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Invited review

Tumor necrosis factor and cancer, buddies or foes?[1]

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Abstract

Tumor necrosis factor (TNF) is a multifunctional cytokine that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death. As a pro-inflammatory cytokine, TNF is secreted by inflammatory cells, which may be involved in inflammation-associated carcinogenesis. TNF exerts its biological functions through activating distinct signaling pathways such as nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK). NF-κB is a major cell survival signal that is anti-apoptotic, whereas sustained JNK activation contributes to cell death. The crosstalk between the NF-κB and JNK is involved in determining cellular outcomes in response to TNF. In regard to cancer, TNF is a double-dealer. On one hand, TNF could be an endogenous tumor promoter, because TNF stimulates the growth, proliferation, invasion and metastasis, and tumor angiogenesis of cancer cells. On the other hand, TNF could be a cancer killer. The property of TNF in inducing cancer cell death renders it a potential cancer therapeutic, although much work is needed to reduce its toxicity for systematic TNF administration. Recent studies have focused on sensitizing cancer cells to TNF-induced apoptosis through inhibiting survival signals such as NF-κB, by combined therapy. In this article we provide an overview of the roles of TNF-induced signaling pathways in cancer biology with specific emphasis on carcinogenesis and cancer therapy.

Introduction

Tumor necrosis factor (TNF, also referred to as TNFα) was identified in the late1970s as a cytokine produced by immune cells having a capacity to suppress tumor cell proliferation and induce tumor regression[1,2]. TNF is a protein consisting of 157 amino acids and is synthesized as a membrane-bound protein (pro-TNF) that is released by TNF-converting enzyme (TACE)-mediated cleavage. Since the TNF gene was cloned in 1984, extensive research has revealed a variety of roles for TNF under physiological conditions such as in body development and immunity, and in pathological responses such as inflammation, tumor growth, transplant rejection, rheumatoid arthritis, and septic shock[3]. On the cellular level TNF exerts its effects through its receptors to activate distinct signaling pathways that regulate cell survival, proliferation, or death. Accordingly, complicated roles for TNF in cancer have emerged. On one hand, its anticancer property is mainly through inducing cancer cell death, a process that could be used for cancer therapy. On the other hand, TNF stimulates proliferation, survival, migration, and angiogenesis in most cancer cells that are resistant to TNF-induced cytotoxicity, resulting in tumor promotion. Thus, TNF is a double-edged sword that could be either pro- or anti-tumorigenic. In this review, we focus on the roles and mechanism of TNF in cancer biology with specific emphasis on carcinogenesis and cancer therapy.

TNF-induced signaling pathways

There are 2 receptors for TNF; namely, TNF receptor 1 (TNFR-1, p55 receptor) and TNFR-2 (p75 receptor). TNFR-1 is ubiquitously expressed, whereas TNFR-2 is mainly expressed in immune cells[3]. Although both the receptors bind TNF, the main receptor mediating the

cellular effects of TNF in most cell types is TNFR-1. TNFR-1 is a death domain (DD)-containing receptor with an extracellular domain (ECD), a transmembrane domain (TMD), and an intracellular domain (ICD). TNFR-1 is an important member of the death receptor family that shares the capability of inducing apoptotic cell death. TNFR-2 does not possess a DD, although it can mediate a cell death signal, which may be indirect through TNFR-1[4].

The pathways mediated by TNFR-1 have been extensively studied. Upon binding by TNF, which is a natural homotrimer, TNFR-1 forms a homotrimer to recruit the TNFR-associated death domain (TRADD) through the homologous binding of the DD of both proteins. TRADD serves as a platform to recruit downstream adaptor proteins to generate signals for distinct signaling pathways. These adaptor proteins include receptor interacting protein (RIP), TNFR-associated factor 2 (TRAF-2), and Fas-associated death domain (FADD) that further recruit key molecules that are responsible for intracellular signaling to activate NF-κB, mitogen-activated protein kinases (MAPK), and cell death, respectively (Figure 1).

TNF induces NF-κB activation TNF-induced NF-κB activation is initiated by activation of inhibitor of κB (IκB) kinase (IKK). During TNFR-1 signaling IKK is recruited to the TNFR-1 signaling complex (Complex I), which consists of TRADD, TRAF2, and RIP. IKK is activated by a RIP-dependent mechanism that involves MEKK3, TAK1, and TAB2[5,6]. The activated IKK phosphorylates IκB, which retains NF-κB in the cytoplasm, to trigger its rapid polyubiquitination followed by degradation in the 26S proteasome. This process causes the NF-κB nuclear localization signal to be exposed, allowing its nuclear

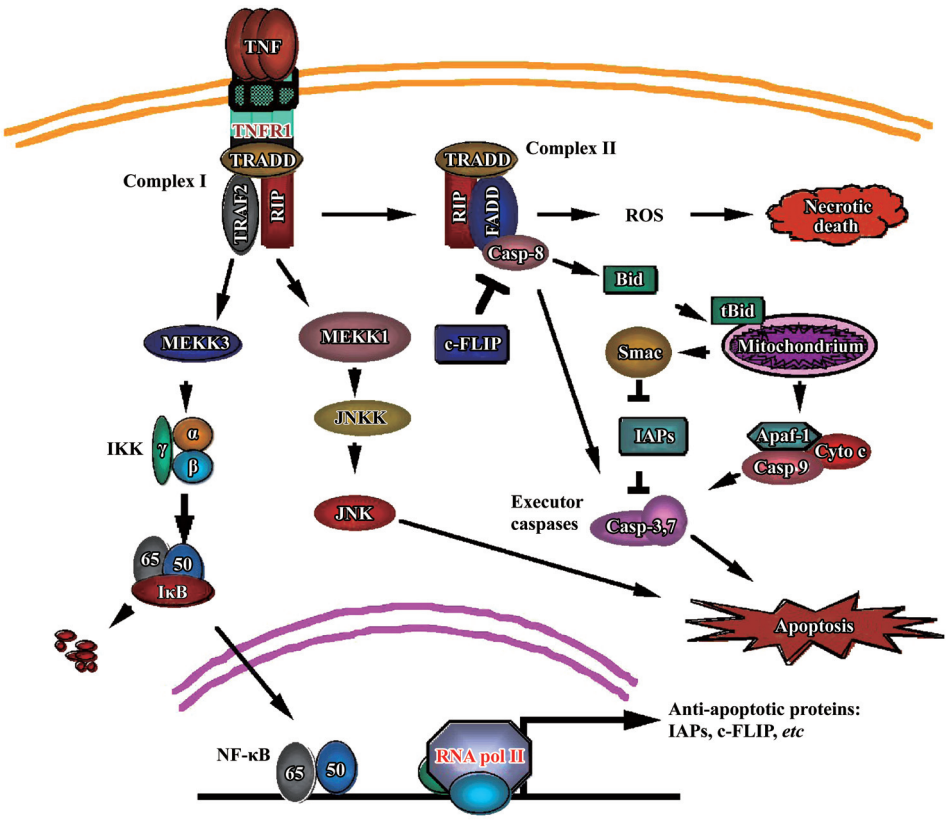

Figure 1. TNFR1 mediated signaling pathways. The binding of the TNF trimer to TNFR1 causes trimerization of TNFR1, resulting in formation of the Complex I consisting of TNFR1, TRADD, TRAF2 and RIP. Complex I mediates the NF-κB activation pathway through the MEKK3-IKK-IκB- NF-κB cascade, leading to expression of a battery of genes including those encoding antiapoptotic factors such as IAP and c-FLIP. The JNK activation pathway is also mediated by Complex I. Sustained JNK activation is apoptotic. The internalization of the TNFR1 complex ensures formation of Complex II that contains RIP, TRADD, FADD and Caspase 8. Caspase 8 is autoactivated to trigger activation of the executor caspases -3, and -7, resulting in apoptosis. Cleavage of Bid by caspase 8 activates the mitochondrial apoptosis pathway that involves release of cytochrome c and Smac/DIABLO from mitochondria. Cytochrome c binds Apaf1 to activate caspase 9-mediated activation of executor caspases. The NF-κB activated factor c-FLIP suppresses caspase 8 activation while IAP inhibit executor caspases. Smac released from the mitochondria suppresses IAP to release the apoptosis brake. Complex II also mediates a necrotic cell death through ROS. See text for details.

translocation to promote transcription of its target genes. Among NF-κB's target genes, A20, cIAP-1, cIAP-2, Bcl-xL, XIAP, and IEX-1L are found to have anti-apoptotic properties[7]. Induction of the antioxidant manganese superoxide dismutase (MnSOD) by NF-κB is also implicated in anti-apoptotic and -necrotic cell death[8]. The transcriptional activity of NF-κB is further regulated by phosphorylation and acetylation, which modulate the DNA binding by NF-κB and interaction with transcriptional co-activators and/or co-repressors[4].

TNF induces MAPK activation TNF is able to activate MAPK (extracellular signal-regulated protein kinases [ERK], JNK and p38). In most cell types, JNK is the main MAPK induced by TNF[4,9]. TNFR-1-mediated JNK activation is transduced by TRAF2 and RIP through sequential phosphorylation of the MAP kinase module, MAP3K-MAP2-MAPK[9,10]. MAP3Ks for JNK include members of the MAP/ERK kinase kinase (MEKK) family such as apoptosis signal-regulating kinase 1 (ASK1), mixed lineage kinase (MLK), and transforming growth factor activated kinase 1 (TAK1). Genetic disruption of MEKK1 in mice abrogates TNF-activated JNK. There are 2 MAP2Ks (JNKK1/MKK4/SEK1 and JNKK2/MKK7) that activate JNK. The 2 JNK kinases (JNKKs) phosphorylate JNK at Thr183 and Tyr185, leading to its activation[11]. The role of JNK activation in cell death regulation is controversial, but recent studies suggested that sustained JNK activation, which is suppressed by NF-κB, is pro-apoptotic[12,13]. As is similar to JNK, the activation of ERK and p38 by TNF involves TRAF2 and RIP[9].

TNF induces cytotoxicity As a death receptor TNFR-1 signals cells to die. It is well established that TNF induces apoptosis in a variety of cell types. This pathway is initiated by TNFR1 internally signaling Complex I to form Complex II that consists of TRADD, RIP, FADD, and caspase-8. Caspase-8 is auto-activated to trigger activation of the executor capsases-3, and -7, and the endonucleases, resulting in destruction of cell component proteins, fragmentation of DNA, and, eventually, apoptotic cell death. This death receptor-mediated apoptosis pathway is also called the extrinsic apoptosis pathway. TNF-induced apoptosis also uses the mitochondria-mediated (intrinsic) apoptosis pathway. This is achieved by caspase-8 activating BCL-2 interacting domain (Bid), a BH3-only Bcl2 family member. Cleavage of Bid by caspase-8 generates tBid, which migrates to the mitochondria and causes loss of mitochondrial membrane potential, and release of cytochrome c and second mitochondria-derived activator of caspase (Smac)/ direct IAP binding protein with low pI (DIABLO) from mitochondria to the cytosol. Cytochrome c binds to apoptotic protease activating factor 1 (Apaf-1) and pro-caspase-9 to form apoptosome, resulting in caspase-9-mediated activation of the executor caspases[14]. Smac binds to and inhibits the inhibitor of apoptosis proteins (IAP, including c-IAP1, c-IAP2, X-linked inhibitor of apoptosis protein [XIAP], and survivin), releasing the brake to accelerate apoptosis. In some cancer cells this apoptosis pathway is suppressed partly through the suppression of caspase-8 by cellular FLICE inhibitory protein (c-FLIP), a caspase-8 homolog that competes with caspase-8 for binding to FADD, and suppression of the mitochondrial pathway by the anti-apoptotic Bcl2 family members[15].

Despite the well-known Complex II-mediated apoptosis pathway, an additional TNF-induced apoptosis pathway has recently been discovered. Although the exact mechanism has not yet been determined, it is clearly distinct from the well-known pathway that is mediated by FADD, RIP dispensable, and can be suppressed by c-FLIP. The new pathway is mediated by RIP and FADD, independent of TRADD, and is suppressed by c-IAP[16,17]. Both the pathways use caspase-8 as the initiator caspase to activate the apoptotic execution enzymes. Interestingly, in some cancer cells, this new apoptosis pathway is partially or completely suppressed[16].

In addition to apoptosis, TNF can also induce necrotic cell death. Reactive oxygen species (ROS) play a critical role in mediating necrotic cell death because ROS scavenger BHA can effectively block this pathway[18]. This TNF-induced necrosis requires RIP kinase activity[19,20]. Furthermore, this pathway involves RIP-dependent activation of the NADPH oxidase Nox1[21]. However, because this pathway is also damaged in some cancer cells, these cells are capable of evading all of the TNF-induced cell death pathways, resulting in their malignant proliferation. Understanding the mechanism behind this capability would improve TNF's anticancer value.

The aforementioned crosstalk among the TNF-induced pathways plays a key role in the biological effects of TNF on cancer. For example, NF-κB suppresses the apoptotic JNK activation and mediates expression of anti-apoptotic and antioxidant genes, blocking cell death and facilitating cancer cell proliferation[4,8,12]. Activation of caspase-8 causes cleavage of the key NF-κB mediator RIP to shut off the cell survival signal while it concurrently promotes the apoptosis pathway[22]. The mitochondria-released Smac suppresses IAP, releasing the apoptosis brake. Therefore, the balance of TNF-induced survival- and death-signaling

is pivotal in determining the fate of TNF-responding cells. Modulating this balance could help to prevent cancer development and facilitate using TNF for cancer therapy.

TNF and carcinogenesis

A growing body of epidemiological and clinical data supports the concept that chronic inflammation promotes tumor development and progression. As a major pro-inflammatory cytokine, TNF is able to act as an endogenous tumor promoter to bridge inflammation and carcinogenesis. Indeed, recent reports have shown that TNF is involved in all aspects of carcinogenesis as summarized below: cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis.

Elevated TNF expression levels in tumor patients Numerous reports have shown that the serum TNF concentration is increased in different cancer patients[23,24]. TNF expression was also expressed at higher levels in various pre-neoplastic and tumor tissues[23–25]. Further, the increased TNF expression level in pre-cancerous and tumor cells was associated with the progression of malignant diseases such as chronic lymphocytic leukemia, Barrett's adenocarcinoma, prostate cancer, breast cancer, and cervical carcinoma[23,24,26–28]. The serum TNF concentration was markedly decreased during chemotherapy in breast and prostate cancer patients, the extent of which was well-correlated with the extent of therapy responses, suggesting that serum TNF level could be an indicator for chemotherapy response and prognosis[23,26,29]. Serum TNF might also be a risk factor for colorectal neoplasia because it is associated with several known risk factors such as age, smoking, and adiposity, and with a higher prevalence of colorectal adenomas[30] (Table 1). These findings indicate that TNF may be involved in tumor development and could be used as an indicator of cancer risk, therapy response, and prognosis for cancer patients.

TNF promoter polymorphisms and tumor risk Single nucleotide polymorphisms (SNP) in the promoter region of the TNF gene have been intensively studied for susceptibility to numerous cancers. In recent years, several SNP have been identified as being involved in regulating TNF expression. Among these SNP, one at position –308 in the TNF promoter (–308G/A) was found to be associated with susceptibilities to various types of cancer. Individuals carrying an AA/GA genotype at TNF-308 had higher TNF expression levels and an increased cancer risk, including non-Hodgkin's lymphoma[31,32], hepatocellular carcinoma[33], gastric cancer[34], invasive cervical cancer[35], ulcerative colitis-associated colorectal cancer[36], and non-small cell lung cancer[37]. The –308AA/GA genotype is also associated with the severity of disease in non-small cell lung cancer[37]. In a case-control study carried out in a large north European population, the –308A allele was associated with the presence of vascular invasion of breast cancer[38]. TNF (–308A) was also related to non-Hodgkin's lymphoma outcome, as freedom from progression was lower and overall survival were shorter in patients with TNF (–308A)[39]. These results suggest that the –308A allele promotes cancer development and progression. On the contrary, the –238A allele in the

Table 1. Evidence of TNF promoter polymorphisms and expression levels associated with malignant tumors.

	Type of tumors	References
Elevated expression		
Tumor progression	chronic lymphocytic leukemia, Barrett's adenocarcinoma, prostate cancer, breast cancer, and cervical carcinoma	[23−28]
Lower therapy response	breast and prostate cancer	[23, 26, 29]
Higher cancer risk	colorectal adenomas	[30]
Promoter polymorphisms		
-308 A allele		
increased cancer risk	non-Hodgkin's lymphoma, hepatocellular carcinoma, gastric cancer, cervical cancer, ulcerative colitis-associated colorectal cancer, lung cancer, and breast cancer	[31−39]
-238 A allele		
decreased cancer risk	lung cancer, gastric cancer, cervical cancer, colorectal cancer, and renal cell carcinoma	[37, 40]
-857 T allele		
decreased cancer risk	maltoma and non-Hodgkin's lymphoma,	[41, 42]
increased cancer risk	B-cell lymphoma	[43]

TNF-promoter is associated with lower risk of cancers. Individuals with –238AA/GA genotypes have decreased susceptibility to lung cancer, gastric cancer, uterine cervical cancer, colorectal cancer, or renal cell carcinoma[37,40]. The different effect of these 2 SNP on cancer provides genetic evidence for the involvement of TNF in carcinogenesis. Carrying the TNF-857T allele, another SNP associated with decreased TNF expression, also confers decreased risk to maltoma[41] and non-Hodgkin's lymphoma[42]. However, the TNF-857T allele was also found to be associated with increased risk to B-cell lymphoma[43]. Thus, the SNPs may influence the susceptibility to cancer, which may be associated with altered TNF production or a neighboring gene in tight-linkage disequilibrium. These reports indirectly suggest that TNF has a tumor-promoting role and that TNF promoter SNPs could be a predictor for cancer risk (Table 1).

The role of TNF in tumor promotion and growth

The tumor promoting role of TNF has also been demonstrated in various mouse tumor models. There is mounting evidence indicating that pathophysiological concentrations of endogenous TNF promote tumor genesis and growth. In a colitis-associated colon carcinogenesis mouse model, mice lacking TNFR-1 or blocking TNF function with the neutralizing antibody etanercept markedly reduced azoxymethane (AOM) and dextran sulfate sodium (DSS)-induced colonic inflammation and attenuated subsequent tumor formation[44]. A similar observation was made in the 7,12-dimethylbenz (α) anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol 13-acetate (TPA)-promoted mouse skin tumorigenesis model, in which blocking or suppressing TNF conferred resistance to skin carcinogenesis[45,46]. The critical role of TNF in tumor promotion was further confirmed in TNF knockout ($TNF^{-/-}$) mice expressing both cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 ($TNF^{-/-}$ K19-C2mE mice), which showed significant suppression of hyperplastic tumors with reduced cell proliferation compared with TNF wild-type (+/+) K19-C2mE mice[47]. It was found that protein kinase C (PKC) ε activation is an initial signal in TPA-induced shedding of TNF from epidermal keratinocytes[48]. TNF may not be involved in tumor initiation because the frequency of DNA adducts formation and c-Ha-ras mutation is the same in wild-type and $TNF^{-/-}$ mouse epidermis after DMBA treatment. Instead, TNF may mediate tumor promotion via activating NF-κB[49,50] or a PKCα- and AP-1-dependent pathway[51]. TNF treatment dose-dependently increased NF-κB activity and tumor promotion in mouse epidermal JB6 cells[49]. NF-κB activation is critical for TNF-induced tumor promotion, because in the transformation-resistant JB6 mouse epidermal cells (P-cells) TNF was unable to induce NF-κB activation[50]. TNF-induced NF-κB inhibits carcinogen-induced cytotoxicity, thereby increasing the pool of mutated cells that are susceptible to malignant transformation[52]. Activating the PKCα- and activator protein 1 (AP-1)-dependent pathways mediated the induction of a specific subset of AP-1 responsive genes such as granulocyte-macrophage colony-stimulating factor (GM-CSF), matrix metalloproteinases (MMP)-9, and MMP-3 that are important for tumor development[51]. Nuclear factor of activated T-cells 3 (NFAT3) activation may also contribute to TNF-induced anchorage-independent epidermal Cl41 cell growth, at least partially through inducing COX-2 expression[53]. In addition, TNF may promote tumor development via inducing secretory leukocyte protease inhibitor (SLPI)[54], platelet-type 12-lipoxygenase (p12-LOX)[55], or activation-induced cytidine deaminase (AID)[56]. It is also reported that TNF promoted cancer development through inducing gene mutations and DNA instability by upregulating ROS production in the cells[57,58] (Table 2).

Table 2. Roles and molecular targets of TNF in promoting carcinogenesis.

	Molecular targets	References
Tumor promotion and growth	NF-κB	[49, 50, 52]
	PKCα, AP-1	[51]
	NFAT3	[53]
	SLPI, p12-LOX, AID, ROS	[54−58]
Tumor angiogenesis	IL-8, VEGF, HGF	[59−61]
Tumor invasion and metastasis	MMPs, α2β1 integrin, MIF, MMP inducer, CXCR4, CP-1, IL-8, intercellular adhesion molecule-1, LOX-1	[65−78]

TNF and tumor angiogenesis, invasion, and metastasis It is becoming clear that TNF is involved in tumor angiogenesis, invasion, and metastasis. TNF upregulated expression of angiogenic factors, including interleukin (IL)-8 and vascular endothelial growth factor in U251 glioma cells[59]. TNF secretion by ovarian cancer cells stimulated a constitutive network consisting of cytokines, chemokines, and angiogenic factors that promoted colonization of the peritoneum and neovascularization for developing tumor deposits[60]. In a lung metastasis mouse model, TNFR-1-mediated signals were able to maintain tumor neovascularization at least partly through inducing hepatocyte growth factor (HGF) expression, which was assumed to support lung metastasis[61]. Both exogenous and macrophage-produced TNF accelerated the epithelial-mesenchymal transition (EMT), which characterized the progression of carcinoma and was linked to the acquisition of an invasive phenotype[62–64]. TNF enhanced the invasiveness of tumor cells through inducing MMP-2, -3, -9, and -12[65–67] or α2β1 integrin[68]. TNF activated focal adhesion kinase (FAK), which was a pivotal mediator for MMP-9 production in cholangiocarcinoma cells[69]. Other mechanisms may also underlie TNF-induced tumor cell invasion, including NF-κB- and JNK-mediated upregulation of macrophage migration-inhibitory factor (MIF) and extracellular MMP inducer in tumor cells[70]. In addition, TNF enhanced a variety of tumor cells to adhere to the mesothelium *in vitro* and increased tumor migration and metastasis *in vivo*, partly through NF-κB-dependent induction of the chemokine receptor CXCR4, upregulation of monocyte chemoattractant protein-1 (MCP-1), and IL-8 and intercellular adhesion molecule-1 in cancer cells[71–77]. Upregulation of lectin-like oxidized-low-density lipoprotein (oxLDL) receptor-1 (LOX-1) by TNF in endothelial cell was also shown to promote the adhesion and trans-endothelial migration of MDA-MB-231 breast cancer cells[78], which may be involved in colonizing tumor cells at remote sites during metastasis (Table 2).

A potential anti-oncogenic effect of TNF Although most studies showed that TNF promoted tumorigenesis, TNF exhibited an anti-oncogenic effect in some experimental systems. Despite the reported role of TNF in chronic inflammation, $TNF^{-/-}$ mice were significantly more susceptible to 3′-methylcholanthrene (MCA) -induced skin sarcoma than wild-type mice, suggesting that TNF protects the host against MCA-induced sarcoma formation[79]. A similar effect of TNF was observed in a xenografted glioma mouse model[80,81]. The antitumor role of TNF may involve immune responses that prevent tumor formation, for example, promoting tumor stroma destruction by cytotoxic T lymphocyte (CTL) or tumor infiltrating macrophages[80,82,83] and activating tumor-infiltrating dendritic cells (DC), thereby triggering a potent adaptive immune response leading to tumor rejection[84]. Using gene knock-out mice that were null of TNFR-1, -2, or both, it was found that TNFR-2 expressed in host innate immune cells is sufficient to mediate the antitumor effect of TNF. A large number of macrophages were recruited by the TNFR-2-mediated production of nitric oxide (NO), which efficiently inhibited angiogenesis in the tumor[85].

How the pro- or anti-tumorigenesis roles of TNF are regulated is currently unclear, but could be attributed to differences in organs, cell contexts, and carcinogens. For example, TNF-induced NF-κB activity has contradictory effects in organs with different regenerating rates (ie, anti-tumorigenic in rapid regenerating liver while pro-tumorigenic in slow regenerating colon)[86]. Whether anticancer immunity is involved may also contribute to the discrepancy[80,82,83]. The contradictory roles of TNF in tumorigenesis raise questions regarding the use of anti-inflammatory or anti-TNF agents for cancer prevention. Indeed, data from the Wegener's Granulomatosis Etanercept Trial demonstrate that combining TNF inhibitor etanercept with cyclophosphamide increased, rather than decreased, the cancer risk beyond that observed with cyclophosphamide alone[87]. Therefore, due to the complexity of the roles of TNF in carcinogenesis, more studies are needed to clarify the mechanism behind the development of different cancers before TNF modulating approaches can be used for cancer prevention. Also, caution and surveillance of cancer risk are necessary when inhibiting TNF inhibition is applied for inflammatory disease therapy.

TNF and cancer therapy

Although TNF has cytotoxic, cytostatic, and immunomodulatory effects on malignant tumors, using TNF as a chemotherapeutic drug has been hampered by its deleterious side effects, including systemic shock and widespread inflammatory responses. In addition, many cancer cells are resistant to TNF-induced cytotoxicity. Therefore, the challenge is to maximize the tumor-selective cytotoxicity and ameliorate the side effects of TNF.

TNF mutants with higher antitumor activity and lower systemic toxicity One attempt to overcome the systematic toxicity is to mutate TNF by molecular engineering. It was reported that a lysine-deficient mutant TNF (mTNF-K90R) had higher affinities to both TNFR-1

and -2, a higher *in vitro* bioactivity, a considerably longer plasma half-life, and an *in vivo* antitumor therapeutic window 60-fold greater than that of the wild-type TNF[88]. TNF mutants deficient in their lectin-like activity retained their anti-tumor activities, including necrotic and tumoristatic activities and anti-angiogenic activity, but exhibited reduced systemic toxicity and pro-metastatic capacity[89]. Another TNF mutant in which amino acids Arg, Lys, and Arg substituted for Pro, Ser, and Asp at TNF positions 8, 9, and 10 , and Phe substituted for C terminal Leu157, along with deletion of the first 7 N-terminal amino acids, also showed a more effective cytotoxicity than wild-type TNF[90]. Therefore, molecular modification of TNF may pave the way for the use of TNF in tumor therapy, although the mechanism by which the reduction of systematic side effects by the TNF mutations is achieved requires further study.

Sensitizing cancer cells to TNF-induced cytotoxicity With the clarification of the mechanisms underlying the tumor cell's resistance to TNF, a number of new strategies for sensitizing tumor cells to TNF-induced cell death have been explored. As TNF-activated NF-κB is known to be the major mechanism by which tumor cells escape TNF-induced cytotoxicity, the effect of NF-κB blockage on TNF sensitivity has been studied extensively in a variety of experimental systems. Numerous agents, including naturally occurring and synthetic compounds, were shown to sensitize tumor cells to TNF-induced cell death through inhibiting NF-κB activation. Combining these compounds with TNF led to synergistic cytotoxicity in tumor cells[91–97]. Because Akt contributes to TNF-induced NF-κB activation in lung cancer cells, we investigated and found that concurrently suppressing NF-κB and Akt synergistically triggers TNF-induced cytotoxicity in lung cancer cells[98]. Some compounds enhanced TNF-induced cell death in an NF-κB-independent manner via downregulating the protein level of c-FLIP or increasing expression of both TNF-R1 and cathepsin B[99,100].

In addition, TNF can be used as an adjuvant reagent to promote the anti-cancer effect of chemotherapy agents such as doxorubicin[101], sensitize low epidermal growth factor receptor (EGFR)-expressing carcinomas to anti-EGFR therapy[102], or overcome acquired resistance to EGFR tyrosine kinase inhibitor in non-small-cell lung cancer cells[103]. The combination of TNF and chemotherapeutic agents has been shown to be an effect therapeutic strategy for many tumors by increasing tumor sensitivity to treatment.

Local administration of TNF in an isolated limb or organ setting Due to its systemic toxicity, the clinical use of TNF has been limited to its administration through sophisticated locoregional drug-delivery systems in patients with certain types of organ-confined solid tumors. TNF administration through isolated limb perfusion (ILP) for regionally advanced melanomas and soft tissue sarcomas of the limbs was demonstrated to be safe and efficient. When combined with the alkylating agent melphalan, ILP produces a high objective response rate with acceptable local toxicity and negligible systemic toxicity[104,105]. Several reports showed that the inclusion of TNF in ILP appears to be a very efficacious procedure to obtain local control and achieve limb salvage in patients with metastasized, bulky, limb-threatening tumors[105,106]. The action of TNF-based ILP may be twofold: first, there is an increased endothelium permeability, leading to enhanced chemotherapy drug uptake within the tumor tissue; second, a selective destruction of the tumor vasculature that suppresses the tumor's blood supply occurs. Downregulation of the vascular endothelial growth factor (VEGF) receptor fetal liver kinase-1 (Flk-1) on tumor endothelium may underlie the mechanism of the tumor vasculature targeting effect of TNF[107,108]. Other TNF-based ILP regimens, including combination with hyperthermia or doxorubicin, also were reported with promising results[109,110]. In addition to ILP, TNF-based isolated hepatic perfusion (IHP) for hepatic metastases and intratumoral administration of TNF into the post-operative tumor cavity for patients with malignant glioma are also shown to be safe and effective therapies[111,112]. However, the effectiveness of including TNF in ILP was challenged by results from a randomized prospective multi-institutional trial that showed that TNF did not significantly enhance short-term (up to 3 month) response rates over melphalan alone in locally advanced extremity melanoma[113]. Nevertheless, although more careful studies are required, locoregional drug-delivery of TNF alone or in combination with other therapeutic agents could be a potential tumor therapy approach.

In addition, tumor-targeted delivery of TNF can substantially maximize the local concentration targeting tumor cells and significantly minimize the doses of TNF, thereby effectively reducing the systemic toxicity.

TNF gene therapy driven by inducible promoters One new strategy is the selective delivery of TNF to tumors using an adenoviral vector, for which TNFerade has been extensively studied. In this replication-incompetent adenoviral vector, expression of human TNF is under the control of the early growth response 1 (Egr-1) promoter, which could be activated by ionizing

radiation (IR) and diverse anticancer agents including cisplatin, cyclophosphamide, doxorubicin, 5-fluorouracil, gemcitabine, and paclitaxel, allowing temporal and spatial control of TNF release[114–116]. A potent anticancer activity was observed without significant systemic toxic effects using this vector[114]. The combination of TNFerade and temozolomide or IR significantly improved antiglioma efficacy in 2 glioma xenograft models[117]. The TNFerade vector was activated by resveratrol and can be given to patients who are intolerant of radiation or cytotoxic therapy[118]. TNFerade also reduced metastasis in the syngeneic B16F10 murine melanoma model[119]. Further modification of the adenoviral vector allowed higher specificity in tumor targeting through a combination of modifying the viral capsid and restricting tumor-specific TNF expression. The capsid-modified adenoviral vector ensures high selectivity for targeting the α(v)β(3/5) integrin-positive ovarian cancer cells. Meanwhile, the promoter of the tumor antigen MUC-1 in this vector triggered TNF expression in MUC1-positive tumor cells. When this vector was used in ovarian cancer xenografted models, an effective anti-tumor activity was achieved with no obvious toxicity. Thus, combining capsid modification and transcriptional regulation of TNF expression is a promising strategy for developing TNF-based cancer therapy[120]. An infected cell protein 34.5 (ICP34.5)-deleted oncolytic herpes simplex virus (HSV) vector was found to provide localized TNF delivery and anti-tumor effects[121]. Additionally, the membrane-bound TNF was shown to be ideal for cancer gene therapy because it displays better therapeutic activity and lower side effects[122]. Generation of a TNF mutant that is resistant to cleavage by TACE may be a useful approach to increase locally delivered anticancer therapy value of TNF.

Antibody-directed tumor delivery of TNF Another avenue for improving selective localization of TNF at the tumor site is to generate a recombinant protein that fuses TNF to a monoclonal antibody that specifically targets a tumor antigen. These fusion proteins, immunocytokines, were composed of TNF and an antibody or antibody fragment targeting various tumor antigens such as HER-2/neu, gp240 antigen, EGFR, or fibronectin[123–126]. When systemically given, these immunocytokines were shown to specifically deliver TNF to tumors, which resulted in pronounced anti-tumor activity and lowered side effects. In addition, a different strategy using a bispecific antibody (BsAb) that simultaneously recognizes a cancer antigen and TNF was explored and showed some anticancer effects when combined with radiotherapy[127].

Vascular targeting by TNF coupled to tumor-homing peptides TNF is able to cause selective destruction of tumor-associated blood vessels. This therapeutic property was improved by fusing TNF with peptides, such as CNGRCG or ACDCRGDCFCG, that bind tumor blood vessels. Intramuscular administration of plasmid vectors encoding CNGRCG-TNF (NGR-TNF) or ACDCRGDCFCG-TNF (RGD-TNF) inhibited the growth of tumors implanted at distant sites from the plasmid injection site, indicating that vascular targeting was achieved[128]. The combination of NGR-TNF or RGD-TNF with various chemotherapeutic drugs, including doxorubicin, melphalan, cisplatin, paclitaxel, and gemcitabine, enhanced the anticancer response but did not increase toxicity[129–131]. Neovascular targeted TNF may improve drug delivery to tumors by altering vascular permeability[132]. Local induction of interferon (IFN)-γ may play a crucial role in tumor vascular targeting by NGR-TNF[133]. Therefore, vascular targeting by TNF could be a novel strategy for increasing the therapeutic index of chemotherapeutic drugs.

Shielding or encapsulation of TNF for reducing toxicity Surface-shielded or encapsulated TNF expressing plasmid or TNF protein could be another approach to improve its anticancer value. It was found that systemic administration of the surface-shielded TNF expressing vector using transferrin-polyethylenimine (Tf-PEI) resulted in preferential expression of TNF in tumor cells, induced pronounced hemorrhagic tumor necrosis, and inhibited tumor growth in 3 murine tumor models. Neither a detectable change in TNF serum levels nor systemic TNF-related toxicity was observed[134]. Similarly, encapsulating TNF in pegylated liposomes (TNF-PEGL) dramatically improved the serum circulation time and accumulation of TNF in tumor tissue and increased the anticancer efficacy of radiation or chemotherapy in advanced solid tumors[135,136]. TNF can also be encapsulated in nanoparticles to maximize tumor damage and minimize systemic toxicity[137,138].

Enhancing the antitumor adaptive immune responses of TNF As an important mediator of innate immunity, TNF is capable of activating T cells and DCs to enhance host antitumor adaptive immune response. Administration of adenovirus-encoded TNF together with TNF-gene-engineered DCs elicited tumor-specific cytotoxic T cells and evoked prominent tumor suppression[139,140]. TNF behaved as an immunoadjuvant through inducing major histocompatibility complex (MHC) class I molecules or maintaining the maturation status of DC[141,142]. Similarly,

TNF gene-engineered CD8+ cytotoxic T cells had enhanced cytotoxicity and prolonged survival *in vivo* after adoptive transfer, and exerted a stronger antitumor immunity against the immunosuppressive IL-10-secreting B16 lung tumors in mice[143]. Injecting a TNF-engineered tumor vaccine into tumors strongly augmented the overall antitumor effectiveness through development of systemic antitumor immunity[144,145]. Anchoring the soluble TNF via a biotin-avidin-biotin bridge to the membrane of apoptotic melanoma cells was also found to enhance tumor immunogenicity[146].

Anti-TNF treatment in cancer prevention and therapy Because TNF is implicated in tumor growth under certain circumstances, anti-TNF treatment has been tested in some experimental systems for cancer prevention and therapy. TNF could be blocked with anti-TNF neutralizing monoclonal antibodies, soluble TNF receptors, or TNF autovaccination. Inhibiting TNF using the monoclonal antibody infliximab or recombinant human soluble TNFR-2 exerted strong antitumoral effects in mice with pancreatic tumors[147]. In phase I and II clinical trials, both infliximab and etanercept achieved prolonged disease stabilization in patients with metastatic breast cancer, recurrent ovarian cancer, or immunotherapy-resistant or refractory renal cell carcinoma[148–150]. TNF neutralization also significantly prevented the development of malignant pleural effusion in mice[151]. In addition to infliximab and etanercept, immunizing C57BL/6 mice with TNF autovaccine produced a 100-fold antibody response to TNF and significantly reduced both the number and size of metastases of the B16F10 melanoma cells[152]. However, due to the complexity of the role TNF plays in carcinogenesis, the potential tumor-promoting effect of TNF-modulating strategies should be carefully evaluated. Although TNF blockers did not increase overall tumor risk in patients with rheumatoid arthritis, a recent study showed that TNFR-1-mediated signals in antigen presenting cells and TNFR-2-mediated signals in T cells are critically required for effective tumor immune surveillance[153,154]. Thus, caution should be paid to anti-TNF therapy, as it might dampen tumor surveillance to favor cancer development.

Conclusions and perspectives

With better understanding of the molecular mechanisms of TNF-induced cellular signaling, it is becoming clear that TNF plays a major role in the development of different types of cancer. Thus, TNF could be a molecular target for cancer prevention. The cancer cell killing and anticancer immunity modulation properties of TNF render it a potential cancer therapeutic, which has already achieved promising results when used locally. However, the severe systematic toxicity of TNF substantially diminishes the enthusiasm for its application in a clinical setting, although direct tumor-targeting and modification of TNF partly overcome this hurdle. Recent reports found that induction of TNF at a low concentration by Smac mimetics effectively killed cells derived from different cancer types, suggesting that the anticancer activity of TNF could be achieved at non-toxic concentrations[16,155,156]. Thus, efforts should be made to characterize the TNF-resistance mechanism in order to establish effective means to potentiate TNF killing cancer cells. With such strategies, the TNF doses could be significantly reduced to the non-toxic levels that can be well tolerated by cancer patients. Finally, due to the dual and organ-specific roles of TNF in carcinogenesis, TNF-modulating approaches should be carefully evaluated and monitored in regard to cancer therapy and prevention.

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