing the likelihood of improved immune recognition and cytotoxic killing of tumor targets, respectively.

The EMT is a process by which epithelial cells lose their polarization and cell-cell contacts and undergo remarkable morphologic changes switching from an epithelial cobblestone phenotype to an elongated fibroblastic phenotype. The EMT provides for the evolution of cancer cells to the metastatic phenotype and contributes to their invasiveness, stemness and drug resistance. In a recent study, the IFN-α-inducible protein-27 was associated with the EMT marker vimentin in ovarian cancer (panel 6, Fig. 2). This phenomenon finally led to chemoresistant cells with a cancer stem cell (CSC) phenotype. CSCs are defined as the reservoir of a chemoresistant niche within the tumor and the driving force for tumor relapse. Mounting observations indicate a potential contribution of
Type-I-IFN signaling in the generation and/or maintenance of CSCs (panel 7, Fig. 2). Indeed, IFN-α was reported to affect the migration and invasion of pancreatic ductal adenocarcinoma cells through the upregulation of specific CSC markers such as CD24, CD44 and CD133. In addition, it was recently shown that TLR3 stimulation on somatic cells caused global changes in the expression of epigenetic modifiers leading to enhanced chromatin remodeling, nuclear reprogramming, cell plasticity, pluripotentiality, transdifferentiation and even malignant transformation. In line with these data, experiments in breast cancer cells put in evidence that NF-κB and β-catenin signaling downstream of TLR3 promoted the enrichment of a subset of cells with CSC phenotype. Similarly, in the haematopoietic stem/progenitor cell (HSPC) compartment, chronic Type-I-IFN stimulation resulted in HSPC loss of quiescence and dysfunction. This phenomenon was mainly due to Type-I-IFN-induced accumulation of reactive oxygen species (ROS). Additional indirect proofs of the tumor growth promoting role of Type-I-IFNs come from recent studies showing that, in cancer cells, Type-I-IFNs upregulated the ISG programmed death-ligand (PD-L)174 (panel 8, Fig. 2). PD-L1 is a cell-surface molecule expressed by most tumor cells that mediates inhibitory signals toward CTLs and thus plays a major role in cancer immune-evasion through CTL exhaustion. It is tempting to speculate that sustained therapeutic responses could rely on the combination of Type-I-IFNs or Type-I-IFN-inducing therapies with antibodies targeting the PD1-PD-L1 axis. Accordingly, a recent study from Shen et al. demonstrated that the oncolytic vesicular stomatitis virus engineered to constitutively express IFN-β had significant anti-leukemia activity, which was further enhanced when combined with an anti-PD-L1 antibody. These observations lend further support to the double-edged sword of Type-I-IFNs in controlling tumor growth and promoting tumor escape. Further insights are needed to decipher the mechanisms through which Type-I-IFNs may paradoxically favor tumor progression, which will certainly have a great impact in the clinical use of Type-I-IFNs.

Cancer-extrinsic effects of Type-I-IFNs

In addition to the direct impact on cancer cells, Type-I-IFNs have extrinsic effects on tumors by regulating processes such as angiogenesis and immunity. Type-I-IFNs have been long recognized as powerful angiogenesis inhibitors. The effects of Type-I-IFNs on the vasculature have been mainly attributed to the downregulation of vascular endothelial growth factor (VEGF) expression as well as to the impairment of endothelial cell proliferation and migration (panel 9, Fig. 2). Seminal experimental findings from Schreiber’s group strongly suggest that, although the immune system plays a major part in restraining the development of cancer, it may also promote the emergence of tumors that escape immune control. According to the immune-editing model, malignant cells, initially held in check by immune-surveillance means, can grow into clinically manifest tumors provided that (1) they lose the cancer molecular determinants that make them recognizable by immune-effectors (immune-selection), or (2) they actively counteract immune responses (immune-suppression). Immuno-editing consists of three phases: first, at an early stage, malignant cells are recognized and eradicated by immune-effector cells (elimination); second, at a later stage small tumors are still held in check by increasingly less proficient immune responses (equilibrium); and finally, neoplastic cells lose their antigenic properties or establish potent immune-suppressive networks, thus avoiding any control (escape). Most noteworthy, Dunn et al. proved that Type-I-IFNs intervene in all these three phases. They demonstrated that endogenously produced Type-I-IFNs were required, in immunocompetent mice, to reject highly immunogenic 3’-methylcholanthrene (MCA)-induced sarcomas and to prevent the outgrowth of primary carcinogen-induced tumors. Furthermore, they observed that several MCA-induced sarcomas from Ifnar−/− mice were rejected in a T cell-dependent manner in wild-type mice, which suggests that tumors arising in the absence of Type-I-IFN responsiveness are more immunogenic than tumors growing in IFNAR competent hosts.

The earliest indication that Type-I-IFNs could stimulate extrinsic antitumor effects was reported in a mouse model of lymphocytic leukemia, in which it was shown that survival rates were increased by administering crude (mixed-type) IFN preparations, irrespective of whether tumor cells themselves were intrinsically sensitive to the anti-proliferative actions of these IFN preparations. From then, an impressive number of instrumental studies in both mice and humans unveiled the plethora of mechanisms by which Type-I-IFNs act on immune cells to mount a strong antitumor response. In the early 1990s, Ferrantini and colleagues showed that highly metastatic Friend leukemia cells genetically modified to secrete IFN-α1 exhibited a marked loss of their tumorigenic potential when injected into syngeneic immunocompetent mice, and inhibited the growth of metastatic parental cells in transplantation assays mainly through CD8+ CTLs. Despite these encouraging data, the clinical development of Type-I-IFNs remained underappreciated for many years. In the past two decades, the findings that IFN-α induced the differentiation/activation of DCs (panel 10, Fig. 2) in both mice and humans have spurred the idea of new immunotherapeutic regimens. Today, new attention is given to Type-I-IFNs as crucial factors bridging innate and adaptive immunity. Several studies support the importance of Type-I-IFNs as a stimulus for the production of various cytokines (e.g., TNF, IL-1, IL-6, IL-8, IL-12 and IL-18) by macrophages (panel 11, Fig. 2), and as factors that markedly affect DC-mediated TAA retention and cross-priming (panel 12, Fig. 2) and stimulate antibody-dependent cellular cytotoxicity on established B16 murine melanoma liver micrometastases. Furthermore, Type-I-IFNs were reported to play a major role in the development and differentiation of the Th1 subset, as well as in the generation, activity, expansion and long-term survival of CTLs (panel 13, Fig. 2). Type-I-IFNs are also responsible for the activation of tumoricidal NK cells (panel 14, Fig. 2), which represent one of the host key mechanisms to preempt tumor growth. More recently, the role of Type-I-IFNs in the immunometabolism - which is an emerging field investigating the interplay between immunological and metabolic processes - gained increasing appreciation (panel 15, Fig. 2). A substantial number of evidence indicates that signaling downstream of PRRs induces changes in core metabolism of DCs and macrophages, which are crucial in shaping their function and fate. In macrophages, Type-I-IFNs downstream of TLR3...
induced a shift in the balance of lipid metabolism away from de novo cholesterol and fatty-acid synthesis in favor of the uptake of exogenous lipids. This immunometabolic circuit is critical for host immune responses. In line with this discovery, TLR9 stimulation in pDCs led to an autocrine IFNAR signaling resulting in an increased fatty-acids oxidation and oxidative phosphorylation, which is key for pDC immune functions. In addition, fasting or the administration of caloric restriction mimetics has been shown to improve the efficacy of immunogenic chemotherapy correlating with the depletion of immunosuppressive regulatory T (Treg) cells from the TME. Notably, Type-I-IFNs are known to negatively regulate the proliferation and activity of Treg cells (panel 16, Fig. 2) and other immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs; panel 17, Fig. 2). Undoubtedly, understanding the multilevel interactions between metabolic, immunologic and Type-I-IFN nets will offer additional tools to manage beneficial and detrimental Type-I-IFN immune effects and reshape the way Type-I-IFN-IFNAR axis can be exploited therapeutically during infection and cancer.

The role of Type-I-IFNs in anticancer therapy

Although soon after their discovery the antiviral activity of Type-I-IFNs attracted widest interest, the first US Food and Drug Administration (FDA) approval for IFN-α2, in 1986, was for cancer treatment (Fig. 3). Even before recombinant IFNs were available, reduction of disease morbidities with partially purified IFN-α was reported in several studies performed in patients with hairy-cell leukemia and chronic myelogenous leukemia. In both cases, however, over time more effective therapeutic regimens than IFN have been devised (e.g., the targeted inhibitor of the activated BCR-ABL tyrosine kinase Imatinib). In following clinical studies, the therapeutic effectiveness of IFN-α2 either as unmodified recombinant proteins or pegylated variants in inducing at least partial disease regression was reported for other hematological and solid tumors including myelomas, lymphomas, melanomas, Kaposi’s sarcoma and renal-cell and bladder carcinoma. To date, IFN-α2 is still commonly used combined with IL-2 in immunotherapeutic regimens for metastatic renal-cell carcinomas and cutaneous melanoma. In addition, more than 100 clinical trials are currently underway worldwide using IFN-α2 as monotherapy or in combination regimens for both hematological and solid malignancies (for further details, please refer to https://clinicaltrials.gov/ and ref. 101).

A wide range of conventional chemotherapy, radiotherapy and immunotherapy, including oncolytic virotherapy, currently licensed for use in humans are particularly successful if they induce tumor-targeting immune responses. The current view is that therapeutic agents must induce a sort of “viral mimicry”, i.e., a combination of stress signals that are usually linked to viral infection, such as Type-I-IFNs, and are believed to contribute to their clinical effectiveness. We recently showed that Type-I-IFNs lie at the nexus that controls immunogenic cell death (ICD) and constitutes a hallmark of successful chemotherapy. In particular, we showed that the treatment of various tumor types (e.g., MCA205-fibrosarcomas and AT3-breast carcinoma) with anthracyclines or oxaliplatin gave rise to the rapid production of Type-I-IFNs, thus mimicking the immune reactions evoked by viruses. We also elucidated the mechanism of Type-I-IFN-mediated ICD demonstrating that (1) hit dying cancer cells emit self-nucleic-acids (especially single-stranded RNAs) in the TME, which are sensed by TLR3 on surrounding yet viable cells and (2) released Type-I-IFNs act as the primum movens for the sequential events bridging innate and cognate antiviral immunity through a specific ISG signature that includes soluble chemotactic mediators such as the C-X-C motif chemokine ligand (CXCL)10. This is crucial for the recruitment, selection and differentiation/maturation of engulfing cells thus dictating the immunogenic outcome of cell death. Corroborating this evidence, the efficacy of anthracyclines was lost upon co-administration of anti-IFNAR or anti-IFN-α/β neutralizing antibodies. Importantly, in breast cancer patients, increased expression levels of the ISG MX dynamin-like GTPase (MX)1 predicted the likelihood of response to anthracycline-based treatment in neoadjuvant and adjuvant settings. In previous studies, Type-I-IFNs were described as crucial mediators of the off-target immunomodulatory effects of cyclophosphamide, an alkylating agent inducing ICD responsible for the expansion of memory CD4+ and CD8+ T cells as well as of DCs. In patients with hematological malignancies, the administration of high-dose cyclophosphamide induced a rapid, transient and broad transcriptional modulation on peripheral blood mononuclear cells resulting in DNA damage, cell death and, noticeably, a Type-I-IFN signature. This promoted the establishment of a systemic sterile inflammatory response characterized by the release of endogenous adjuvant signals able to enhance the efficacy of immunotherapy. Similar to chemotherapy, radiation therapy was also reported to increase the levels of Type-I-IFNs and CXCL10 in the TME. In this study, CXCL10 was shown to promote tumor CD8+ T cell-homing and cytolytic activity. Subsequent observations revealed that radiation-mediated antitumor immunity in immunogenic tumors requires a functional cytosolic DNA-sensing pathway upstream of Type-I-IFNs. Accordingly, Hartlova and colleagues recently found that in the absence of ataxia-telangiectasia mutated (ATM, which is an apical component of the DNA damage response), the accumulation of DNA lesions generated spontaneously or provoked by irradiation induced Type-I-IFNs via STING-mediated signaling. Type-I-IFNs in turn primed the innate immune system for a rapid and amplified response to microbial and environmental threats. In addition, Type-I-IFNs boosted the antineoplastic activity of antibodies specific for oncogenic receptors, such as epidermal growth factor receptor (EGFR) and human EGFR (HER)2, mobilizing DCs to cross-present TAAs to CTLs. However, despite these observations strongly support the antitumor and immune-stimulatory effects of Type-I-IFNs, paradoxical proofs of a dichotomous, detrimental tumor growth-promoting role for these cytokines are also reported. In this context, some harmful effects seem to depend on the ability to induce immune-checkpoint pathways as a major mechanism of immune resistance, particularly against CTLs.
| Year | Event | Details |
|------|-------|---------|
| 1950 | Discovery of Type-I IFN antiviral activity in mice | PMID: 14156587 |
| 1960 | Production and purification of Type-I IFNs by human leukocytes | PMID: 4290664 |
| 1970 | Identification of two distinct antigenic species of human Type-I IFNs: fibroblast (IFN-β) and leukocyte (IFN-α) IFNs | PMID: 49055 |
| 1980 | Isolation, cloning and sequencing of human Type-I IFNs: IFN-α1, IFN-α2, IFN-β (PMID: 6150984; PMID: 6159625) | |
| 1990 | Characterization of the transcriptional activator ISGF3 | PMID: 2236065 |
| 2000 | FDA approval of recombinant IFN-α2b for chronic granulomatous disease | PMID: 11807032 |
| 2010 | Introduction of highly glycosylated human IFN-α as a new therapeutic candidate | PMID: 20067809 |
| Now | Use of recombinant IFN-α2b to potentiate the antitumor effects of epigenetic drugs in colorectal cancer | PMID: 27028869 |

Figure 3. Timeline of IFN discovery and clinical use. The discovery of IFNs evolved from studies of viral interference beginning in 1950. Since then, great attention has been devoted to the molecular understanding and clinical use of IFNs for virus-related and -unrelated malignancies. DC: dendritic cell; FDA: Food and Drug Administration; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; ICD: immunogenic cell death; IFNs: interferons; IFNAR: IFN-α/β receptor; IFNGR: IFN-γ receptor; IPS-1, IFN-beta promoter stimulator-1; IRF: IFN regulatory factor; ISG: IFN-stimulated gene; ISGF3: IFN-stimulated gene factor 3; JAK: Janus kinase; MDA5: melanoma differentiation-associated protein 5; RIG-I: retinoic acid-inducible gene-I; SCID: severe combined immunodeficiency; STAT: signal transducer and activator of transcription; TLR3: Toll-like receptor 3.
specific for TAAs. As reported above, Type-I-IFNs upregulate PD-L1 in tumor cells, which can lead to T cell exhaustion. It remains a central goal of studies on tumor immunity to elucidate the multitude of molecular nets activated by Type-I-IFNs. Big issues to solve are when and through which pathways Type-I-IFNs counteract or promote tumor growth. These insights will likely pave the way to more effective IFN-based immunotherapies.

Conclusions and perspectives

Type-I-IFNs are among the most pleiotropic cytokines and are produced and sensed by almost every cell type in the body. The discovery of Type-I-IFN role in cancer immune-surveillance at first, and cancer immune-editing later, made these cytokines and the immune sensing networks that drive their production very attractive for deeper investigation in preclinical and clinical contexts. As cancer-related genomic information is constantly published, it is emerging that Type-I-IFNs can be produced by, and act on, both malignant and immune cells, thus eliciting immune responses via tumor cell-intrinsic or -extrinsic means. Type-I-IFNs, either naturally produced, exogenously administered or induced by chemotherapy, radiotherapy or oncolytic virotherapy exert all biologic effects through the action of ISGs. Therefore, efforts to decipher the specific functions of individual ISGs on the reciprocal crosstalk between cancer cells and immune cells may likely help fulfill IFN therapeutic efficacy and identify predictive biomarkers of response. Taken into account the dual role of Type-I-IFNs in containing and favoring tumor growth, it will be important to understand which subtype of, at which time point and through which pathways Type-I-IFNs cease to be immune effectors and flip to become immune suppressors and CSC promoters. The limited efficacy of Type-I-IFNs in cancer medicine may likely reflect this gap of knowledge.

Matter-of-factly, Type-I-IFNs have more than reached the potential envisioned by early discovering virologists, however answering these questions will certainly have a tremendous impact on tumor immunology and biomedicine.

Disclosure of potential conflicts of interest

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