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TNF-α in Cancer Treatment: Molecular Insights, Antitumor Effects, and Clinical Utility

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Key Words. Cancer • TNF-α • TNFR-1 • Tumor vasculature • Isolated limb perfusion

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Discuss the role of TNF-a in cancer survival and apoptosis.
2. Describe the mechanism of chemotherapy potentiation by TNF-a.
3. Explain the selective targeting of tumor vasculature by TNF-a.
4. Discuss TNFR-1 and TNFR-2 signaling.

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ABSTRACT

Tumor necrosis factor alpha (TNF-α), isolated 30 years ago, is a multifunctional cytokine playing a key role in apoptosis and cell survival as well as in inflammation and immunity. Although named for its antitumor properties, TNF has been implicated in a wide spectrum of other diseases. The current use of TNF in cancer is in the regional treatment of locally advanced soft tissue sarcomas and metastatic melanomas and other irresectable tumors of any histology to avoid amputation of the limb. It has been demonstrated in the isolated limb perfusion setting that TNF-α acts synergistically with cytostatic drugs. The interaction of TNF-α with TNF receptor 1 and receptor 2 (TNFR-1, TNFR-2) activates several signal transduction pathways, leading to the diverse functions of TNF-α. The signaling molecules of TNFR-1 have been elucidated quite well, but regulation of the signaling remains unclear. Besides these molecular insights, laboratory experiments in the past decade have shed light upon TNF-α action during tumor treatment. Besides extravasation of erythrocytes and lymphocytes, leading to hemorrhagic necrosis, TNF-α targets the tumor-associated vasculature (TAV) by inducing hyperpermeability and destruction of the vascular lining. This results in an immediate effect of selective accumulation of cytostatic drugs inside the tumor and a late effect of destruction of the tumor vasculature. In this review, covering TNF-α from the molecule to the clinic, we provide an overview of the use of TNF-α in cancer starting with molecular insights into TNFR-1 signaling and cellular mechanisms of the antitumor activities of TNF-α and ending with clinical response. In addition, possible factors modulating TNF-α actions are discussed. *The Oncologist* 2006;11:397–408

INTRODUCTION

Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine involved in apoptosis, cell survival, inflammation, and immunity acting via two receptors [1, 2]. Currently it

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is used in cancer treatment in the isolated limb perfusion (ILP) setting for soft tissue sarcoma (STS), irresectable tumors of various histological types, and melanoma intransit metastases confined to the limb [3]. TNF-α was isolated in 1975 from the serum of mice treated with bacterial endotoxin as the active component of "Coley's toxin" and was shown to induce hemorrhagic necrosis of mice tumors [4, 5]. It was almost a century ago that William Coley, a surgeon from New York, observed high fever and tumor necrosis in some cancer patients treated with his bacterial filtrate ("Coley's mixed toxins") [6]. A decade after its isolation, TNF-α was also characterized as "cachectin" and as T-lymphocyte differentiation factor [7, 8]. In 1984, the human *TNF*-α gene was cloned [9, 10], and a range of clinical experiments were set up, leading to a license from the European Agency for the Evaluation of Medicinal Products (EMEA) for the treatment of limb-threatening STS in an isolated perfusion setting [11].

TNF-α and TNF Receptor 1 Signaling

TNF-α is a 17-kDa protein consisting of 157 amino acids that is a homotrimer in solution. In humans, the gene is mapped to chromosome 6 [12]. Its bioactivity is mainly regulated by soluble TNF-α–binding receptors. TNF-α is mainly produced by activated macrophages, T lymphocytes, and natural killer (NK) cells. Lower expression is known for a variety of other cells, including fibroblasts, smooth muscle cells, and tumor cells. In cells, TNF-α is synthesized as pro-TNF (26 kDa), which is membrane-bound and is released upon cleavage of its pro domain by TNF-converting enzyme (TACE) [13].

As mentioned above, TNF-α acts via two distinct receptors [14]. Although the affinity for TNF receptor 2 (TNFR-2) is five times higher than that for TNFR-1 [15], the latter initiates the majority of the biological activities of TNF-α. TNFR-1 (p60) is expressed on all cell types, while TNFR-2 (p80) expression is mainly confined to immune cells [16]. The major difference between the two receptors is the death domain (DD) of TNFR-1 that is absent in TNFR-2. For this reason, TNFR-1 is an important member of the death receptor family that shares the capability of inducing apoptotic cell death [17]. Besides this apoptotic signaling, TNFR-1 is widely studied because it is a dual role receptor: next to induction of apoptosis, it also has the ability to transduce cell survival signals. Although signaling pathways are well defined nowadays, the life-death signaling regulation is still poorly understood [18, 19]. The TNFR-1 signaling pathways are depicted in Figure 1. Upon binding of the homotrimer TNF-α, TNFR-1 trimerizes, and silencer of death domain (SODD) protein is released [20]. TNFR-associated death

the adaptor proteins receptor interacting protein (RIP), TNFR-associated factor 2 (TRAF-2), and Fas-associated death domain (FADD) [21]. In turn, these adaptor proteins recruit key molecules that are responsible for further intracellular signaling. When TNFR-1 signals apoptosis, FADD binds pro-caspase-8, which is subsequently activated. This activation initiates a protease cascade leading to apoptosis, also involving the mitochondria and with caspases as key regulators [22]. The ultimate event in this apoptotic signaling is the activation of endonucleases, like EndoG, resulting in DNA fragmentation. Alternatively, when TNFR-1 signals survival, TRAF-2 is recruited to the complex, which inhibits apoptosis via cytoplasmic inhibitor of apoptosis protein (cIAP). The binding of TRAF-2 initiates a pathway of phosphorylation steps resulting in the activation of cFos/cJun transcription factors via mitogen-activated protein kinase (MAPK) and cJun N-terminal kinase (JNK) [23]. The major signaling event of TRAF-2 and RIP is the widely studied activation of nuclear factor kappa B (NF-κB) transcription factor via NF-κB–inducing kinase (NIK) and the inhibitor of κB kinase (IKK) complex [24]. Both the NF-κB and cFos/cJun transcription factors induce transcription of antiapoptotic, proliferative, immunomodulatory, and inflammatory genes. NF-κB is the major survival factor in preventing TNF-α–induced apoptosis, and inhibition of this transcription factor may improve the efficacy of apoptosis-inducing cancer therapies [25]. NF-κB activation in many human malignancies is aberrant or constitutive, and its role in the regulation of the apoptosis–proliferation balance in tumor cells indicates its role in oncogenesis [26, 27]. For further details on the dual signaling of TNFR-1, see Figure 1.

Implications for Cellular Mechanisms Underlying TNF-α Effects During Solid Tumor Treatment

It is widely known that TNF-α induces hemorrhagic necrosis in a certain set of tumor types. To investigate the underlying mechanisms of TNF-α action during ILP of solid tumors in humans, we set up perfusion models in rats and reported that hemorrhagic necrosis was much greater in tumors treated with TNF-α and chemotherapeutic drugs [28]. In addition, we showed a synergistic antitumor effect of the combination treatment with TNF-α and chemotherapeutic drugs [29]. In contrast, TNF-α alone induced only a mild central necrosis, and there was no objective tumor response observed. The same rat models also revealed that the addition of TNF-α improved the accumulation of chemotherapeutic drugs selectively in the tumor up to three- to sixfold. The augmented uptake of melphalan added to the molecular properties of this small molecule (distribution by gradient

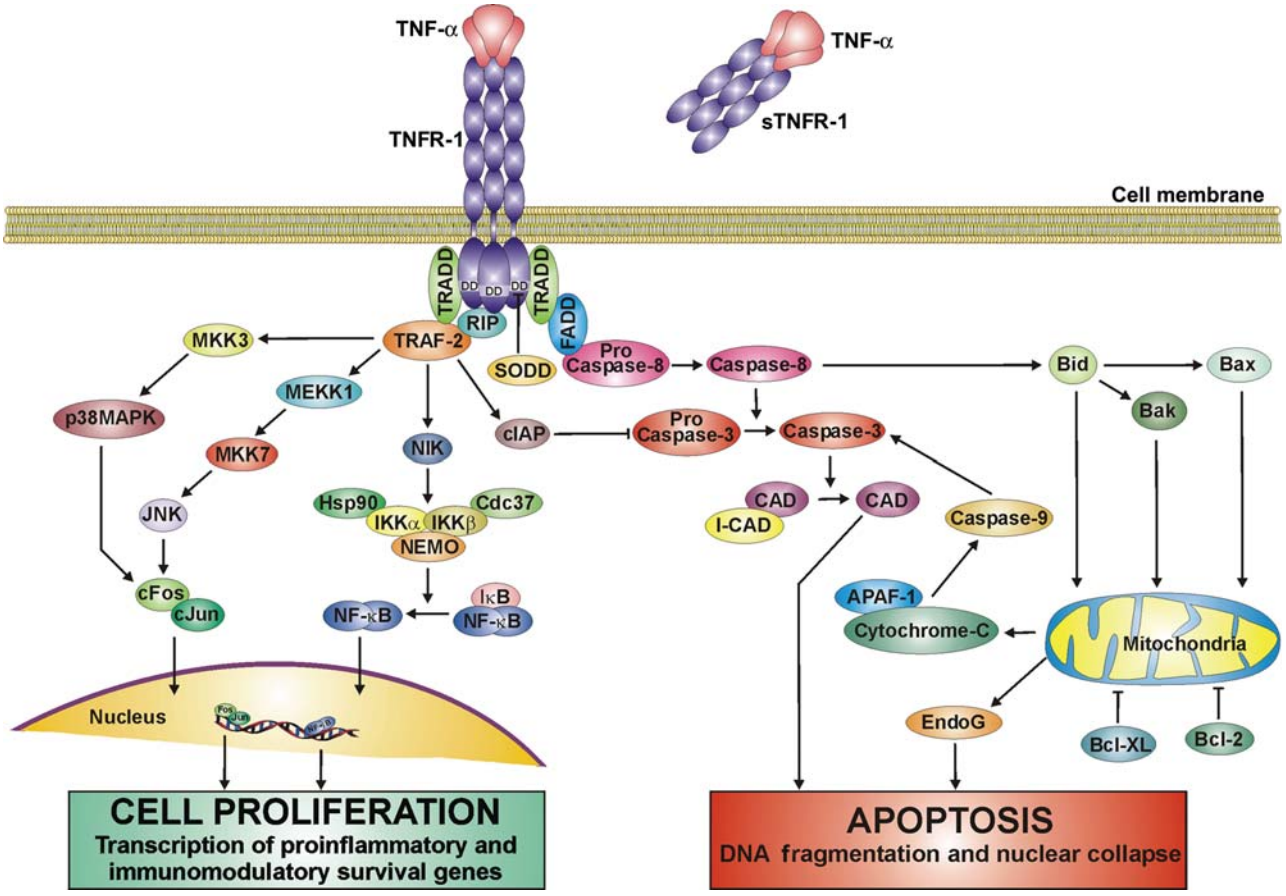

Figure 1. Tumor necrosis factor receptor 1 (TNFR-1) signaling pathway. Tumor necrosis factor alpha (TNF-α) activates both survival and proliferation pathways along with apoptotic pathways via TNFR-1. The separate pathways are well defined, while the survival-death balance regulation remains unclear. Abbreviations: APAF-1, apoptosis protein activating factor 1; Bcl-2, B-cell lymphoma 2; Bid, Bak, Bax, and Bcl-XL, mitochondrial proteins of the Bcl-2 family; CAD, caspase-activated DNAse; Caspase-3/8/9, cysteine aspartase (apoptotic protease) 3/8/9; Cdc37, co-chaperon of HSP90; cIAP, cytoplasmic inhibitor of apoptosis; cFos/cJun, transcription factors; DD, death domain; EndoG, mitochondrial DNAse; FADD, Fas-associated DD; HSP90, heat shock protein 90; I-CAD, inhibitor of CAD; IκB, inhibitor of NF- κB; IKKα/β, IκB kinase; JNK, cJun n-terminal kinase; MEKK1, mitogen-activated protein kinase/extracellular signal–related kinase kinase 1; MKK3/7, MAPK kinase 3/7; NEMO, NF-κB essential modulator; NF-κB, nuclear factor kappa B transcription factor; NIK, NF-κB inducing kinase; p38MAPK, p38 mitogen-activated protein kinase; RIP, receptor interacting protein; SODD, silencer of DD; sTNFR-1, soluble TNFR-1; TNF-α, tumor necrosis factor alpha; TNFR-1, TNF receptor 1; TRADD, TNF receptor-associated DD; TRAF-2, TNF receptor-associated factor-2.

tions close to the 50% inhibitory concentration (IC_{50}) in STS cells in vitro [30, 31]. These levels result in tumor cell kill in the ILP setting, and melphalan can distribute within the well-perfused parts of the tumor even though the intratumoral pressure is high. This selective uptake of melphalan by the tumor was also observed when other vasoactive drugs were used in the ILP setting (see below). It is important to note that the cell lines we used were not sensitive to TNF-α in vitro, which is in accordance with other reports describing a lack of effect of TNF-α and no synergism with cytotoxic drugs in cell lines [32, 33]. Next to these ILP data, studies in mice and rats showed that a systemic low dose of TNF-α augments the antitumor activity of pegylated liposomal doxorubicin [34, 35]. These observations are comprehensible clues that mechanisms underlying the TNF-α effect during solid tumor treatment cannot be caused by a direct cytotoxic or cytostatic effect of TNF-α toward the tumor cells. It was suggested that, rather than tumor cells themselves, cells of the tumor stroma may be responsible for the observed antitumor effect of TNF-α in patients. This hypothesis was confirmed by data from mice experiments revealing that TNF-α had a cytotoxic effect on tumor vasculature [36].

Angiogenesis and Tumor-Associated Vasculature

Angiogenesis, the formation of new blood vessels from preexisting ones, has become a major field of research, mainly in cancer [37]. Angiogenesis is essential for a tumor to pro-

vide the tumor cells with oxygen and essential nutrients for growth and to metastasize hematogenically [38]. A growing tumor activates surrounding vessels by secreting angiogenic factors, thereby changing the dormant tumor phenotype toward an angiogenic one, the so-called "angiogenic switch" [39]. Activated endothelial cells have to migrate toward the tumor along a newly formed matrix, the components of which are synthesized by themselves, tumor cells, and other cells such as macrophages and fibroblasts [40]. Figure 2 shows schematically the process of tumor angiogenesis, which can be divided into four different stages. A small, dormant tumor (stage 1) can, depending on the nature of the tumor and its microenvironment, make the angiogenic switch to ensure exponential growth. The tumor secretes growth factors to activate endothelial cells of surrounding vessels (stage 2). Upon activation, these endothelial cells start to migrate and proliferate toward the tumor. Only one endothelial cell starts an angiogenic sprout and develops into an endothelial tip cell migrating along the extracellular matrix (ECM) and guiding the following so-called stalk endothelial cells (stage 3) [41]. Finally, the growing tumor is connected to the vasculature (stage 4). In addition to growth and proliferation, the tumor can metastasize. Malignant tumor cells, by invasion of the vessels, ECM degradation, attachment, and homing to target sites can form distal metastases [42]. The process of tumor angiogenesis results in a tumor-associated vasculature (TAV) that is rather chaotic, both in structure and function. In comparison with normal vessels, tumor vessels have a noncontinuous endothelium, an enlarged basal membrane, and an aberrant pericyte coverage [43]. Frequently in tumors, the vascular hierarchy of arterioles, capillaries, and venules is absent, resulting in loosely associated pericytes [44]. From animal experiments, it is known that pericytes are present in small tumors and more abundant in large tumors [45]. The contribution of pericytes to (anti)-angiogenic therapies is currently an attractive focus of research. On one hand, these characteristics impair tumor blood flow, delivery of oxygen, and therapeutics to the tumor cells and vessel functionality, but on the other hand, these differences may be used as a target. The solid tumors treated by ILP with TNF-α have a massive vascular structure consisting of vessels with a phenotype specific to tumor vessels, although detailed study needs to clarify the exact contribution of the TAV to the observed antitumor responses.

Activity of TNF-α in Solid Tumors: Hypothetical Mechanism

The vascular differences mentioned above are depicted in Figure 3A. These differences are responsible for a more leaky vasculature in the tumor, with average intraendothelial gaps of 400 nm, depending on the tumor type [46]. Blood cells such as lymphocytes and monocytes easily adhere and extravasate into the tumor. We speculate that the endothelial cells of the tumor vessels, compared with normal vessels, have an upregulation of TNFR-1 on their membranes, which may be dependent on TNFR-1–upregulating factors produced by vessel-surrounding cells like tumor cells and macrophages. This upregulation, along with the specific architecture of the

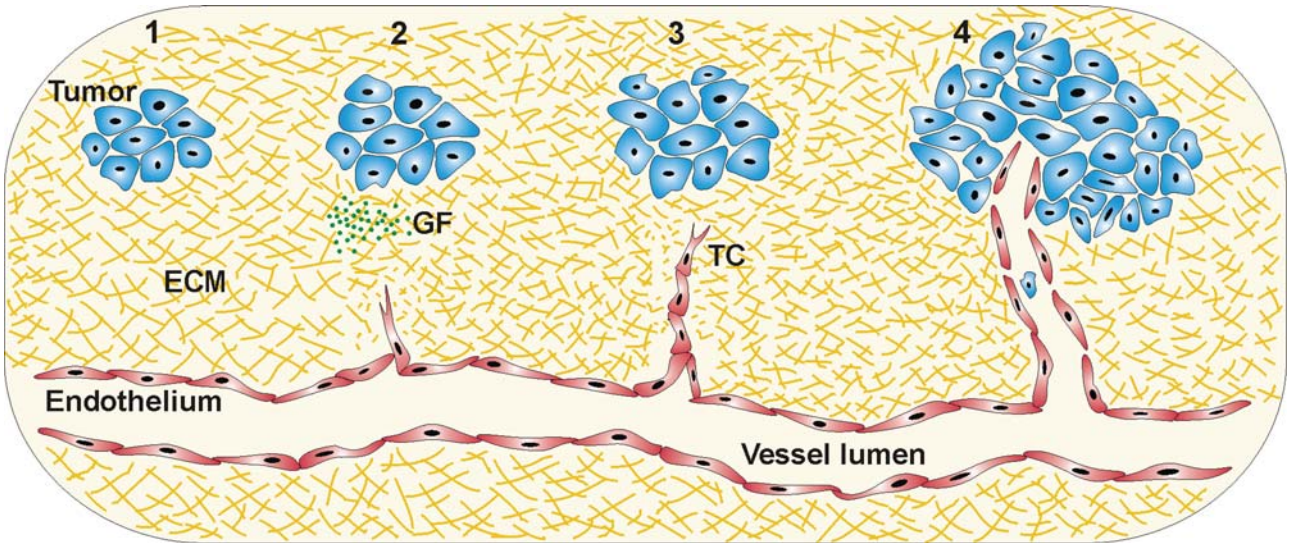

Figure 2. The sequential steps during tumor angiogenesis. The dormant tumor in stage 1 starts to secrete angiogenic growth factors (GF) after its "angiogenic switch", which is accomplished by an imbalance in pro- and antiangiogenic factors. These GFs activate endothelial cells of surrounding vessels, and these cells start to migrate (stage 2) and proliferate toward the tumor. An endothelial tip cell (TC) is guiding this sprouting process (stage 3). In stage 4, the novel sprout has formed a lumen and the tumor is connected to the vasculature, thereby ensuring its growth and enabling it to metastasize hematogenically. Abbreviation: ECM, extracellular matrix.

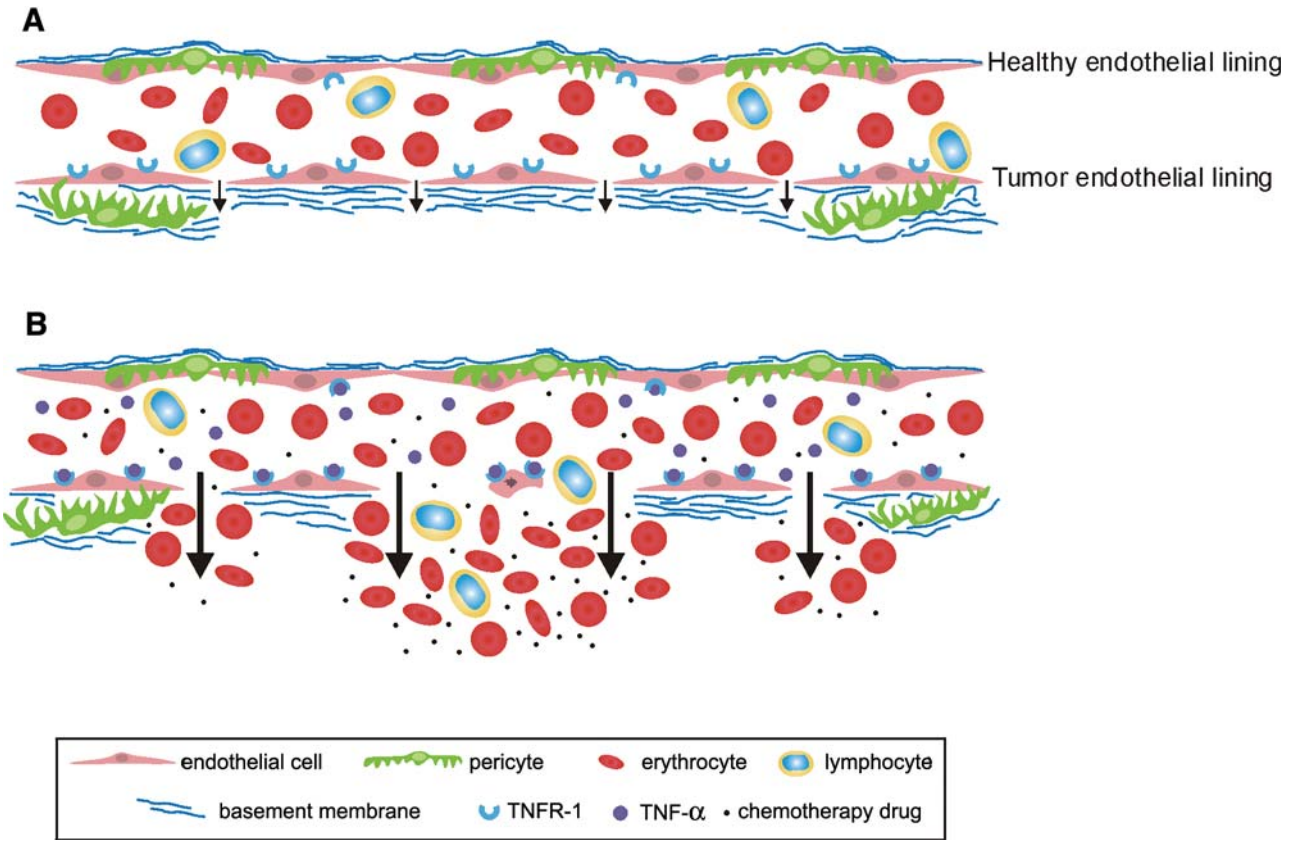

Figure 3. Differences between healthy and tumor endothelium and a proposed mechanism of the tumor necrosis factor alpha (TNF-α) effect. **(A):** Schematic representation of a vessel with healthy endothelial lining (upper) and tumor endothelial lining (lower). Healthy endothelium is a continuous lining of endothelial cells, covered with pericytes and with a thin basement membrane. The permeability is low, and extravasation is tightly organized. In contrast, tumor endothelium does not consist of a continuous lining of endothelial cells; it lacks pericyte coverage, and the basement membrane is thickened. This phenotype results in greater permeability of the tumor vessel (small arrows). We hypothesize that tumor endothelium exhibits a higher TNFR-1 expression level because of TNFR-1–upregulating factors produced by vessel-surrounding cells. **(B):** Upon TNF-α treatment with isolated limb perfusion, the healthy endothelium stays intact because it is TNF-α insensitive by its lack of TNFR-1 expression on the membrane. Tumor endothelium binds TNF-α, which affects the endothelial phenotype and induces apoptosis in some endothelial cells. These two processes result in an enormous induction of vessel permeability (big arrows). As a result, the chemotherapy drug is well distributed throughout the tumor, and a strong extravasation of erythrocytes results in massive hemorrhagic tumor necrosis. The selectively targeted tumor vessels are no longer functional and regress.

endothelial lining, defines the tumor vessels as a specific target for TNF-α treatment (Fig. 3B). When TNF-α is administered via ILP to treat solid tumors, it binds soluble receptors, and because of the high dosage, TNFR-1 receptors on tumor endothelial cells become occupied. Healthy endothelium, in contrast, also binds TNF-α; however, because of a lower number of membrane-bound TNFR-1 receptors (most TNFR-1 is stored in the golgi apparatus [47]), there is no toxicity. We propose that this TNF-α to TNFR-1 binding results in hyperpermeability of the tumor vessels, and erythrocytes and other blood cells extravasate. The strong extravasation of erythrocytes results in massive hemorrhagic necrosis of the tumor. As a result of the direct cytotoxicity of high-dose TNF-α to endothelial cells, some of these cells undergo apoptosis, and

ity (Fig. 3B). Several studies have shown that a lower dose of TNF-α results in comparable responses [48, 49], suggesting that a lower dose still may induce these antivascular effects. The healthy vessels, however, stay intact; no apoptosis and no extravasation occurs. The observed synergistic activity of TNF-α and chemotherapeutic drugs is a consequence of this double-induced hyperpermeability. This hyperpermeability throughout the tumor facilitates the augmented accumulation and distribution of the drug in the tumor, resulting in better exposure of the tumor cells to the cytostatic agent [30]. This double-induced hyperpermeability, along with the dual targeting—the TAV (by TNF-α) and the tumor cells (by the chemotherapy drug)—is one explanation for the observed synergistic response of tumors to TNF-α and chemotherapy

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