Interferon-α and its effects on cancer cell apoptosis (Review)

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Abstract. Interferon (IFN)-α is a cytokine that exhibits a wide range of biological activities and is used in various cancer treatments. It regulates numerous genes that serve roles in antiviral, antiproliferative and proapoptotic activities. For decades, one of the main aspects of clinical oncology has been the development of anticancer therapeutics that promote the effective elimination of cancer cells via apoptosis. However, the updated available information concerning IFN-α-induced cancer cell apoptosis needs to be assembled, so as to provide an improved theoretical reference for the basic scientific research and clinical treatment of malignant tumors. Therefore, the present review focuses on the potential effects of IFN-α in inducing cancer cell apoptosis. The biological characteristics of IFN-α, the apoptotic signaling pathways and molecular mechanisms of apoptosis caused by IFN-α are discussed in different types of cancer cells. The present review provided a comprehensive understanding of the effects of IFN-α on cancer cell apoptosis, which will aid in developing more efficient strategies to effectively control the progression of certain cancers.

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1. Introduction

Interferons (IFNs) are produced by the innate immune system via Toll-like receptor (TLR) stimulation and other signaling cascades (1). According to the primary structure of IFNs and their impact on three dimeric target receptors, IFNs can be classified into several types and families. There are three main classes of IFNs in humans: IFN-α, -β and -γ. Among them, IFN-α and -β belong to the type I IFNs. IFN-α is primarily secreted by monocytes/macrophages and can also be synthesized by B cells and fibroblasts, whereas IFN-β is mainly produced by fibroblasts. IFN-α and -β bind to the same receptor and are widely distributed, including on monocytes/macrophages, B cells, T cells, platelets, epithelial cells, endothelial cells and cancer cells. Human IFN-α subtypes share ~50% sequence identity and IFN-α2 is ~20% identical to IFN-β. IFN-α and -β have 186-190 amino acids (aa) and have a cleavable signal peptide, which forms a secreted protein of 165 or 166 aa (2,3).

IFN-α not only serves a vital role in modulating the immune system and inducing antiviral innate immune responses, but it also serves an important role in antitumor therapy (4-6). Numerous mechanisms have been proposed for the anticancer effect of IFN-α, including the induction of cell apoptosis, which is initiated via the extrinsic signaling pathway, the intrinsic mitochondrial signaling pathway or the stress kinase signaling pathway (7). Due to its antitumor properties, IFN-α has been widely used for the clinical treatment of malignancies, such as renal cell cancer (RCC), hepatocellular carcinoma (HCC), malignant melanoma and cervical cancer (8-10). For a long time, most of the reviews on type I IFNs mainly focused on IFN-β, and less attention was paid to IFN-α (11-15). Therefore, in the present review, a brief overview of the proapoptotic effects of IFN-α in various cancers will be provided and the existing literature on the signaling pathways and molecular mechanisms of IFN-α-induced cancer cell apoptosis will be explored so as to supplement and improve IFN-α-related reviews.

2. Biological characteristics and subtypes of IFN-α

IFN-α exhibits a wide variety of direct and/or indirect biological properties, including antiproliferative and antiviral properties, stimulating the cytotoxic activity of different host-immune cells, inducing proapoptotic genes/proteins, upregulating major histocompatibility complex class I antigens and tumor-associated surface antigens, suppressing

antiapoptotic genes, inhibiting angiogenesis and modulating cell differentiation (9,10,16-19). It can therefore be hypothesized that IFN-α is an important agent for treating various infectious diseases.

In total, there are 13 IFN-α subtypes expressed from 14 human IFN-α genes. The IFN-α subtype generated from the IFN-α13 gene is identical to that generated from the IFN-α1 gene. Therefore, there are 12 different IFN-α subtypes in humans (20-22). All IFN-α subtypes have a high structural similarity, including the length of the protein and the absence of introns. Out of the 12 IFN-α subtypes 11 are 166 aa in length with a molecular weight of ~20 kDa (IFN-α2 is 165 aa due to the deletion of D44). Their protein sequence is highly conserved and the identity score among the IFN-α subtypes ranges from 76-96% (23,24). Each IFN-α subtype exhibits different activities, which include antiproliferative and antiviral activities, as well as promoting the cytotoxic activities of T cells and natural killer (NK) cells (25). For example, most subtypes of IFN-α (IFN-αA, B, C, D, F, I and K) are capable of boosting NK cells. However, IFN-αJ exhibits virtually no NK cell activity but has potent antiviral and antiproliferative activities, which suggests that it has an antagonist effect on NK cell activity via inhibiting other IFN-α subtypes to stimulate NK cells.

3. IFN-α signaling and its regulation

Similar to other type I IFNs, IFN-α exerts biological effects by binding to a specific receptor known as the IFN-α/β receptor subunit (IFNAR)1/IFNAR2 heterodimer on the surface of target cells (23,26). Upon binding, the downstream molecules Janus kinase (JAK)1 and tyrosine kinase 2 are activated, which results in the recruitment of STAT1 and STAT2 to the cytoplasmic tail of the receptor and therefore forms STAT1/STAT2 heterodimers that can translocate into the nucleus. Subsequently, the heterotrimeric transcriptional complex IFN-stimulated gene (ISG) factor 3 (ISGF3) is formed by STAT1/STAT2 heterodimers combining with IFN-regulatory factor (IRF)9. Upon binding to specific DNA response elements, ISGs can be transactivated by ISGF3 (Fig. 1). IFN-α also stabilizes other STAT homodimers or heterodimers, including the CRK-like proto-oncogene adaptor protein/STAT5 heterodimer and NF-κB. Moreover, IFN-α signaling can activate the PI3K signaling pathway. IFN-α can also activate VAV guanine nucleotide exchange factor 1, which elicits a broad response involving numerous transcription factors, such as tumor protein p53, MYC, ETS transcription factor ELK1 and the STAT1/STAT2 heterodimer (1,27,28).

A number of compounds can affect the expression or signaling of IFN-α, which therefore impacts its underlying biological functions. RO8191 (CDM-3008), an orally administrable low-molecular weight compound, is a potent IFN receptor agonist. It mimics IFN-α via the direct binding of IFNAR2, which activates ISG expression and JAK/STAT phosphorylation (29,30). Small ubiquitin-related modifier (SUMO)ylation has been reported to suppress type I IFN (IFN-α and -β) responses. However, TAK-981, a selective small-molecule inhibitor of SUMOylation, pharmacologically reactivates IFN-α and -β signaling. It was previously demonstrated that *in vivo* treatment of wild-type mice with

TAK-981 upregulates the gene expression of IFN-α and -β in blood cells and splenocytes (31). Tilorone dihydrochloride is the first synthetic, orally active, low-molecular weight compound that can significantly induce IFN-α *in vivo* within 24 h of administration (32,33). It was previously reported that in patients with Sézary syndrome, TLR7/8 agonists induce inflammatory cytokines. In this study IFN-α, -β and -γ and a TLR9 agonist efficiently induced IFN-α and IFN-β, even though this positive association was not demonstrated for other cytokines (34). Oligo-deoxy-nucleotides with a CpG motif and double/multi-stranded structure-forming sequences, function as TLR9 agonists and increase the expression of IFN-α (35). The small-molecule STAT3 inhibitor FLLL32 is hypothesized to selectively bind to JAK2 and the STAT3 Src homology 2 domain, which serve vital roles in STAT3 dimerization and the signaling pathway. FLLL32 can downregulate STAT3 phosphorylation via interactions with IL-6 and IFN-α (36,37). IRF3 can regulate bacterial and viral innate immune responses via the modulation of the secretion of type I IFNs. Thymoquinone, a black cumin-derived compound, suppresses the IRF3-mediated expression of IFN-α and -β by suppressing TANK-binding kinase 1 (38). Moreover, abnormal IFN-α signaling is associated with numerous immune diseases, such as chronic infection, inflammation and autoimmune disease (39). Therefore, the integrated modulation of the IFN-α response is important to maintain a balance between IFN-α-mediated protective effects and cell toxicity due to dysregulated IFN-α signaling.

4. Apoptosis

Apoptosis, a type of programmed cell death, is of great significance for cell development and maintaining tissue homeostasis. It is a complex and signal-regulated process involving the participation of numerous molecules (40). Apoptotic events are mainly performed by the caspase protease family (cysteine-aspartic-specific proteases). According to their functions, the caspases can be categorized into three groups: i) Apoptotic executioner caspases; ii) apoptotic initiator caspases; and iii) inflammatory caspases (41). Caspase-1, -4, -5, -11, -12, -13 and -14 belong to the inflammatory caspases, which are associated with inflammation. The apoptotic initiator caspases are important for interactions with upstream adaptor molecules. All apoptotic initiator caspases possess long pro-domains, which contain caspase activation and recruitment domains (caspase-2 and -9) or death effector domains (caspase-8 and -10) (42). The apoptotic executioner caspases (caspase-3, -6 and -7) are typically processed and activated via upstream caspases and perform apoptosis by cleaving cellular components. Once the apoptotic signaling pathways are activated a caspase cascade will occur (43). It is currently considered that at least three signaling pathways are related to the occurrence of apoptosis: i) The intrinsic (mitochondrial) signaling pathway; ii) the extrinsic (death receptor) signaling pathway; and iii) the endoplasmic reticulum (ER) stress-related signaling pathway, of which the first two are recognized as the main apoptotic signaling pathways in most cells (44).

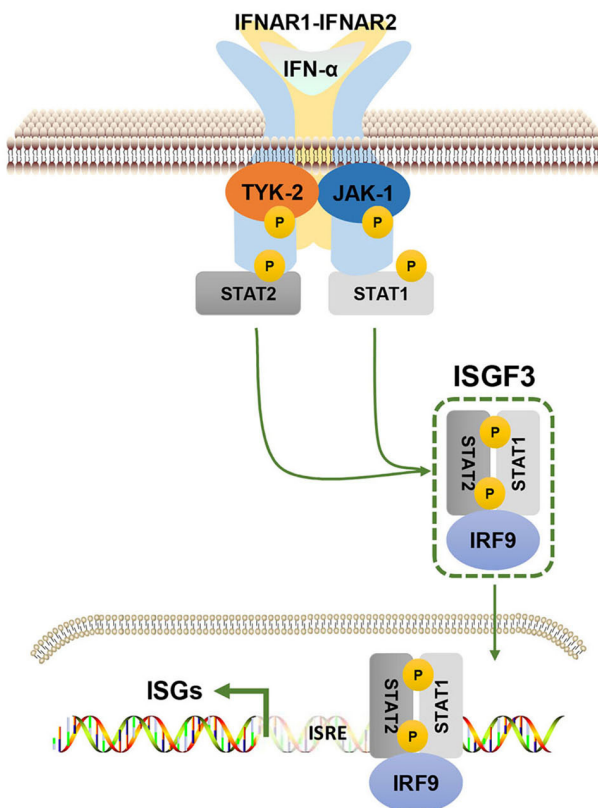*Extrinsic (death receptor) signaling pathway*. The extrinsic signaling pathway is associated with the ligation of the

Figure 1. Canonical IFN-α signaling pathway. IFN-α binds to IFNAR1/IFNAR2 and subsequently activates JAK-1 and TYK2, which are two members of the JAK family. This subsequently leads to the phosphorylation of STAT1 and STAT2. The pSTAT1/pSTAT2 heterodimeric complex combines with IRF9 to form an ISGF3 complex. ISGF3 binds to the homologous DNA sequence of ISRE to directly activate the transcription of ISGs. IFN-α, interferon-α; IFNAR, IFN-α/β receptor subunit; JAK, Janus kinase; TYK2, tyrosine kinase 2; p, phosphorylated; IRF9, IFN regulatory factor 9; ISG, IFN-stimulated gene; ISGF3, ISG factor 3; ISRE, IFN-sensitive response element.

tumor necrosis factor (TNF) receptor (TNFR) superfamily (TNFRSF), including TNFRSF1a, TNFRSF21, TNFRSF25, TNFRSF10a/b and TNFRSF6. This signaling pathway includes receptor oligomerization and the recruitment of death domain (DD)-containing adaptor proteins to the aggregated receptor domains via DD/DD interactions. These adaptor proteins include a death-effector domain module, which recruits procaspase-8 and -10 to induce a death-inducing signaling complex that regulates oligomerization and consequently activates caspase-8 and -10. The activated caspase-8 and -10 cleave additional downstream caspases, such as caspase-3, -6 and -7, which triggers the morphological hallmarks of apoptosis, such as apoptotic body formation, DNA fragmentation, cytoplasmic condensation and cytoskeletal collapse (45-47).

Intrinsic (mitochondrial) signaling pathway. The intrinsic apoptotic signaling pathway is triggered in response to stress stimuli such as heat, γ-irradiation, UV radiation, growth-factor deprivation, viral virulence factors, certain oncogenic factors and DNA-damaging agents (48). These stressors are driven by different intracellular components that relay signals to mitochondria, which result in a change in the mitochondrial membrane permeability (MMP) that is primarily modulated

by Bcl-2. The MMP promotes the secretion of cytochrome *c* from the mitochondria, which subsequently interacts with apoptotic protease-activating factor-1 (Apaf-1) and induces nucleotide exchange activity. This therefore results in the formation of the homo-heptameric Apaf-1 complex, namely the apoptosome. Procaspase-9 is cleaved and activated by the apoptosome. The apoptosome complex and caspase-9 can also form the holoenzyme, which activates the downstream effectors caspase-3 and -7 (49-52).

ER stress-related signaling pathway. The ER is important for protein modification, folding and synthesis and it is also the main reservoir of Ca^{2+}. An increase in unfolded proteins or a calcium imbalance leads to ER stress and the unfolded protein response (UPR) to maintain normal cellular function. However, the prolonged activation of the UPR may initiate apoptosis if ER protein homeostasis is not restored (41). Intracellularly the ER is the main store of Ca^{2+} ions. Stress-induced apoptosis involves the release of Ca^{2+} from the ER into the cytosol. Moreover, ER stress specifically activates mouse caspase-12 (that is equal to human caspase-4). The activated caspase-12 translocates from the ER into the cytosol and subsequently cleaves procaspase-9, which results in caspase-3 activation (53,54). Furthermore, the C/EBP homologous protein is also responsible for ER stress-induced apoptosis (55,56).

5. Mechanisms of IFN-α-induced cancer cell apoptosis

Regardless of tissue histology or cell type, apoptosis can be induced by almost all IFN subtypes, including IFN-α. Furthermore, IFN-α is involved in Fas-associated via death domain (FADD)/caspase-8 signaling, the disruption of the MMP, the release of cytochrome *c* from mitochondria and the activation of the caspase cascade, which suggests that diverse strategies can be applied for cancer treatment (16,57). Possible mechanisms of IFN-α alone or in combination with other drugs to induce apoptosis in different cancer cell types will be discussed in this section (Table I; Fig. 2).

HCC. HCC is a commonly used cancer model to study the mechanism of apoptosis caused by IFN-α. Previous studies have reported the involvement of TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis following IFN-α stimulation in HCC. TRAIL is a type of proapoptotic protein that can activate caspase-8 via interacting with the TRAIL receptor, which consequently initiates apoptosis. Shigeno *et al* (58) demonstrated that IFN-α pretreatment could enhance the TRAIL-induction of Hep3B and HuH-7 cell apoptosis, in which IFN-α increased the expression of TNFRSF10B. However, this study also demonstrated that IFN-α pretreatment also suppresses the TRAIL-regulated activation of NF-κB. In addition to TRAIL, promyelocytic leukemia protein (PML) is also involved in IFN-α-induced HCC apoptosis (59). TRAIL functions as a downstream target of PML and both TRAIL and PML serve essential roles in IFN-α-regulated HCC apoptosis. Compared with IFN-α stimulation alone, IFN-α in conjunction with other compounds can enhance cell apoptosis. For example, IFN-α and celecoxib, a cyclooxygenase-2 inhibitor, synergistically increase TRAIL-induced HCC apoptosis, which suggests that this combination may serve as a new

Table I. Main apoptotic signaling pathways and mechanisms targeted by IFN-α in cancer.

Treatments	Apoptotic pathways	Molecules involved	Types of cancer cells	(Refs.)
IFN-α2a	The extrinsic (death receptor) pathway	TRAIL, DR5, NF-κB, and caspase-8	HuH-7 and Hep3B	(58)
IFN-α	The extrinsic (death receptor) pathway	TRAIL and PML	Hep3B, Huh7, Huh6, HepG2, Chang and CEM	(59)
IFN-α/celecoxib	The extrinsic (death receptor) pathway	TRAIL, DR4, DR5, PARP, caspase-3, and caspase-8	SMMC-7721, HepG2, and HLCZ01	(60)
IFN-α/aspirin	The intrinsic (mitochondrial) pathway	Caspase-3, caspase-9, Bax, JAK1, STAT1, and XAF1	Bel-7402 and MHCC97L	(61)
IFN-α2b	The intrinsic (mitochondrial) pathway	TGF-β1, ROS, JNK, FoxO3a, PUMA, and cholesterol	Preneoplastic rat hepatocytes	(62-64)
IFN-α	The intrinsic (mitochondrial) pathway; the ER stress-related pathway	Caspase-3, Bim, PARP, cytochrome *c*, and caspase-4	HeLa	(67)
IFN-α2a	The intrinsic (mitochondrial) pathway	Bid, Bak, and AIF	OVCAR3	(68)
IFN-α2a/IFN-γ/IL-4-PE	Not mentioned	JAK, STAT1, STAT6, PARP, caspase-3, and caspase-7	OVCAR-5	(69)
IFN-α2b	The extrinsic (death receptor) pathway	ING4, caspase-3, caspase-8, PARP, and Fas/FasL	A375 and HT-144	(53)
IFN-α/bortezomib	The extrinsic (death receptor) pathway	caspase-3, caspase-7, caspase-8, caspase-9, PARP, Fas, and FADD	A375, HT-144, B16F1, JB/MS, 1259 MEL, 18105 MEL, and MEL 39	(70)
IFN-α	The intrinsic (mitochondrial) pathway	Bak, Bim, cytochrome *c*, caspase-2, caspase-3, caspase-8, caspase-9, AIF, JAK1, and mTOR	NCI-H929 and U266	(57,73)
IFN-α/TRAIL	The extrinsic (death receptor) pathway	Caspase-3, caspase-8, PARP, and ERK	A-498, ACHN, and 786-O	(75)
IFN-α/Smac mimetic BV6	The extrinsic (death receptor) pathway	RIP1, FADD, caspase-8, caspase-9, and caspase-3	CaKi1, CaKi2, KTCTL2, KTCTL26, KTCTL30, A498, 769P, and 786O	(76)
IFN-α/Smac mimetic BV6	The extrinsic (death receptor) pathway	TNF-α, TNFR1, and IRF1	MV4-11, OCI-AML3, Molm13, MonoMac6, and NB4	(77)

IFN-α, interferon-α; TRAIL, TNF-related apoptosis-inducing ligand; DR4/5, death receptor 4/5; NF-κB, nuclear factor κB; PML, promyelocytic leukemia; PARP, cleaved poly (ADP-ribose) polymerase; Bax, Bcl 2-associated X; JAK, janus kinase; STAT, signal transducer and activator of transcription; XAF1, XIAP-associated factor 1; TGF-β1, transforming growth factor-β1; FoxO3a, forkhead box O transcription factor 3a; PUMA, p53 upregulated modulator of apoptosis; Bim, Bcl-2-like protein 11; Bid, BH3 interacting-domain death agonist; Bak, Bcl-2 homologous antagonist/killer; AIF, apoptosis-inducing factor; IFN-γ, interferon γ; IL-4-PE, IL-4 fused to *Pseudomonas* exotoxin; ING4, inhibitor of growth family member 4; FasL, Fas ligand; FADD, Fas-associated death domain protein; mTOR, mammalian target of rapamycin; ERK, extracellular signal-regulated kinase; RIP1, receptor-interacting protein 1; TNF-α, tumor necrosis factor-α; TNFR1, TNF receptor 1; IRF1, interferon regulatory factor 1.

therapeutic option for TRAIL-resistant cancer (60). Moreover, STAT1 can regulate the proapoptotic effect of IFN-α. Aspirin may increase the antitumor efficacy of IFN-α on hepatoma cells via activating the JAK1/STAT1 signaling pathway, which improves IFN-α gene and protein therapy (61).

Furthermore, in addition to promoting apoptosis in multiple types of HCC cells, IFN-α can also cause apoptotic events in rats in early-stage hepatocarcinogenesis. In a model described in a previous study, it was demonstrated that IFN-α2b initiates the intrinsic apoptotic cascade by inducing hepatocytes to produce reactive oxygen species (ROS) and TGF-β1, which ultimately leads to cell death (62). This previous study also demonstrated that the endogenous production of ROS caused by IFN-α2b-activated JNK in rat preneoplastic liver was
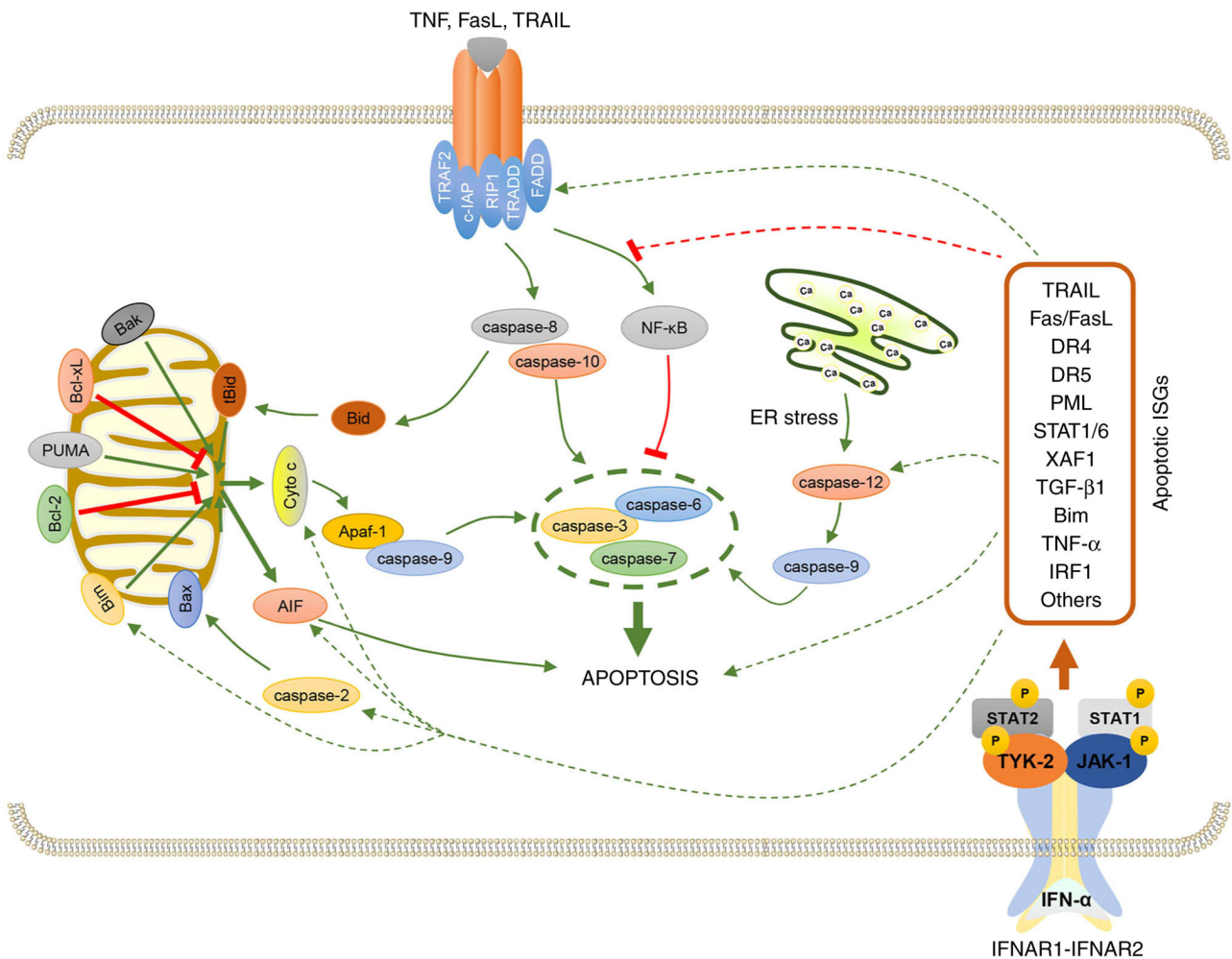
Figure 2. Mechanism of IFN-α-induced apoptosis. The apoptotic effects of IFN-α result from the induction of ISGs. For example, IFN-α upregulates TRAIL, FasL and TNF-α, which bind to the corresponding receptors and activate caspase-8 and -10. The activated caspase-8 and -10 subsequently activate caspase-3, -6 and -7, which results in cell apoptosis. IFN-α also induces other proapoptotic proteins such as PML, STAT1, STAT6, XAF1, TGF-β1, Bim and IRF1, which regulate apoptosis. These underlying regulatory mechanisms are demonstrated in the figure by the green and red dotted arrows. IFN-α, interferon-α; ISGs, IFN-stimulated genes; TRAIL, TNF-related apoptosis-inducing ligand; FasL, Fas ligand; TNF, tumor necrosis factor; PML, promyelocytic leukemia; XAF1, XIAP-associated factor 1; Bim, Bcl-2-like protein 11; IRF1, interferon regulatory factor 1.

responsible for the transcriptional activity and nuclear translocation of FoxO3a. FoxO3a positively modulates the expression of proapoptotic Bcl-2 protein family members, such as p53 upregulated modulator of apoptosis (PUMA), which triggers the mitochondrial apoptotic signaling pathway (63). There is also a correlation between IFN-induced apoptosis and lipid metabolism (64). Treatment with IFN-α2b, decreases the synthesis of liver cholesterol and increases its secretion, which is required for IFN-α2b to promote cell apoptosis. These aforementioned data have demonstrated the complicated role of IFN-α2b in the early development of HCC.

Cervical and ovarian cancers. Cervical and ovarian cancers are the two most common types of female malignant tumors, which severely affect the mental and physical health of women. In the last decade, antitumor research based on IFN-induced apoptosis of these two types of cancer has been ongoing (65,66). IFN-α promotes HeLa cell apoptosis via the activation of both ER stress-induced and intrinsic mitochondrial signaling pathways (67). The activation of caspase-3, the secretion of cytochrome *c* from mitochondria, the downregulation of

Bcl-extra-large (Bcl-xL) and the upregulation of Bcl-2-like protein 11 (Bim) and cleaved poly(ADP-ribose) polymerase (PARP) are observed following IFN-α treatment, which suggests that the intrinsic apoptotic signaling pathway is activated. Furthermore, caspase-4, which is responsible for ER stress-induced apoptosis, is activated following treatment with IFN-α. In ovarian cancer OVCAR3 cells, IFN-α2a-induced apoptosis is regulated by apoptosis-inducing factor (AIF) signaling. IFN-α2a treatment results in the cleavage of BH3 interacting domain death agonist that activates mitochondrial Bcl-2 homologous antagonist/killer to impair the integrity of the mitochondrial membrane, which leads to AIF secretion. AIF induces nuclear fragmentation and cell apoptosis after being translocated from the mitochondria to the nucleus, which indicates a novel mitochondria-associated apoptotic signaling pathway (68). In a previous study, the combination of IL-4-*Pseudomonas* exotoxin, IFN-γ and IFN-α resulted in increased apoptotic cell death in ovarian cancer. This mechanism of the synergistic anticancer effect is dependent on IFN-mediated JAK/STAT signaling and the consequent activation of apoptosis-related molecules, including caspase-3, -7

and PARP (69). These aforementioned studies have provided a theoretical basis for the immunotherapy of cervical cancer and ovarian cancer based on IFN-α.

Melanoma. Melanoma is the most severe type of skin cancer and is resistant to existing therapies. The combination of IFN-α and other drugs has been proven to significantly enhance cell apoptosis in melanoma. Cai *et al* (70) reported that inhibitor of growth family member 4 (ING4) overexpression potentially improves the effects of IFN-α2b and induces melanoma cell apoptosis. This study also demonstrated that ING4 overexpression reduces the expression levels of caspase-3, -8 and PARP and increases the expression levels of cleaved caspase-3, -8, cleaved PARP and Fas/Fas ligand (FasL), which indicates the involvement of the Fas/FasL-mediated death receptor apoptotic signaling pathway. Similar, the combination of bortezomib and IFN-α leads to enhanced apoptotic cell death in melanoma cell lines (71). Moreover, decreased levels of the apoptosis-antagonizing proteins myeloid leukemia-1 and Bcl-2 are detected following treatment with IFN-α and bortezomib, which suggests that the intrinsic apoptotic signaling pathway is promoted via the modulation of protein targets in the mitochondria. However, bortezomib in combination with IFN-α stimulates the extrinsic signaling pathway of apoptosis via the activation of FADD-induced caspase-8. Therefore, a combination of IFN-α and other drugs may be effective against apoptosis in melanoma cells.

Multiple myeloma. IFN-α has been used in the treatment of several hematological neoplasia, including multiple myeloma (72). In human myeloma H929 and U266 cell lines, apoptosis induced by IFN-α results in phosphatidylserine exposure, MMP loss, Bak conformational change, Bim upregulation, reduced levels of cytochrome *c* release from the mitochondria and a low rate of caspase activation, as well as AIF release. Moreover, PUMA levels increase following IFN-α treatment, whereas PUMA knockdown has no effect on IFN-α-induced apoptosis, which suggests that PUMA is not required for IFN-α triggered apoptosis. Furthermore, IFN-α-induced apoptosis is completely inhibited by JAK1, whereas rapamycin, an mTOR inhibitor, mitigates apoptosis in U266 cells but potentiates it in H929 cells. The potentiating action of rapamycin on H929 cell apoptosis is related to the upregulation of Bim levels induced by IFN-α (73). A previous study reported that IFN-α-induced U266 cell apoptosis is related to the activation of caspase-2, -3, -8 and -9. The activation of caspase-3 relies on the activities of caspases-8 and -9 and caspase-8 lies upstream of IFN-α-related caspase cascades. The interaction between the Fas-receptor and its ligand is independent of IFN-α-induced apoptosis (57). These data have demonstrated that IFN-α induces apoptosis in myeloma cells via the activation of the mitochondrial pathway and that inhibitors of mTOR or JAK1 may facilitate IFN-α maintenance therapy in patients with multiple myeloma.

RCC. RCC is the third most common urological cancer and has a poor prognosis. Researchers have long been committed to the study of treatment strategies against RCC, including the application of IFNs (74). Although IFN-α can directly promote apoptosis in various cancer cell lines, as demonstrated in the aforementioned sections, it is currently used to treat RCC mainly in combination with other antineoplastic agents. Clark *et al* (75) demonstrated that TRAIL and IFN-α act synergistically to induce RCC cell death. IFN-α on its own does not cause RCC cell apoptosis as there is no effect on the expression of TRAIL or death receptors and other known mediators of the intrinsic and extrinsic apoptotic signaling cascades, including caspase-3, -8, PARP and Bcl-2 family proteins. However, the extracellular signal-regulated kinases (ERKs) are prominently activated upon IFN-α treatment alone or in combination with TRAIL. The apoptotic synergy between TRAIL and IFN-α is due at least in part to the activation of ERK mediated by IFN-α.

IFN-α together with BV6, which antagonizes inhibitor of apoptosis proteins, displays cooperative antitumor activity in different cancer cell lines. In RCC cells, BV6/IFN-α have a significant antitumor effect, including in reducing cell viability and inducing apoptosis (76). Molecular studies have reported that the scaffold function of receptor-interacting protein 1 (RIP1) is important for BV6/IFN-α-induced apoptosis. BV6 and IFN-α work together to induce caspase activation by forming a cytosolic cell death complex (caspase-8, FADD and RIP1). The synergistic effect of IFN-α and BV6 in acute myeloid leukemia cell death has also been identified (77). BV6 and IFN-α cooperate to enhance the expression of TNF-α. As they are secreted into the supernatant they initiate a TNFR1 loop that triggers cell apoptosis. IFN-α/BV6-induced cell apoptosis is also dependent on IRF1. This combination approach of IFN-α and BV6 may serve as a potential strategy to induce apoptosis in cancer cells.

The activation of effector caspases can be achieved by the convergence of the extrinsic and intrinsic apoptotic signaling pathways. Crosstalk between these two signaling pathways has previously been reported. For example, caspase-3, -6 and -7 are involved in the execution phase of apoptosis via both the intrinsic and extrinsic signaling pathways (41). In the intrinsic pathway, caspase-3 and -7 are proteolytically activated by caspase-6. Subsequently, caspase-8 is cleaved or translocated into the nucleus to cleave its target substrates, which results in cell death. Therefore, caspase-8 cleavage and apoptosis are markedly attenuated via the inhibition of caspase-6 activity in cells, which indicates that caspase-8 is mainly activated by caspase-6 *in vivo* (78-80). The association between these two signaling pathways demonstrates that stress-inducers or chemotherapeutic agents may sensitize cells to death ligand-induced apoptosis. This information is important to determine the proapoptotic and antitumor mechanisms of IFN-α.

6. Conclusions and future prospects

The aim of the present review was to assess the scientific advances made concerning IFN-α-induced cancer cell apoptosis. In most cases, IFN-α needs to be used in combination with other drugs or molecules in order to have an improved antitumor effect. This information will provide a focus area for future research into the clinical application of IFN-α in cancer treatment.

Over the past decade, numerous clinical trials involving IFN-α have been implemented worldwide for use in different

types of cancer (81-85). However, the mechanisms of IFN-α antitumor activity do not only include the proapoptotic effects mentioned in the present review, but also consist of various other functions, including antiproliferation, immunological and regulatory effects. These other areas still require further research. Furthermore, it is necessary to clarify the mechanisms of IFN-α toxicity so that IFN-α can be safely used as an antitumor agent either alone or in combination with other anticancer drugs (86,87). In-depth consideration of these aspects may help establish eligibility criteria for cancer therapy.

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Authors' contributions

WS and YW designed the framework and theme of the review. WS retrieved the literature and wrote the first draft. XY and YF participated in writing the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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