The Role of TNF in Cancer

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Abstract Tumor necrosis factor (TNF) is an extraordinarily pleiotropic cytokine with a central role in immune homeostasis, inflammation, and host defense. Dependent on the cellular context, it can induce such diverse effects as apoptosis, necrosis, angiogenesis, immune cell activation, differentiation, and cell migration. These processes are of great relevance in tumor immune surveillance, and also play crucial roles in tumor development and tumor progression. It is therefore no surprise that TNF in a context-dependent manner displays pro- and antitumoral effects. Modulation of the activity of the TNF–TNF receptor system thus offers manifold possibilities for cancer therapy. In fact, TNF in combination with melphalan is already an established treatment option in the therapy of advanced soft tissue sarcoma of the extremities and many preclinical data suggest that TNF neutralization could also be exploited to fight cancer or cancer-associated complications.

Abbreviations AOM: Azoxymethane; APC: Antigen presenting cell; COX-2: Cyclo-oxygenase-2; cIAP1/2: Cellular inhibitor of apoptosis protein-1/2; DMBA: 7,12-di-methylbenz[α]-anthracene; DSS: Dextran sulfate sodium salt; EMT: Epithelial–mesenchymal transition; ERK: Extracellular-regulated kinase; FADD: Fas-associated death domain; GSK3: βGlycogen synthase kinase; ILP: Isolated limb perfusion; JNK: cJun N-terminal kinase; LMCV: Lymphocytic choriomeningitis virus; MCA: 3´-Methylcholanthrene; MCP1: Monocyte chemoattractant protein-1; MDR2: Multidrug resistance p-glycoprotein 2; MMP-9: Matrix metalloprotease-9; OA: Ocadaic acid; PDAC: Pancreatic ductal adenocarcinoma; p38 MAPK: p38 mitogen-activated protein (MAP) kinase; RANK: Receptor activator of NF-kappaB; TPA: 12-0-Tetradecanoyl-phorbol-13-acetate; TRPV1; Transient receptor potential channel vanilloid type 1; VEGFR2: Vascular endothelial growth factor (VEGF) receptor

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1 Introduction

The prime sources of TNF are activated immune cells, especially macrophages and T-cells, but it can also be produced by a variety of other cell types including fibroblasts and tumor cells (Wajant et al. 2003). TNF is a trimeric type II transmembrane protein consisting of about 80 amino acid residues comprising proline-rich cytoplasmic domain involved in membrane trafficking and receptor binding-induced reverse signaling, a single transmembrane domain (TM) and an extracellular domain containing the characteristic TNF homology domain (THD), which is separated from the TM by a stalk region (Bodmer et al. 2002). The THD mediates trimerization of the molecule and is also responsible for receptor binding. The stalk region contains a processing site for the matrix metalloprotease TNFα converting enzyme (TACE)/ADAM17, allowing the release of a soluble trimeric form of TNF (Fig. 1). Transmembrane TNF, as well as soluble TNF, interacts with

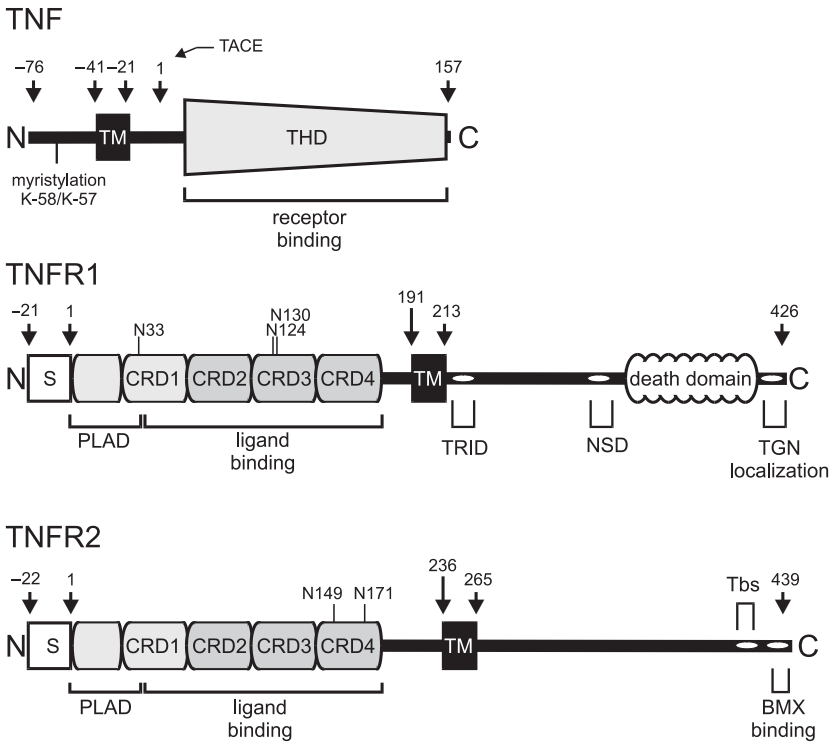

Fig. 1 Human TNF, TNFR1, and TNFR2. Amino acid numbering refers to the mature receptors and processed soluble TNF, respectively. Myristylated lysine residues in TNF and glycosylated asparagine residues of TNFR1 and TNFR2 are indicated. *S* signal peptide, *CRD* cysteine rich domain, *NSM* neutral sphingomyelinase activating domain, *PLAD* pre-ligand binding assembly domain, *Tbs* TRAF2 binding site, *TGN* trans-Golgi network, *THD* TNF homology domain, *TM* transmembrane domain, *TRID* TNFR1 internalization domain. For details see text

two distinct receptors, TNFR1 and TNFR2, both belonging to the TNF receptor superfamily (Wajant et al. 2003). Importantly, binding of transmembrane TNF results in strong activation of each of the two TNF receptors, while binding of soluble TNF triggers only TNFR1 signaling (Grell et al. 1995). TNFR1 and TNFR2 belong to different subgroups of the TNF receptor superfamily. TNFR1 contains a death domain (DD) in the cytoplasmic part and interacts by virtue of this module with cytoplasmic death domain-containing adapter proteins (Fig. 1). In contrast, TNFR2 lacks a death domain and is instead able to directly interact with adapter proteins of the TNF receptor associated factor (TRAF) family by a short TRAF binding site (Bodmer et al. 2002; Wajant et al. 2003). Notably, TNFR1 also exploits TRAF adapter proteins for signal transduction, but does not directly interact with these molecules (Fig. 2). TNF binding to TNFR1 results in the recruitment of the death domain-containing adapter protein TRADD (TNF receptor associated death domain) and the death domain-containing serine–threonine kinase RIP (receptor interacting protein). The latter together with TRAF2, which is indirectly recruited in the TNFR1 signaling complex due to its association with TRADD, recruit and activate the inhibitor of IκB kinase (IKK) complex, the crucial bottle neck for activation of the classical NFκB pathway (Perkins 2007; Wajant et al. 2003). TRAF2 and RIP also mediate activation of the cJun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinase cascades. TRAF2 strongly binds to cIAP1 (cellular inhibitor of apoptosis protein-1) and cIAP2 and therefore also directs these caspase inhibitory E3 ligases into the TNFR1 signaling complex (Wang et al. 1998; Fig. 2). There is evidence that these proteins fulfill two distinct functions: first, they contribute to NFκB activation by ubiquitination of RIP and second they prevent activation of caspase-8 and thus prevent TNFR1-induced apoptosis (Wang et al. 1998).

By help of its death domain TNFR1 also induces, by less understood mechanisms, the activation of acidic sphingomyelinase and the extracellular-regulated kinase (ERK) signaling pathway (Schwandner et al. 1998). By death domain-independent mechanisms, TNFR1 is also linked to the stimulation of neutral sphingomyelinase and again the ERK signaling pathway (Adam-Klages et al. 1996). TNFR1 can also induce cell death by two distinct pathways, both emerging from its death domain. First, TNFR1 induce necrosis, which is mediated by RIP- and TRAF2-driven excessive generation of reactive oxygen species and subsequent prolonged JNK signaling (Lin et al. 2004). Second, TNFR1 induce caspase-mediated apoptosis, which involves the death domain-containing adapter proteins TRADD and FADD (Fas-associated death doamin) and also the FADD-associated initiator caspase, caspase-8 (Wajant et al. 2003). Notably, there is evidence that the pro-apoptotic interplay of TRADD, FADD, and caspase-8 takes place in a secondary cytoplasmic multiprotein complex that is formed after release from TNFR1. This complex might also contain TRAF2, RIP, and the TRAF-associated IAP proteins cIAP1 and cIAP2 and might also recruit the caspase-8 inhibitory FLIP protein. Dependent from the presence of the inhibitory FLIP and IAP proteins, the cytoplasmic complex is able to process and activate caspase-8 and apoptotic cell death (Muppidi et al. 2004). There is also evidence that TNFR1-induced apoptosis in contrast to

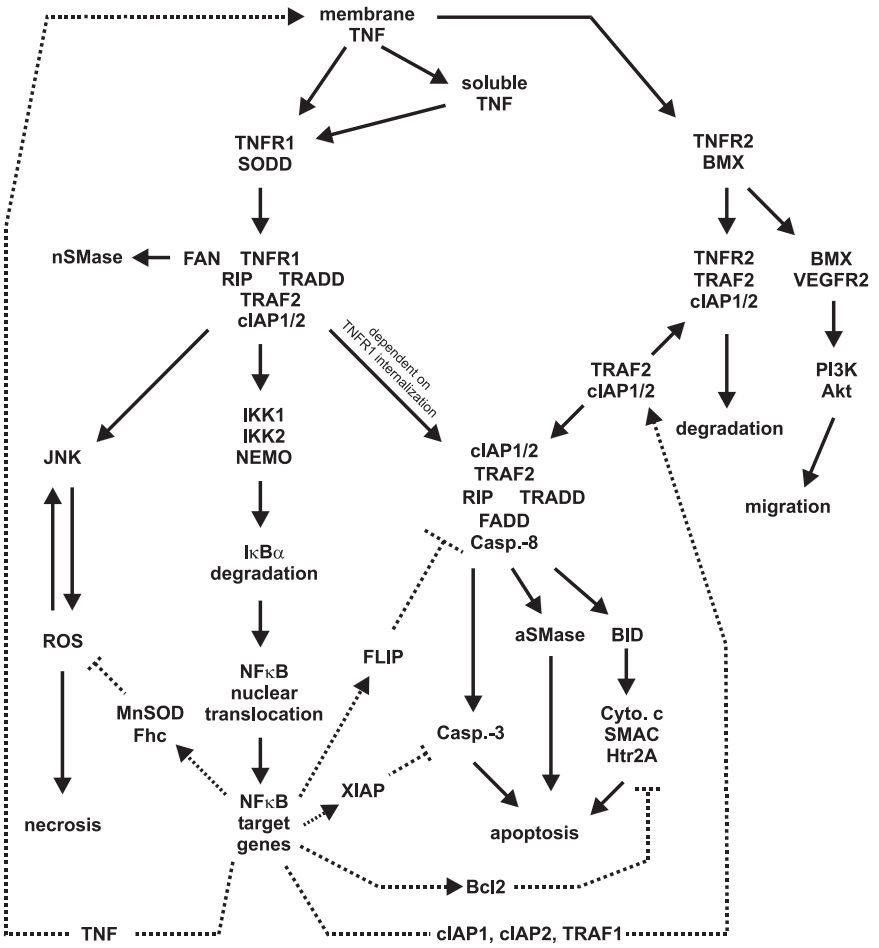

Fig. 2 The TNF signaling network. Dotted lines refer to TNF receptor-induced events requiring protein synthesis. For details see text

TNFR1-induced NFκB activation depend on internalization of the receptor signaling complex. How the later finding match together with the formation of the proapoptotic secondary complex is currently not clearly understood (Schütze et al. 2008). As caspase-8 can cleave RIP, the apoptotic pathway actively represses necrosis induction (Lin et al. 1999). Consequently, TNF-induced necrosis become especially apparent in cells treated with pharmacological inhibitors of caspases or cells that for other reasons are resistant against caspase-mediated apoptosis. TNFR2 can also activate the classical NFκB signaling pathway and the various MAP kinase cascades, but the underlying signaling mechanisms are mainly unknown (Wajant et al. 2003). Activated TNFR2 interacts with TRAF2, which secondarily recruits

TRAF1, cIAP1, and cIAP2 into the TNFR2 signaling complex (Fig. 2), but the relevance of these components of the TNFR2 complex for activation of the aforementioned pathways has not been clarified so far (Rothe et al. 1994, 1995). The TNFR2-TRAF2 interaction came along with translocation into lipid rafts and depletion of cytosolic TRAF2 pools (Fotin-Mleczek et al. 2002). The latter is enhanced with time by lipid raft-associated proteasomal degradation of TRAF2 (Fotin-Mleczek et al. 2002; Li et al. 2002; Wu et al. 2005). In accordance with the crucial role of TRAF2 and the associated IAP proteins in preventing TNFR1-induced caspase-8 activation, TNFR2-induced depletion and degradation of TRAF2 result in strong enhancement of TNFR1-induced apoptosis (Weiss et al. 1997; Chan and Lenardo 2000; Fotin-Mleczek et al. 2002). TNFR2 further constitutively associates with the tyrosine kinase BMX (Pan et al. 2002). TNFR2 stimulation results in interaction of BMX with the receptor tyrosine kinase VEGFR2 (vascular endothelial growth factor (VEGF) receptor-2) and reciprocal transphosphorylation of the two molecules (Zhang et al. 2003). Phosphorylated BMX than serves as a docking site for the p85 subunit of PI3 kinase, leading to the activation of the Akt signaling pathway. This way TNFR2 can mediate proliferation and migration of endothelial cells (Pan et al., 2002; Zhang et al., 2003).

2 Antitumoral Effects of TNF

The literature on TNF is clearly dominated by its pleiotropic proinflammatory functions and its crucial role in autoimmune pathologies such as rheumatoid arthritis, Crohn's disease, and psoriasis. As it is already evident from its name, however, TNF has been originally identified as a tumor necrosis inducing factor (Wang et al. 1985). The observation that microbial challenge or treatment with microbial compounds, such as LPS, lead to the protection of mice against experimental cancer resulted in the identification of TNF as the main responsible factor. Notably, TNF has been independently identified as cachectin, a proinflammatory factor that mediates cancer-associated fatigue and muscle wasting (Beutler et al. 1985). Early efforts to use recombinant TNF in tumor therapy, prompted by the before mentioned findings, failed, however, due to the severe inflammatory side effects associated with systemic TNF receptor activation. Nevertheless, safe local administration of TNF in combination with melphalan by isolated limb perfusion (ILP) is now an established treatment option for locally advanced soft limb sarcomas, and a variety of preclinical (van Horssen et al. 2006) and clinical studies are underway aiming antibodies to restrict TNF activity to the tumor area or to inhibit the therapy limiting side effects of TNF without affecting its antitumor properties (see chapter by Gerspach et al). Notably, it turned out that the before mentioned antitumoral effects of TNF in ILP are not caused by its capability to trigger apoptotic and necrotic signaling pathways, but is instead rather an indirect consequence of its inflammation-related capability to regulate endothelial permeability. Thus, TNF induces hyperpermeability in the tumor-associated vessels facilitating tumor entry of blood cells, and also enhances

accumulation of melphalan, together yielding a strong antitumoral effect (van Horssen et al. 2006).

A more "natural" antitumoral role of TNF has been further observed in mice expressing the p53 and retinoblastoma protein inhibitory SV40 large T antigen under control of the rat insulin promoter (RIP-Tag2 mice), causing multistage carcinogenesis (Müller-Hermelink et al. 2008). Transfer of in vitro activated T antigen-specific Th1 $CD4^+$ cells in RIP-Tag2 mice enhanced their survival time and inhibited tumor growth and angiogenesis in a TNF- and IFNγ-dependent manner. Notably, this inhibitory effect was not related to apoptosis induction. When either IFNγ action was blocked by antibodies or TNFR1 activation were prevented by crossing RIP-Tag2 mice with TNFR1 knockout mice, the Tag-specific Th1 cells enhanced tumor development (Müller-Hermelink et al. 2008). In a variation of this model that is based on the use of RIP-Tag2 mice that has been crossed with mice carrying the lymphocytic choriomeningitis virus (LMCV) glycoprotein (GP) under control of the RIP promoter, there was further evidence for a role of $CD8^+$ T-cells in tumor control (Calzascia et al. 2007). Adoptively transferred GP-specific $CD8^+$ T-cells showed significant reduced proliferation after recovering from pancreatic draining lymphe nodes when they lack TNF or TNFR2 expression, but behaved normal when TNFR1 was absent. Thus, TNFR2-mediated costimulation seems to be important in this model to ensure proper activation of tumor antigen-specific $CD8^+$ T cells. On the other site, TNFR1 expression in the host cell was also necessary in this study to reach optimal activation of the adoptively transferred GP-specific $CD8^+$ T-cells pointing to an additional role of TNF in this model related to TNFR1 signaling in antigen presenting cells (APC; Calzascia et al., 2007).

3 Pro-Tumoral Functions of TNF

3.1 TNF and Skin Carcinogenesis

First genetic evidence pointing to a role of TNF in malignancy was obtained by analysis of experimental skin carcinogenesis. Mice of different genetic background that were sequentially treated with a single dose of the carcinogen 7,12-di-methylbenz[α]-anthracene (DMBA) and 2–3 months with 12-0-tetradecanoyl-phorbol-13-acetate (TPA) or ocadaic acid (OA) develop in up to 100% of challenged mice papillomas. In this two step model of skin carcinogenesis, DMBA acts as a tumor initiator causing genetic alterations, while TPA or OA promote tumor development by facilitating the dominant growth of cells bearing appropriate mutations. In TNF-deficient mice or mice treated with a TNF-neutralizing monoclonal antibody, the DMBA/TPA model of skin carcinogenesis, however, results in reduced papilloma development and diminished papilloma numbers per mouse (Moore et al. 1999; Suganuma et al. 1999). While analysis of DNA-adduct formation

delivered no evidence for a role of TNF in DMBA-induced tumor initiation, TPA-induced activation of PKCα and AP1 are reduced in TNF-deficient mice, pointing to role in tumor promotion (Arnott et al. 2002). Analysis of TNFR1 and TNFR2 knockout mice further revealed that both TNF receptors contribute to DMBA/TPA-induced carcinogenesis with a more important role of TNFR1 (Arnott et al. 2004). TNF is upregulated in the epidermis within hours after TPA treatment, and TNF-deficient mice show much lower infiltration of neutrophils and eosinophils after TPA treatment than wild-type mice. TPA-induced TNF production, epidermal hyperplasia, and leukocyte infiltration are blocked in skin derived from transgenic mice expressing the NFκB inhibitory Smad7 protein (Hong et al. 2007). In accordance with evidence suggesting that clonal expansion of mutated follicular stem cells underlies carcinogenesis in the DMBA/TPA model (Binder et al. 1997), transcription of matrix metalloprotease-9 (MMP-9) is induced in follicular epithelial cells early in TPA promotion and after repeated TPA treatments also in interfollicular keratinocytes (Scott et al. 2004). Notably, migration of keratinocytes in vitro is dependent on endogenous TNF and MMP-9 (Scott et al. 2004). Thus, the tumor promoting activity of TPA considerably relies on induction of TNF and TNF-dependent pro-inflammatory events. Transformation and anchorage-independent growth of the epidermal cell line CI41 by benzo[α] pyrene-7,8-diol-9,10-epoxide, a carcinogenic metabolite of benzo[α]pyrene, which has been implicated in smoking-related cancer, also base significantly on TNF induction (Ouyang et al. 2007). However, TNF not necessarily has an obligate role in carcinogenesis. In fact, 3′-methylcholanthrene (MCA)-triggered formation of fibrosarcoma has been found to be significantly enhanced in TNF-deficient mice (Swann et al. 2007). The qualitatively contrasting effects of TNF in the DMBA/TPA and MCA model of carcinogenesis might be related to the strength of the associated inflammatory processes. While the DMBA/TPA model is strongly associated with inflammation, development of MCA-related tumors has only a minor inflammatory component.

3.2 TNF and Hepatic Carcinogenesis

Compensatory hepatocyte proliferation together with suppression of apoptosis occurs regularly after liver injury by non-genotoxic compounds and can induce preneoplastic alterations that might ultimately result in tumor formation. The injury-induced regenerative response includes expansion of a progenitor cell compartment called oval cells that become either hepatocytes or biliary epithelial cells. Upon liver damage induced with a choline-deficient ethionine-supplemented (CDE) diet, TNF can be found in oval cells and infiltrated leukocytes. More relevant, TNFR1-deficient mice show reduced oval cell number after 2–4 weeks of CDE diet and less liver tumors after long time diet, while TNFR2-deficient mice showed no changes compared to wt mice (Knight et al. 2000). Thus, these data suggest that TNF contributes to liver carcinogenesis early in the preneoplastic

phase by driving oval cell proliferation (Fig. 3). TNF and NFκB activation in hepatocytes have also been identified as crucial factors accelerating tumor progression in hepatocarcinogenesis, which spontaneously occur in multidrug resistance p-glycoprotein 2 (Mdr2)-knockout mice. In this model hepatocytes display enhanced proliferation associated with increased hyperploidy, parenchymal infiltration of TNF expressing inflammatory cells, and dysplasia, which over an adenoma-like intermediate step progress into hepatocellular carcinoma at an age of about 7 months (Pikarsky et al. 2004). Experiments with transgenic Mdr2-knockout mice allowing tetracyclin-regulated expression of a nondegradable deletion mutant of IκBα in hepatocytes showed that NFκB signaling in the latter is dispensable for the occurrence of the early neoplastic events while it is required during tumor progression to prevent apoptosis (Pikarsky et al. 2004). As treatment with TNF-blocking antibodies displayed a similar effect, it is tempting to speculate that TNF facilitates tumor progression in this model by inducing NFκB-regulated anti-apoptotic proteins in hepatocytes (Fig. 3).

3.3 TNF and Gastrointestinal Carcinogenesis

The importance of chronic inflammation for gastrointestinal carcinogenesis is particularly evident for gastric cancer and colitis-associated cancer. While gastric cancer is associated with chronic inflammation related to infections with *Helicobacter pylori*, the latter primarily develops in patients suffering from ulcerative colitis and is responsible for up to 5% of colorectal cancers (Correa 2003). In a mouse model of ulcerative colitis-associated carcinogenesis, the incidence of tumors induced by a single challenge with the pro-carcinogen azoxymethane (AOM) is enhanced by triggering colitis-like ongoing colonic inflammation by repeated cycles of dextran sulfate sodium salt (DSS) administration. Based on a former study showing a crucial role of NFκB activity in the AOM/DSS model of colon carcinogenesis (Greten et al. 2004), it has been recently demonstrated that tumor incidence and tumor-associated symptoms such as body weight loss and diarrhea are reduced in TNFR1-deficient mice (Fig. 3; Popivanova et al. 2008). TNF is not or poorly expressed in untreated or AOM challenged mice, but become readily detectable after DSS-induced inflammation in infiltrating mononuclear cells (Popivanova et al. 2008). TNF was also observed in colon biopsies from patients with active ulcerative colitis or colorectal cancer (Popivanova et al. 2008). While apoptosis in AOM/DSS-induced cancer was not affected in TNFR1 knockout mice, expression of cyclo-oxygenase-2 (COX-2) and the chemokines CXCL-1/KC and monocyte chemoattractant protein-1 (MCP1) as well as tumor infiltration by their target cells (neutrophils and macrophages) were strongly reduced. The relevance of this inflammatory defect was further substantiated by the observation that chimeric mice with transplanted TNFR1-deficient bone marrow cells showed a strongly reduced tumor incidence in the AOM/DSS model compared with chimeric mice that have

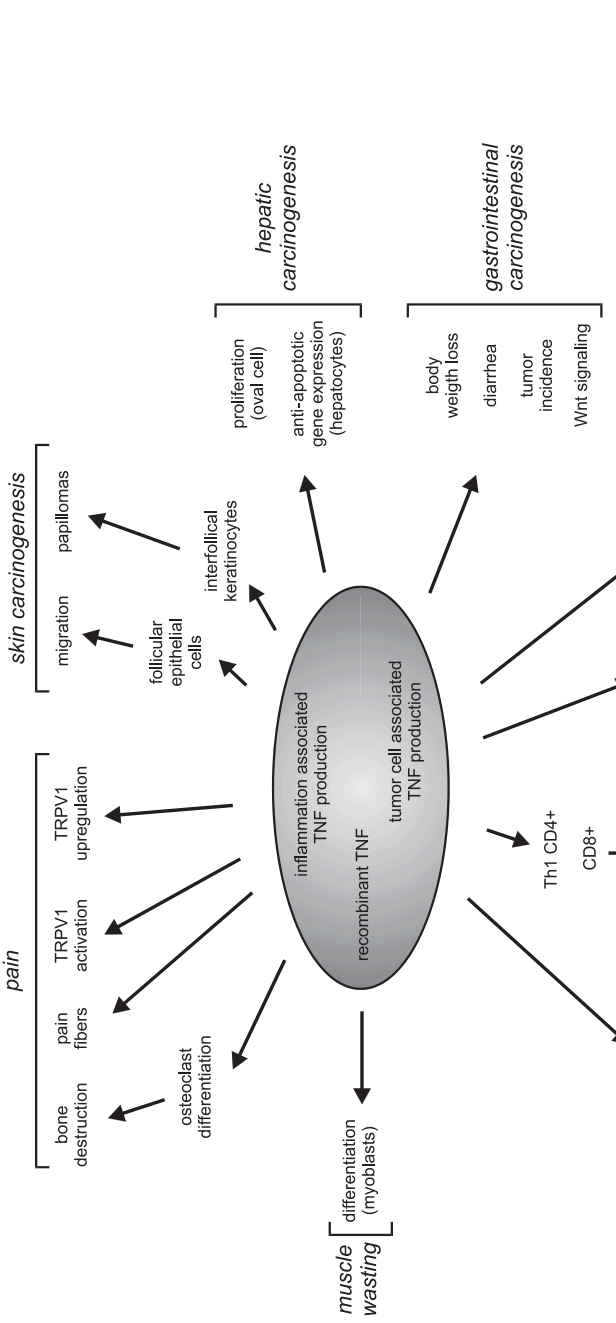

Fig. 3 Cancer-related effects of TNF. In accordance with its immunostimulatory functions, TNF can contribute to tumor suppression by the immune system. However, these capabilities can be hijacked by cancer cells in later stages of tumorigenesis to drive, for example, migration and angiogenesis or to evade apoptosis induction. TNF is also crucially involved in cancer-associated pain. The main sources of TNF in cancer-related processes are tumor infiltrating leukocytes, tumor-associated fibroblasts, and the tumor cells itself. TNF might also be delivered exogenously during isolated limb perfusion

received bone marrow cells from wild-type mice. Last but not least, tumor development in AOM/DSS-treated wild-type mice could be significantly inhibited by therapeutic intervention with the TNF antagonist etanercept even if the treatment is not started until the finalization of the AOM/DSS treatment scheme (Popivanova et al. 2008). In accordance with the special role of the Wnt/β-catenin pathway in the development of gastrointestinal cancers, a lower number of adenocarcinomatous lesions and reduced accumulation of β-catenin in the nucleus have also been observed in tumor cells of TNFR1-knockout mice (Popivanova et al. 2008). Furthermore, in transgenic mice that develop dysplastic lesions in the stomach due to expression of Wnt1 in gastric epithelial cells (K19 promoter), β-catenin accumulating cells associate with infiltrated macrophages, which in turn in vitro activate the Wnt/β-catenin pathway in gastric cancer cell lines via TNFR1 stimulation and inhibitory phosphorylation of glycogen synthase kinase (GSK)-3β, the major negative regulator of the Wnt/β-catenin pathway (Oguma et al. 2008).

A crucial role of hematopoietic cell-derived TNF has also been found in a murine metastasis model with the colon cell line CT26. In this model, LPS-induced NFκB-mediated metastasis into the lung was reduced upon transplantation of irradiated mice with TNF-deficient bone marrow cells (Luo et al. 2004). Likewise, pretreatment of CT26 cells with TNF caused increased metastasis in lung and liver after transplantation (Choo et al. 2005). Metastasis driving TNF-activity must not be necessarily initiated by immune cells. In a murine orthotopic xenotransplantation model with pancreatic ductal adenocarcinoma (PDAC) cell lines mimicking tumor recurrence and metastasis after surgical tumor resection, we identified by use of human TNF-specific blocking antibodies a crucial role of tumor cell-derived TNF on recurrent tumor growth and metastasis (Egberts et al. 2008). A pro-metastatic role of TNF was also shown in the B16-F10 melanoma model by the help of a TNF-neutralizing soluble TNFR1 fusion protein, TNF-blocking antibodies, or TNF autoavaccination-induced self anti-TNF antibodies (Waterston et al. 2004; Cubillos et al. 1997). The principle capacity of TNF to promote metastasis has also been shown using treatment with exogenous TNF (Orosz et al. 1993) or tumor cells genetically engineered to express TNF (Qin et al. 1993). In further accordance with a metastasis promoting function of TNF there is evidence from in vitro studies that TNF can induce epithelial–mesenchymal transition (EMT), the hallmark in the progression of benign carcinomas into more aggressive invasive tumors (Bates and Mercurio 2003; Chuang et al. 2008).

4 TNF and Cancer-Associated Pain

TNF not only has crucial roles in tumor development and tumor progression, but is also of relevance in several tumor-associated complications such as bone destruction, cancer-related pain, fatigue, and muscle wasting. Bone destruction is

a major source of tumor-associated pain and is characterized by the imbalanced differentiation and activation of osteoblasts. RANK (receptor activator of NF-kappaB) ligand (RANKL), which is expressed on stroma cells and osteoblasts, and its corresponding receptor RANK, which is expressed on osteoclasts, are the main regulators of osteoclast differentiation and activation (Boyce and Xing 2008). However, there is good evidence from in vitro studies that TNF via its two receptors TNFR1 and TNFR2 can regulate differentiation and activation of osteoclasts independent from the RANKL-RANK system (Kobayashi et al. 2000). Moreover, there is evidence that the latter and TNF can cooperate in osteoclastogenesis (Zhang et al. 2001). However, the TNF–TNF receptor system is not only involved in cancer-related pain due to its osteoclastogenesis promoting function, but might also be directly involved in pain sensing. It has been observed that TNF increases the number of pain signaling neurons (pain fibers or nociceptors) in the tumor area and contributes this way to mechanical and heat hyperalgesia (Constantin et al. 2008). It has been further demonstrated that TNF via TNFR2 sensitizes for signaling via the pain-related TRP channel, transient receptor potential channel vanilloid type 1 (TRPV1) within second via a p38- and PKC-dependent signaling pathway (Constantin et al. 2008). Moreover, TNF again via TNFR2 is involved in the upregulation of TRPV1. The fatigue and muscle wasting effect of TNF is evident from its NFκB-mediated inhibitory effect on MyoD-mediated differentiation of myocytes, which has been shown in vitro and in vivo (Guttridge et al. 2000).

TNF has been implicated in renal cell carcinoma and TNF blockage by Infliximab has been recently investigated in two phase II trials, with patients showing disease progression after cytokine therapy with IFNα and/or IL2 (Harrison et al. 2007). In these studies, 6 of 19 and 11 of 18 patients showed clinical response (mainly stable disease) while the others showed ongoing disease progression (Harrison et al. 2007). Besides mild side effects, the major caution note in these trials was one patient who died due to a non-neutropenic sepsis, a complication that could be related or exacerbated by TNF blockade. Fatigue and muscle wasting can be exacerbated by chemotherapeutic drugs and thus also limit the maintenance of dose-intensity during conventional chemotherapy. This opens the possibility that TNF neutralization improves the tolerability of chemotherapy and allows prolonged treatment with appropriate chemotherapeutic drugs. In fact, it has been recently published that patients with advanced malignancies belonging to a cohort that has been treated with docetaxel and etanercept report an improved fatigue symptom inventory (FSI) score compared to patients of a cohort receiving only docetaxel. Moreover, in 12 of 18 patients treated with doxetaxel and etanercept a clinical benefit was reported (Monk et al. 2006). Because of the absence of a placebo control, however, this pilot study has to be considered with caution, but inspire corresponding clinical studies. Likewise, recent pilot studies with Infliximab showed disease stabilization and mainly good tolerability, but in one study there was also occurrence of severe infections (Tookman et al. 2008; Brown et al. 2008). In a multicenter, phase II, placebo-controlled study, however, where cachexia in patients suffering on advanced pancreatic cancer was

treated with gemcitabine or gemcitabine and infliximab, no statistically significant differences in safety or efficacy were observed compared with the placebo group (Widdenmann et al. 2008).

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