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Anti-cancer therapy with TNFα and IFNγ: A comprehensive review

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Abstract

Tumour necrosis factor alpha (TNFα) and interferon gamma (IFNγ) were originally found to be produced by inflammatory cells and play important roles in the immune system and surveillance of tumour growth. By activating distinct signalling pathways of nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), and JAK/STAT, TNFα and IFNγ were reported to effectively trigger cell death and perform powerful anti-cancer effects. In this review, we will discuss the new advancements of TNFα and IFNγ in anti-cancer therapy.

1 | INTRODUCTION

Tumour necrosis factor alpha (TNFα), a member of the TNF superfamily, was reported to be mainly produced by macrophages and capable of replicating the ability of endotoxin in inducing haemorrhagic tumour necrosis.[1] A number of studies demonstrated it as a potent inflammatory cytokine inducing complex immune responses[2] and also performing anti-cancer effects. TNFα was the first cytokine to be employed for cancer treatment. It exerts anti-tumour activity through complex mechanisms of induction of inflammatory and immune responses, tumour cell apoptosis/necrosis and extensive thrombosis and destruction of tumour vasculature.[3] By now, many studies have been conducted to evaluate the anti-cancer efficacy of TNFα in various tumour types and some are even put into clinical trials.

The other modulator interferon gamma (IFNγ), which is a cytokine, belongs to a type II interferon group and plays critical roles in both host defence and immune regulation. Mature forms of natural human or murine IFNγ comprise of glycosylated polypeptides of 143 and 134 amino acids, respectively and homodimerize to form a non-covalently linked 50 kDa protein. The understanding of cell biology and physiology of IFNγ started from the initial description of its anti-viral activities

produced by phytohaemagglutinin-activated human leucocytes.[4] The later discovery that patients with deficiency in IFNγ production or signalling are highly susceptible to rare mycobacterial infections highlighted the importance of IFNγ in preventing infectious diseases.[5] IFNγ is now clearly depicted to exert its effects by binding to distinct high affinity receptors of IFNGR1 and IFNGR2 and subsequently activate a specific signal transduction pathway termed JAK-STAT pathway to regulate transcription of IFNγ-inducible genes mediating specific IFNγ-dependent cellular responses[6] of apoptosis etc. Studies then focused on a critical role of endogenously produced IFNγ in promoting host responses to tumours, which then evoked many interests on its anti-cancer function and clinical application. Despite the overwhelming evidence indicating the anti-tumour activity of IFNγ, there are still some studies revealing its pro-tumourigenic activities based on the cellular, microenvironment and/or molecular context.[7] Therefore, the anti-cancer therapeutic application of IFNγ should be carefully evaluated.

Despite the promising anti-cancer potential of the two cytokines, their clinical application is still hindered by severe toxicity after systemic administration. Many strategies have been investigated to reduce their systemic toxicity.[8] Fusion proteins consisting of a cytokine and a recombinant peptide are regarded as a novel class of "armed" antibodies acting as delivery vehicles and increasing the therapeutic index of pro-inflammatory cytokines.[9] So far, various ligands targeting tumour-associated antigens have been employed to combine with cytokines as fusion proteins, which help for the specific accumulation of cytokines at tumour sites. But the tumour-targeting and therapeutic effects have variable outcomes and should be evaluated from case to case. For example, some pro-inflammatory cytokines as IL-2, IL-12 and TNFα that fused to L19 (specific to spliced EDB domains) or F8 (specific to spliced EDA domains) exhibited impressive anti-cancer activity with selective uptake at the tumour site, while IL-7, IL-17, IL-15 and IL-18 showed limitations either in tumour-targeting or therapy.[10-12] Our review here will mainly discuss the anti-cancer mechanisms of TNFα and IFNγ and their selective delivery systems and potential clinical application in cancer therapy.

2 | THE ANTI-CANCER ACTIVITY OF TNFα

Tumour necrosis factor alpha consists of 3 non-covalently linked TNFα monomers, ~17.5 kDa each, which forms a compact bell-shaped homotrimer.[13,14] The soluble homotrimeric TNFα can be released via proteolytic cleavage by a metalloprotease, the TNFα converting enzyme. TNFα was reported to bind to 2 receptors, TNFR1 and TNFR2, where TNFR1 is constitutively expressed in most tissues and considered as a death receptor, and TNFR2 is mainly expressed in cells of the immune system.[15] Upon TNFα binding, TNFRs form homotrimers which cause conformational changes to the receptors with a series of intracellular events leading to the activation of 3 major signalling cascades, namely the nuclear factor kappa B (NF-κB) pathway, the mitogen-activated protein kinase (MAPK) pathway and the induction of death signalling.[8]

Tumour necrosis factor alpha plays a paradoxical role in cancer biology in which its induction of cancer cell death or survival depends on the cellular context. TNFα was initially isolated from the sera of mice treated with bacterial endotoxin and it was found to be able to replicate the ability of endotoxin in inducing haemorrhagic tumour necrosis. After that, numerous studies were conducted to investigate its clinical applications especially in cancer therapy. It has been discovered that TNFα can lead to massive haemorrhagic necrosis of transplanted tumours.[2] Although TNFα shows potent anti-tumour activity in various animal cancer models, this cytokine unselectively binds not only to tumour cells and endothelial cells, but also to normal cells and blood vessels, to produce non-specific damage to various cell types. This could cause severe toxicity after systemic administration, even at doses far below the therapeutic window. Various phase I and phase II clinical trials were conducted in the 1980s and 1990s for systemic treatment of recombinant human TNFα (rhTNFα), using TNFα either as a single agent or in combination with other cytokines, chemotherapy or radiotherapy. However, the results were disappointing due to significant toxicities and very limited beneficial outcome.[16] The main clinical trials and toxicities associated with systemic administration with TNFα have been reviewed previously.[16] The common dose limiting side effects include hypotension, rigors, phlebitis, thrombocytopenia, leucopenia and hepatotoxicity. Other general symptoms include fever, fatigue, nausea/vomiting, malaise and weakness, headache, chest tightness, low back pain, diarrhoea and shortness of breath.[16-20] For the above reason, the clinical use of TNFα is now confined to isolated limb perfusion (ILP) in combination with melphalan for soft tissue sarcoma and melanoma. Many efforts have been paid to augment the anti-tumour effect of TNFα while to reduce its systematic toxicity, including passive targeting by PEGylation, cell-based therapy, gene therapy with inducible or tissue-specific promoters, shielding or encapsulation of TNFα, antibody-TNFα conjugate, vascular targeting TNFα coupled to tumour-homing peptides and TNFα mutants.[8,21,22] Lately, it is reported that systemic administration of TNF-expressing tumour cells can reduce the growth of both primary tumours and metastatic colonies in immunocompetent mice by homing to tumours, locally releasing TNFα, damaging neovascular endothelia and inducing massive cancer call apoptosis.[23] At the same time, it can minimize the common side effects. However, more pre-clinical and clinical studies are needed to fully assess the safety and efficacy of this approach.

3 | TNFα AND TUMOUR ANGIOGENESIS

As early as the 1990s, TNFα has been reported to exert synergic anti-tumour effects when combined with other chemotherapeutic drugs. Such synergism is mainly based on the alteration of endothelial barrier function, reduction of tumour interstitial pressure and finally improvement of drug delivery to the tumours.[24,25] It has also been proposed that the anti-tumour activity of TNFα depends on indirect mechanisms of selective obstruction and damage of tumour-associated blood vessels and activation of immune responses rather

than having toxic effects directly on tumour cells.[26-29] Studies then found out that isolated limb or hepatic perfusion with high dose of TNFα in combination with melphalan (a chemotherapeutic drug) produced high complete response rates in patients with melanoma or sarcoma of the extremities,[30,31] as well as regression of bulky hepatic cancer confined to the liver.[32] The micro- and macro-vasculature in tumours was observed to be extensively damaged after isolated perfusion to limbs with TNFα in combination with IFNγ and melphalan.[33]

However, as we mentioned above, TNFα showed non-specificity in cancer therapy, which hampered its systemic administration. Therefore, specially homing TNFα to tumour vessels could be a powerful anti-tumour strategy. By in vivo phage ligand capable of homing to tumour vessels and is first explored to fuse with TNFα to effectively homing TNFα to tumours. By coupling TNFα to CNGRC as a compound of NGR-TNF, it can deliver pictogram doses of TNFα into tumours, which indeed successfully hyper-concentrated TNFα at tumours and enhanced the immunotherapeutic properties of TNFα.[14] Studies investigating the structure-activity and receptor-binding of NGR-TNF fusion proteins showed that NGR peptide did not influence and prevent folding, oligomerization, and the interaction between TNFα with TNFα receptors.[14] Studies were then conducted in the in vivo murine tumour models showing that compared to TNFα, low doses of NGR-TNF could greatly inhibit tumour growth and enhance chemotherapeutic efficacy of doxorubicin and melphalan,[34] indicating that the conjugation with NGR did not influence the biological effect of TNFα in vivo. Besides the direct inhibition of tumour growth by NGR-TNF, many efforts were paid to explore its capacity to improve response to chemotherapy by altering tumour vasculature and tumour microenvironment. Since TNFα itself could alter endothelial barrier function and synergistically improve drug concentration in tumours, one study in 2006 aimed at evaluating the biological effects of NGR-TNF on tumour vasculature at low doses in lymphoma-bearing mice.[35] This study demonstrated an increase in vascular permeability after NGR-TNF treatment. However, two hours after NGR-TNF treatment, there was a decrease in tumour hypoxia and an increase in labelling index of the S-phase marker bromodeoxyuridine, which could lead to increased tumour growth. However, after 1 day of treatment, the in vivo tumour growth decreased, implying that other potentially long-lasting effects of NGR-TNF did occur. This study underlines the importance of timing for the combined treatment of NGR-TNF with other therapeutic agents.

By targeting tumour vessels, NGR-TNF was proven to exert synergistic anti-tumour effects with melphalan, doxorubicin, cisplatin, gemcitabine and paclitaxel in RMA lymphoma-bearing mice.[36] Similar to TNFα, a primary mechanism for NGR-TNF to produce synergic effects with chemotherapeutic drugs was related to disassembly of endothelial VE-cadherin-dependent adherence junctions and alteration of endothelial barrier function in tumours, increase of tumour perfusion and reduction of interstitial pressure. Currently, NGR-TNF, either alone or in combination with chemotherapy, has been tested in various clinical studies in cancer patients.[37,38]

Tumours can develop new strategies to impair effector T lymphocyte function[39] and cause hypoxic microenvironment to form new vessels that are disorganized, tortuous and more leaky than the normal ones. NGR-TNF, on other hand, even at low doses, was identified to upregulate endothelial cell adhesion molecules in tumour vessels and enhance the local production of immunomodulating cytokines in tumour-bearing mice, thereby favouring the extravasation of immune cells and improving therapeutic activity of immunotherapy.[40]

In addition to NGR-TNF, other tumour vessel homing derivatives of TNFα, such as fusion protein with ACDCRGDCFCG or CisoDGRC peptides (both ligands of αv integrins)[41] or with the single chain Fv Ab L19,[42] can be exploited to produce synergic effects with chemotherapeutic drugs and enhance immune response in tumours. One example is the RGD peptide which can recognize various αβ integrins heterodimers.[43] Interestingly, the $\alpha v\beta_3$ heterodimer is overexpressed in blood vessels in tumours. Therefore, this receptor could be exploited as a pharmacological target to deliver cytokines to tumour blood vessels.[44,45] Subnanogram doses of RGD-TNFα prepared by recombinant DNA technology were sufficient to enhance anti-tumour effects in combination with melphalan in subcutaneous murine B16F1 melanomas and RMA-T lymphomas. However, the trimetric RGD-TNFα fusion protein hardly folded in a homogeneous manner due to 4 Cys residues involved in the structure of RGD peptide.[46] In this regard, NGR-TNFα was preferentially chosen for clinical study. Another peptide named RGR selected by phage display in pancreatic tumours showed special affinity to angiogenic vessels in insulinomas.[47] It has been used as a carrier to deliver therapeutic proteins, such as TNFα and IFNγ to the targeted site for cancer therapy. Johansson et al[48] demonstrated that intratumoural low-dose of RGR-TNFα (2 μg over 2 weeks) caused initial vessel activation and stabilization, enhanced vascular functionality, decreased vascular leakiness and T-cell infiltration mediated by $CD8^+$effector cells. Recently, our group has found a tumour vascular-homing peptide TCP-1 (a 9-amino acid cyclic peptide) based on an in vivo phage library screening against an orthotopic colorectal cancer developed in mice.[49] This peptide can specifically recognize the neovasculature of the colorectal tumour but not normal tissues in different organs. Our study showed that TCP-1/TNFα could synergize with 5-FU to inhibit orthotopic colorectal cancer growth. TCP-1/TNFα normalized tumour blood vessels, increased the absorption of 5-FU into the tumour and also facilitated the infiltration of immune cells into the neoplasm.[50] Thus, TCP-1/TNFα could be a novel agent targeting colorectal cancer tumour vessels and improve drug delivery and immune response in tumours.

4 | THE ANTI-CANCER ACTIVITY OF IFNγ

Angiogenesis is a basic process in promoting tumour growth. Numerous studies so far have focused on the angiogenic process, in an attempt to explore new strategies against tumour growth. Angiogenesis has been revealed to be a highly regulated process involving the balance between pro- and anti-angiogenic factors and the interaction between the immune and endothelial cells. Vascular endothelial growth factor (VEGF) is an important pro-angiogenic molecule in the tumour microenvironment, whose upregulation has

TABLE 1 Clinical studies of single agent IFNγ in different types of cancer

Study	Total no. of patients	Phase	Tumour type	Route	Dose of IFN gamma	Schedule	MTD	ORR[a]	Major reported toxicities
Foon[66]	11	N/A	Melanoma Adenocarcinoma lung Multiple myeloma Renal cell carcinoma Giant cell sarcoma Hairy cell leukaemia	IM(6) or IV(5)	0.05-10 mg/m^2	Twice weekly and the IV dose was infused over 5 min	NR	0%	Fever, chills, fatigue, anorexia and granulocytopenia
Kurzrock[67]	10		Renal cell carcinoma; Sarcomas Colon adenocarcinoma Nodular poorly Differentiated lymphocytic lymphoma, Carcinoid Multiple myeloma Adenocarcinoma of the lung	IM and IV	0.01-2.5 mg/m^2	A twice weekly schedule with IM injections alternating with IV bolus injections. A minimum period of 72 h between injections	NR	0%	Fever, chills and fatigue after both routes of administration and granulocytopenia after IM
Muss[65]	15	II	Advanced carcinoma of the breast	IV	2 mg/m^2	Five consecutive days every other week	NR	0%	Flu-like symptoms and nausea, vomiting and anorexia, hepatic toxicity
Boue[68]	29	I	Advanced malignancy	IV	0.01-5 mg/m^2	Every 72 h for 15 days	NR	3.4%	Fever, chills, nausea, vomiting and hypocholesterolaemia
Vadhan-Raj[69]	16	I	Advanced malignancy	IV	0.1, 0.5, or 1.0 mg/m^2/d	Six-hour IV infusions daily, 5 days a week for 2 weeks. After a 2-week rest period, the IV treatment cycle was repeated	NR	12.5%	Fever, chills, fatigue and myalgias
D'Acquisto[70]	27	I	Refractory ovarian carcinoma	IP	0.5-8 IU/m^2	Weekly	NR	0%	Fever, myalgias and flu-like symptoms, transaminase elevation
Lane[71]	16	I	Acquired immunodeficiency syndrome (AIDS) patients with Kaposi's sarcoma	IM and IV	0.001, 0.01, 0.1, or 1.0 mg/m^2	Single dose followed 4 days later by a 10-day course of daily therapy. Following a 1-week washout period, repeated administration by the alternate route	0.1-1.0 mg/m^2	NR	Fever, headache, fatigue, nausea and hepatitis
Yoshida[72]	15	II	Advanced hepatocellular carcinoma	IV	1.6-2.4 × 10^{7}units	Five consecutive days every 2 weeks.	NR	0%	Flu-like symptoms, pyrexia, anorexia, nausea and vomiting, headache, sore throat and hepatotoxicity

(Continues)

TABLE 1 (Continued)

Study	Total no. of patients	Phase	Tumour type	Route	Dose of IFN gamma	Schedule	MTD	ORR[a]	Major reported toxicities
Jett[64]	100	III	Small-cell lung cancer	SC	4×10^6 U/d	Daily for 6 months	NR	0%	Chills, myalgia, lethargy, and alteration of mood-personality
Pujade-Lauraine[61]	108	/	Ovarian cancer with residual disease after first line cisplatin-based chemotherapy	IP	20×10^6 IU/m^2	Twice a week for 3-4 months	NR	31.6%	Fever, flu-like syndrome, neutropenia and liver enzyme disturbances
Giannopoulos[57]	123		Superficial transitional cell carcinoma of the bladder	Intravesical Instillations	1.5×10^7 IU/ instillation	Eight weekly instillations followed by four biweekly and then by eight monthly instillations	NR	NR	Cystitis-like symptoms
Rinehart[73]	13	I/II	Metastatic renal cell carcinoma	IV		Twice weekly	NR	0%	Anorexia, fever and malaise
Quesada[74]	33	II	Metastatic renal cell carcinoma	IM (15) and IV (18)	IM: 0.25-1.0 mg/m^2 IV: 0.01-0.05 mg/m^2	Daily	NR	7% for IM and 6% for IV	Fatigue, anorexia, weight loss, leucopenia, abnormalities in liver function tests and hypertriglyceridaemia
Garnick[75]	42	I/II	Advanced renal cell carcinoma	IV	10-3000 mcg/ m^2	Either a daily 2-h infusion or 24-h infusion for 7 days every 3 weeks for at least 2 cycles. Maintenance program of 5 days of recombinant interferon gamma administered every 3-4 weeks	3000 mcg/ m^2	9.8%	Leucopenia, chills, fevers, rigors and hepatotoxicity
Aulitzky[76]	22	II	Metastatic renal cell carcinoma	SC	100 μg	Once weekly	NR	30%	Fever, fatigue, chills, febrile reactions, fatigue and malaise
Ellerhorst[77]	35	II	Metastatic renal cell carcinoma	SC	100 μg	Once weekly	NR	15%	Low grade fever, chills and myalgias
Gleave[62]	181	/	Metastatic renal cell carcinoma	SC	60 mcg/m^2	Once weekly	NR	4.4%	Chills, fever, asthenia and headaches
Small[78]	207	/	Metastatic renal cell carcinoma	SC	60 mcg/m^2	Once weekly	NR	3%	Chills, fever, asthenia, nausea and headache

(Continues)

TABLE 1 (Continued)

Study	Total no. of patients	Phase	Tumour type	Route	Dose of IFN gamma	Schedule	MTD	ORR[a]	Major reported toxicities
Creagan[79]	28	II	Disseminated malignant melanoma	IM	0.25 mg/m^2 on days 1-7 followed by a daily dose of 0.5 mg/m^2 if tolerated	Daily	NR	11.1%	Moderate to severe fever greater than 37°C (100%), fatigue (59%), chills (37%) and mild to moderate myalgias (64%)
Ernstoff[80]	30	I/II	Metastatic melanoma	IV	3-3000 mcg/m^2 over either 2 or 24 h	Daily	1000 mcg/m^2	6.7%	Fever, chills, myalgias, headache, fatigue, neutropenia, elevations in liver enzymes, tachyarrhythmias and change in mental status
Schiller[81]	89	II/III	Metastatic melanoma	IV	0.01-0.90 mg/m^2	Three times per week for at least 8 weeks or until progressive disease	NR	5%	Fever and chills and hepatic toxicity

[a]Objective response rate calculated using number of patients evaluable for response where available.
ORR: objective response rate; NR: not reported in study; IM: intramuscular; IV: intravenous.

been shown to contribute to tumour-associated angiogenesis, and tumour-associated macrophages (TAMs) are one of the main sources of VEGF.[51] It has been found that IFNγ can reduce the expression of mouse-VEGF, inhibit tumour angiogensis[52] and induce blood vessel destruction and necrosis.[53] Study also revealed that IFNγ could promote monocytes/macrophages infiltrating into tumour tissues and inhibit them to differentiate into TAMs.[52] Therefore, IFNγ reduced angiogenesis by inhibiting TAM differentiation and VEGF expression in the tumour microenvironment.

As early as 1992, it was reported that the administration of recombinant IFNγ and a synthetic lipid A subunit analogue (GLA-60) could inhibit tumour-associated angiogenesis synergistically in C57BL/6 mice, perhaps partially depending on the induction of endogenous TNFα.[54] Other mechanisms have also been proposed for IFNγ to inhibit tumour angiogenesis. For example, IFNγ can induce non-haematopoietic cells to secrete interferon-inducible protein 10(IP-10), leading to blockade of tumour angiogenesis and inhibition of tumour growth.[55] Specially targeting cancer-associated fibroblasts by IFNγ to inhibit fibroblasts-induced tube formation of H5V endothelial cells was reported to inhibit tumour vascularization.[56]

Besides anti-angiogenesis effect, IFNγ could exert its anti-cancer effect by inducing chemokine and cytokine secretion in the tumour microenvironment, as well as upregulating MHC class I and II to stimulate anti-tumour immunity. Studies in recurring superficial transitional bladder carcinoma[57] and ovarian cancers[58] demonstrated significant increases of T cells infiltrating into the neoplasm after administration of IFNγ, which favoured a good prognosis in cancer patients. Moreover, IFNγ itself has direct anti-proliferative activity on ovarian cancer cells by inducing tumour cell growth arrest and apoptosis[59] and could achieve an increased complete/partial response. Several clinical trials have been conducted for IFNγ. It is proven that IFNγ when used as an adjuvant therapy, could prolong the survival in ovarian cancer patients.[60] Also intraperitoneally given, IFNγ has been shown to achieve surgically documented responses by intraperitoneal treatment in the second-line therapy of ovarian cancer.[61] Moreover, when administered intravesically, IFNγ was found to be effective against bladder tumour recurrence.[57] In spite of the encouraging result in the above clinical trials, a lack of beneficial effect was seen in metastatic renal-cell carcinoma,[62] advanced colon cancer[63] or small-cell lung cancer,[64] advanced measurable pancreatic adenocarcinoma and also advanced breast cancer.[65] Thus, the anti-cancer effect is only on certain kinds of cancer if not for all cancers.

Similar to TNFα, systematic administration of IFNγ also faces the same problem of systemic toxicity and low anti-cancer efficacy. The clinical anti-cancer effects of IFNγ are summarized in Table 1. The most common adverse effects are "flu-like," such as fever, headache, chills or fatigue. Other common side effects include diarrhoea, nausea, vomiting and anorexia. Reversible and transient increases in hepatic transaminase and decrease in granulocyte and leucocyte counts were also seen.[65,67,69,82-84] Fusion proteins of IFNγ with NGR to form IFNγ-NGR could successfully target IFNγ to tumour vessels. However, excessive stimulation of IFNγ receptors by frequent administration of low doses of IFNγ-NGR could activate counter-regulatory mechanisms and inhibit ongoing anti-tumour response.[41] It was

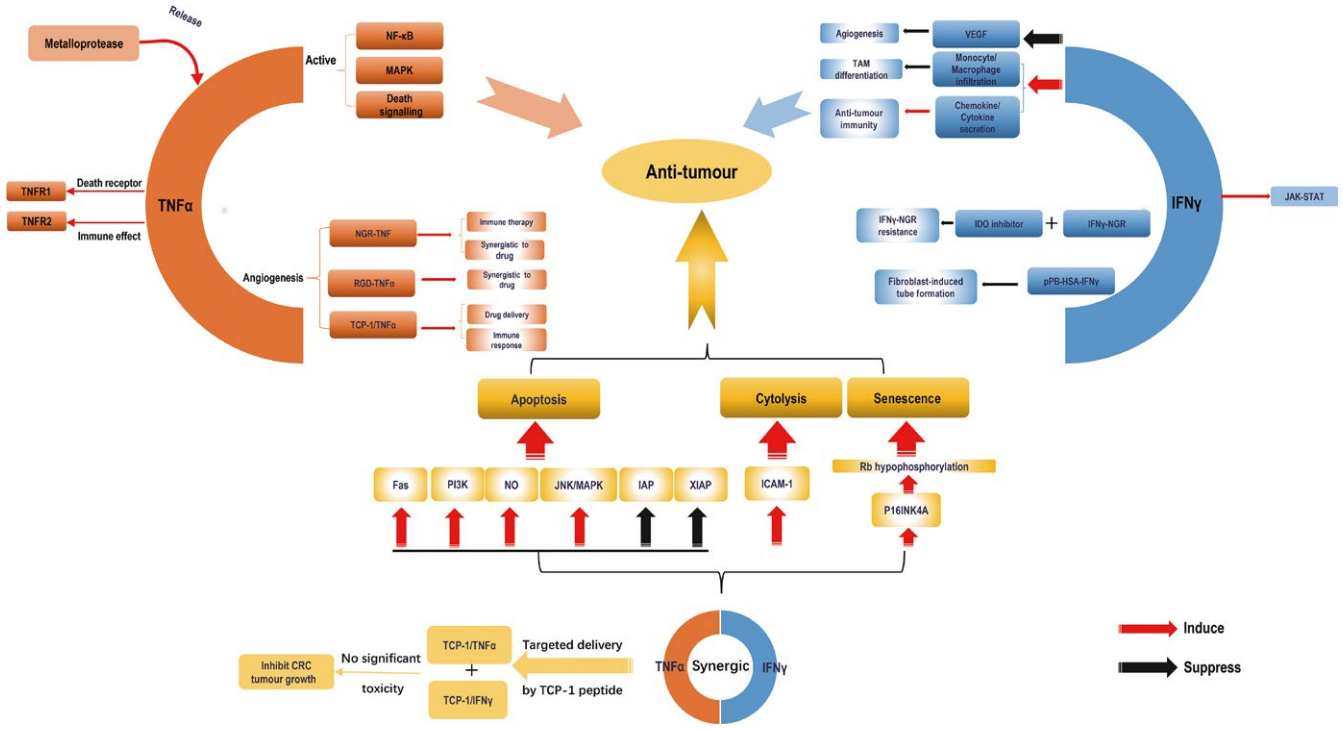

FIGURE 1 The anti-tumour effect of TNFα and IFNγ alone and in combination. The soluble homotrimeric TNFα released by metalloprotease bind to death receptor TNFR1 and immune system receptor TNFR2,which can activate 3 signalling cascades including NF-κB, MAPK and death signalling. The tumour-homing TNFα has significant anti-tumour effect. NGR-TNF can enhance TNFα delivery and immunotherapeutic effect without influencing the biological effect of TNFα in vivo. At the same time, it can also synergize with chemotherapeutic drug. Another peptide RGD can also recognize the tumour blood vessel and RGD-TNFα has synergistic anti-tumour effect with chemotherapeutic drug. Besides, TCP-1/TNFα increased the absorption of drug and immune response in tumours. The 2 receptors IFNGR1/IFNGR2 of IFNγ can activate JAK-STAT pathway to regulate cell apoptosis. Study indicated that IFNγ could reduce the mouse-VEGF and promote monocytes/macrophages infiltrating and chemokine/cytokine secretion to inhibit tumour growth. IFNγ-NGR could target IFNγ to tumour vessels. Combination of IFNγ-NGR with IDO inhibitors could overcome resistance of IFNγ-NGR caused by excessive stimulation of tryptophan catabolism. The pPB-HSA-IFNγ also successfully activated IFNγ-signalling, inhibited the activation and migration of fibroblasts and hampered fibroblasts-induced tube formation of endothelial cells. TNFα combined with IFNγ has been shown to have synergic anti-tumour effect via various pathways. Lately, targeted delivery of TCP-1/TNFα and TCP-1/IFNγ to tumour blood vessel has been demonstrated to significantly inhibit orthotopic colorectal tumour growth without significant systematic toxicity

found that repeated treatment of IFNγ-NGR increases dindoleamine 2,3-dioxygenase (IDO) and caused excessive stimulation of tryptophan catabolism and inhibited anti-tumour immunity.[85] Combination of IFNγ-NGR with IDO inhibitors was then reported to overcome resistance of IFNγ-NGR in nu/nu mice bearing RMA lymphoma.[85] F8 antibody was another ligand specially targeting EDA domain of fibronectin, a tumour-associated antigen expressed in the vasculature and stroma of almost all tumour types. Fusion conjugate of F8 to IFNγ retained the biological activity of both the antibody and the cytokine moiety in vitro,[9] and showed dose-dependent activity with a clear superiority over untargeted recombinant IFNγ.[9] Platelet-derived growth factor-beta receptor (PDGFbR)-binding carrier (pPB-HSA) has been used as fusing peptide to specially target IFNγ to stromal fibroblasts and pericytes (2 components of tumour stroma).The pPB-HSA-IFNγ conjugate successfully activated IFNγ-signalling (pSTAT1α), inhibited the activation and migration of NIH3T3 fibroblasts and hampered fibroblasts-induced tube formation of H5V endothelial cells.[56] This provides new types of drugs to target tumour stromal cells in cancer therapy.

5 | CO-ADMINISTRATION OF TNFα AND IFNγ IN ANTI-CANCER THERAPY

Both TNFα and IFNγ demonstrated inspiring anti-cancer effects in in vitro and in vivo studies. However, both of them when administrated alone presented limited therapeutic responses in clinics. Numerous studies were then conducted to focus on the synergic anti-cancer effects of both TNFα and IFNγ, especially in the induction of cellular apoptosis. As early as 1988, recombinant TNFα and IFNγ have already been reported to induce synergic anti-proliferative effects on human pancreatic tumour cell lines.[86] TNFα combined with IFNγ could accelerate NF-κB-mediated apoptosis through enhancement of fas expression in colon cancer cells.[87] Such effect was also depicted in ewing tumour cells.[88] Nitric Oxide expression and activation of PI3-kinase-dependent signalling cascade were also involved in mediating the synergistic pro-apoptotic effects of TNFα and IFNγ.[89] Kim et al[90] found out that IFNγ sensitizes MIN6N8 insulinoma cells to TNFα-induced apoptosis by inhibiting NF-κB-mediated XIAP upregulation. Kulkarni et al[91] then reported that IFNγ can sensitize the human salivary gland

cell line, HSG, to TNFα-induced activation of dual apoptotic pathways. Hairy cell leukaemia was reported to extremely sensitive to IFNγ, and further studies decoded that exposure of hairy cells (HCs) to IFNγ resulted in a marked increase of TNFα secretion, which was then solidly identified to be attributable to suppression of IAP (inhibitors of apoptosis), a protein known to be regulated by the cytoprotective NF-κB-dependent arm of TNFα signalling.[92] Synergistic activation of JNK/MAPK induced by TNFα and IFNγ to activate apoptosis was observed in pancreatic β-cells via the p53 and ROS pathway.[93]

There are many other mechanisms underlying the synergism between TNFα and IFNγ besides the synergic apoptosis-inducing effects. Studies hypothesized that although TNFα and IFNγ were not required by cytolytic effect on $CD8^+$ T cells (CTLs) for perforin-mediated killing of antigen-expressing tumour cells, tumour antigen-specific CTLs must secrete TNFα and IFNγ for the destruction of tumour stroma.[94] Moreover, TNFα and IFNγ produced by NK cells could induce target cell cytolysis through upregulation of ICAM-1.[95] Lately, TNFα and IFNγ were reported to cooperate together to induce senescence in numerous murine and human cancers by induction of permanent growth arrest in G1/G0, activation of p16INK4a, and downstream Rb hypophosphorylation at serine 795.[96]

Malignant tumours evolve along multistage programs of establishing a tumour stroma, neoangiogenesis and reprogramming of cell metabolism, finally leading to the expression of tumour-associated antigens (TAA).[97] This evolving process mainly depends on innate immune cells to induce aberrant vessel growth and adaptive immune response against TAA, which play important roles in the transition of premalignant dysplasia into carcinoma and further cancer progression.[98,99] Many focuses have been put on CTL to develop tumour immune therapy, and later a more efficient IFNγ-producing $CD4^+$ cell (Th1) was recognized to prevent transplant tumours growth and development by regulating multistage carcinogenesis through cytokine signals. Both tumour necrosis factor p55 receptor (TNFR1) signalling and IFNγ signalling were found to be essential for dominant anti-tumour effects of Tag-specific Th1 cells. Absence of either TNFR1 signalling or IFNγ signalling determined Tag-specific Th1 cells to induce tumour dormancy or promote multistage carcinogenesis,[97] which was another solid evidence supporting the cooperative effects of TNFα and IFNγ in anti-tumour treatment. Our recent study using tumour vasculature homing peptide TCP-1 showed that targeted combination therapy with TCP-1/TNFα and TCP-1/IFNγ could remarkably inhibit orthotopic colorectal tumour growth by inducing tumour necrosis without causing significant systematic toxicity.[100] The anti-tumour effects of TNFα and IFNγ either using alone or in combination are summarized in Figure 1. Our result emphasizes the therapeutic potential of co-administration of targeted TNFα and IFNγ for cancer treatment and the utility of TCP-1 peptide as a tumour-targeting agent in colorectal cancer. Comprehensive toxicity study is still needed before further application of this combination of treatment for type of cancer.

6 | CONCLUSION

Tumour necrosis factor alpha and IFNγ are now affirmed as pro-inflammatory cytokines and also produce effective anti-tumour effects. Their clinical application was limited due to the toxicity and counter-regulatory mechanisms. Such limitations could partially be overcome by fusion of TNFα and IFNγ to peptides or antibodies targeting tumour epithelial, endothelial or stromal cells.[13,101] An alternative strategy of targeted delivery of TNFα by TNF-expressing cancer cells has lately been demonstrated. The safety issues in clinical context await further assessment.[23] The multifunctional properties of TNFα and IFNγ and the newly discovered targeted delivery strategies may well result in a more optimistic clinical applications of these 2 cytokines in cancer treatment in a foreseeable future.

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