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Tumour necrosis factor-α as a tumour promoter

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ABSTRACT

It is becoming more evident that many aspects of tumour promotion arise from persistent and unresolving inflammation. One of the key molecules mediating the inflammatory processes in tumour promotion is the cytokine, tumour necrosis factor-α (TNF-α). Clinically, elevated serum concentrations and increased expression of TNF-α are present in various pre-neoplastic and malignant diseases, compared with serum and tissue from healthy individuals. Although over the last few decades high-dose administration of TNF-α has been used as a cytotoxic agent, recent pre-clinical cancer models have provided critical evidence to support the link between chronic, low level TNF-α exposure and the acquisition of pro-malignant phenotype (i.e., increased growth, invasion and metastasis). Furthermore, sophisticated cellular systems are being utilised to dissect the crucial role TNF-α plays in the communication of stromal/inflammatory cells and tumour cells. Understanding the intricate roles of TNF-α in the process of tumour promotion will assist in the development of novel cancer therapeutics.

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1. Cancer and inflammation

A striking feature of many epithelial cancers is an underlying inflammation, which often predates the disease, functioning as a tumour initiator and promoter and as a key determinant of tumour stroma. Dvorak compared tumours to 'wounds that do not heal', and several recent reviews have revisited and expanded upon these observations.[1–3] More recently, research into the impact of inflammation on carcinogenesis has highlighted the importance of studying the complex interactions of the tumour micro-environment, and in particular the communication between tumour and stromal components.

All tumours comprise a neoplastic clonal cell population in the presence of stromal and infiltrating inflammatory cells that provide sustenance and facilitate the metastatic process of the malignant cell.[4,5] One of the crucial aspects of the tumour micro-environment is the cytokine-mediated communication between the tumour and stromal cells. Cytokines, including tumour necrosis factor-α (TNF-α) have a vast repertoire of activities that permit cell–cell communication at a local tissue level, with the outcome determined by cytokine concentration, milieu and cell type.[6]

Increasing evidence suggests that TNF-α may regulate many critical processes of tumour promotion and progression. In their seminal paper, Hanahan and Weinberg reclassified cancer in terms of six characteristic features or hallmarks that provide the mutated cell with its survival advantage.[7] Although the initial genetic mutations are critical, the ultimate success of the cancer cell is dependent on the acquisition of the following: autocrine growth signals; an insensitivity to anti-growth signals; angiogenesis; the evasion of apoptosis; invasion and metastasis; and unlimited replicative potential. Recent studies have shown that many of these processes are sustained by chronic TNF-α production Fig. 1 and that inhibition of this key pro-inflammatory molecule may lead to novel cancer treatments.

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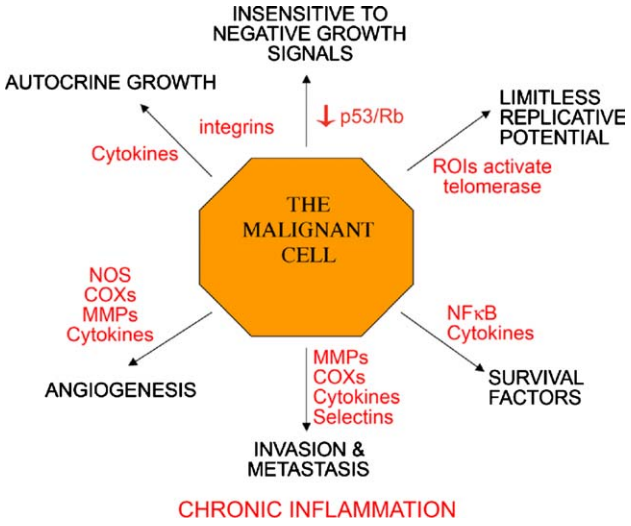

Fig. 1 – Interplay between the hallmarks of cancer, TNF-α and chronic inflammation. Chronic inflammation, a consequence of the dysregulated production of TNF-α promotes tumourigenesis by influencing the six hallmarks described by Hanahan and Weinberg.[7] TNF-α-released from cancer and stromal cells can trigger a range of mediators, including reactive oxygen intermediates (ROIs), inflammatory enzymes (e.g., nitric oxide (NO) synthase, cyclo-oxygenase (COX)), matrix metalloproteinases (MMP), cell adhesion molecules and cytokines.

2. Tumour necrosis factor-α (TNF-α)

TNF-α is a key cytokine involved in inflammation, immunity and cellular organisation.[8] This soluble factor was first isolated from the serum of Bacillus-Calmette-Guerin-infected mice treated with endotoxin, and shown to replicate the ability of endotoxin to induce haemorrhagic tumour necrosis.[9] TNF-α belongs to a large superfamily of ligands that are type II transmembrane proteins. It is a soluble 17 kDa molecule (157 amino acids) that binds as a homotrimer to two distinct homotrimeric receptors on the cell surface: TNFRI (p55 receptor) and TNFRII (p75 receptor).[10] TNF-α is synthesised as a 26 kDa (233 amino acids) membrane-bound pro-peptide (pro-TNF-α) and is secreted upon cleavage by TNF-α – converting enzyme (TACE). The 26 kDa form is also functional, binding to TNFRII via direct cell-to-cell contact. Although activated macrophages are a major source of TNF-α, it can be made by a variety of other cells, including fibroblasts, astrocytes, Kupffer cells, smooth muscle cells, keratinocytes and tumour cells. The signalling cascade downstream of TNFRI and TNFRII is complex, involving multiple adapter proteins, which are recruited upon binding of the TNF-α ligand to its receptor, and regulate at least four distinct pathways: a pro-apoptotic pathway that is induced by binding caspase-8 to FADD; an anti-apoptotic program that is activated by the binding of cellular inhibitor of apoptosis protein-1 (cIAP-1) to TRAF2; AP-1 activation which is mediated through TRAF2 via a JNK-dependent kinase cascade; and NF-κB activation by RIP.[11] For more detailed information about the biology of TNF-α and its signalling pathways the reader is directed to key reviews by Micheau and Tschopp[12] and Wajant.[13]

Evidence for a role of TNF-α in human cancer has been provided by several clinical studies. To date, TNF-α expression has been confirmed in the tumour micro-environment in the following malignancies: breast, ovarian, colorectal, prostate, bladder, oesophageal, renal cell cancer, melanoma, and lymphomas and leukaemias (referenced in).[14] Furthermore, gene and protein expression studies confirm abnormally increased levels of TNF-α in pre-neoplastic lesions such as, *Helicobacter pylori*-positive gastric lesions and inflamed colonic mucosa, compared with normal tissues.[15,16] We have shown, for example, that the dysregulated expression of TNF-α by malignant ovarian epithelium compared with normal ovarian epithelial cells is, in part, due to enhanced stability of TNF-α mRNA in the former.[17] Recent data for gastro-oesophageal cancer reveals a progression in TNF-α and TNFRI epithelial expression along the Barrett's metaplasia–dysplasia–carcinoma sequence (73% of tumours). Using a human gastrointestinal model simulating Barrett's metaplasia (CACO2, a cell line which differentiates into small-intestinal like enterocytes in vitro), Tselepis and colleagues (2002) confirmed that TNF-α could mediate up-regulation of the c-myc oncogene, via β-catenin and a MAPK (p38 and ERK) dependent pathway.[18]

3. The role of TNF-α in cancer – tumour promotion versus tumour necrosis

TNF-α has conflicting roles in cancer, as both a necrotic and promoting/growth factor. The discovery of TNF-α closed a long chapter in the search for a key component of 'Coley's mixed toxins', a crude bacterial filtrate developed by Willam B. Coley, a New York surgeon and pioneer of an early systemic cancer treatment at the turn of the last century. Coley's mixed toxins consisted of a filtrate from cultures of *Streptococcus pyogenes* and *Serratia marcescens* and induced a high fever and tumour necrosis in responding patients, particularly with sarcoma, but also with carcinoma and lymphoma.[19] Detailed investigation of TNF-α-induced haemorrhagic tumour necrosis has shown that it is dependent on two mechanisms: firstly, endothelial cell apoptosis due to deactivation of the integrin $\alpha_v\beta_3$ and disruption of the interface with the extracellular matrix (ECM)[20]; and secondly, an intact immune system requiring T cells to eliminate residual tumour cells.[21] Of note the anti-tumour effect required pharmacological doses of TNF-α that were accompanied by serious toxicity. TNF-α-mediated cytotoxicity involves both the innate and adaptive immune system[22–24] with complex interactions with other cytokines such as IL-2 and IFN-γ.[25–27] An attempt to restrict toxicity with the local administration of high-dose TNF-α in the form of TNFerade™, an adenoviral vector expressing TNF-α under a radiation-inducible Egr-1 promoter, is currently under investigation in phase II trials in patients with solid tumours.[28]

By contrast, low-dose, chronic TNF-α production is a feature of many tumour cells, as described above. TNF-α is rarely cytotoxic to tumour cells in vitro unless an additional stressor (e.g., an RNA and/or protein synthesis inhibitor) is provided.[6] Table 1 summarises the various mechanisms by which TNF-α may promote cancer growth, invasion and metastasis. TNF-α acts as a growth factor in certain tumour types, increasing levels of positive cell cycle regulators (and decreasing levels

Table 1 – Tumour-promoting effects of TNF-α

- Production of nitric oxide (NO) (DNA/enzyme damage, cGMP-mediated tumour promotion)
- Autocrine growth and survival factor for malignant cells
- Activation of E6/E7 mRNA in human papilloma virus (HPV)-infected cells
- Activation of Src kinase activity
- Tissue remodelling via induction of matrix metalloproteinases (MMPs)
- Control of leukocyte infiltration in tumours via modulation of chemokines and their receptors
- Down-regulation of E-cadherin, increased nuclear pool of β-catenin
- Enhance tumour cell motility and invasion
- Epithelial-to-mesenchymal transition
- Induction of angiogenic factors
- Loss of androgen responsiveness
- Resistance to cytotoxic drugs

of cyclin-dependent kinase (cdk) inhibitors), as well as components of growth factor receptor signalling pathways such as ras or c-myc.[17,29,30] Other direct roles for endogenous TNF-α include inducing chemo-resistance in several cancers,[31] and mediating androgen independence in prostate cancer.[32] Lastly, TNF-α promotes both DNA damage and inhibits DNA repair by up-regulating nitric oxide (NO) (non-cGMP)-dependent pathways.[33] Moreover, paracrine TNF-α derived from macrophages stimulated with IFN-γ, enhanced the production of inducible nitric oxide synthase (iNOS) in epithelial cells, resulting in their transformation.[34]

The tumour stroma may be modulated at several levels by TNF-α. Although able to induce collapse of tumour vasculature via down-regulation of $\alpha_v\beta3$ signalling and an increase in angiostatin.[35] TNF-α is also able to promote angiogenesis.[36] Various factors mediate the pro-angiogenic effects of TNF-α, including vascular endothelial growth factor (VEGF) and its receptor (VEGFR2), basic fibroblast growth factor (bFGF), IL-8, platelet-activating factor, ephrin A (a ligand for the Eph family of receptor tyrosine kinases), NO, E-selectin, intercellular adhesion molecule 1 (ICAM-1) and thymidine phosphorylase.[37–41] The paradoxical effects of TNF-α on tumour vasculature may reflect the difference in chronic synthesis, favouring angiogenesis, and acute high-dose local administration, which triggers vascular thrombosis.

TNF-α promotes further tumour remodelling by stimulating fibroblast and macrophage activity, tumour cell motility and tumour invasion via the induction of matrix metalloproteinases (MMPs).[42–44] Recent work by Hagemann and colleagues, has shown that non-contact co-culture of ovarian and breast cancer cells with macrophages increases the invasiveness of tumour cells via TNF-α-dependent activation of JNK and NF-κB pathways. Furthermore, the inhibition of the NF-κB pathway by TNF-α-neutralising antibodies or by pharmacological or genetic blockade of NF-κB (i.e., proteasome inhibitor, RNAi or overexpression of IκB) inhibited tumour cell invasiveness.[45] TNF-α also regulates production of the chemokine CXCR4 in an NF-κB-dependent manner, thereby contributing to the possible dissemination of tumours such as ovarian cancer.[46] Finally, TNF-α has also been shown to modulate epithelial-to-mesenchymal transition (EMT) in a model of colorectal cancer. EMT, a feature of many carcinomas and synonymous with the acquisition of an invasive phenotype, was induced following incubation of macrophages with colonic tumour spheroids.[47] TGF-β and macrophage-derived TNF-α was instrumental in this process, with TNF-α also signalling to the tumour epithelial cells to produce TNF-α in an autocrine fashion.

4. Pre-clinical evidence supporting the tumour-promoting role of TNF-α

Evidence has accumulated in recent years implicating endogenous TNF-α in tumour development and metastasis.[48,49] Moore and colleagues (1999) showed that TNF-$\alpha^{-/-}$ mice had a 10-fold reduction in skin tumours compared with wild-type mice during initiation with a carcinogen (DMBA) and repeated application of a tumour promoter (TPA). Although TNF-α was important for de novo carcinogenesis, the later stages of tumour progression were similar in both TNF-$\alpha^{-/-}$ and wild-type mice. Recent work suggests that the resistance of TNF-$\alpha^{-/-}$ mice to skin carcinogenesis is related to a temporal delay in the activation of protein kinase Cα (PKCα) and AP-1, key TPA-responsive signalling molecules.[50] This appears to affect the expression of genes involved in tumour development (e.g., GM-CSF, matrix metalloproteinase (MMP)-3 and MMP-9), which are suppressed in the TNF-$\alpha^{-/-}$ mice compared with wild-type animals exposed to carcinogen. TNFRI$^{-/-}$ mice and, to a lesser extent, TNFRII$^{-/-}$ mice, also demonstrate resistance to skin carcinogenesis.[51]

The tumour-promoting role of TNF-α was similarly investigated in models of hepatic carcinogenesis,[52] in which both TNFRI$^{-/-}$ and TNFRII$^{-/-}$ mice have proven useful in elucidating the mechanism of action involved.[53] TNFRI$^{-/-}$ mice displayed reduced oval cell (hepatic stem cell) proliferation during the pre-neoplastic phase of liver carcinogenesis, correlating with fewer tumours than wild-type mice. Liver tumourigenesis was unaffected in TNFRII$^{-/-}$ mice. Kitakata and colleagues (2002) used TNFRI$^{-/-}$ mice to show that endogenous TNF-α was critical in promoting liver metastasis following intrasplenic administration of a colonic adenocarcinoma cell line. Although there was no difference in the size of the splenic tumour, greater than 90% of wild-type mice developed liver metastases compared with less than 50% of TNFRI$^{-/-}$ mice. Furthermore, TNFRI$^{-/-}$ mice also displayed a reduction in the volume of intrahepatic metastases. In wild-type mice, TNF-α mRNA and protein was induced at 3 d around the hepatic central and portal veins, and at 24 d in hepatic metastases in wild-type animals. The difference in metastatic behaviour was accounted for by enhanced vascular cell

adhesion molecule 1 (VCAM-1) mRNA and protein expression in the livers of wild-type animals, which was significantly attenuated in TNFRI$^{-/-}$ mice.[54] In a related study, the epidermal growth factor receptor (EGFR) inhibitor gefitinib suppressed the intrahepatic metastasis of a hepatocellular cell line with down-regulation of TNF-α-inducible molecules including, integrin α_v, MMP-9 and fibronectin.[55] Lastly, the fatal lymphoproliferative disorder that develops in Fas ligand-deficient mice was attenuated by crossing these animals with TNF-α$^{-/-}$ mice.[56] Here, TNF-α may have induced the expression of chemokines, which regulate trafficking and accumulation of tumour cells into lymph nodes.

Transgenic mice overexpressing TNF-α have been unsuitable for studying the role of TNF-α in tumourigenesis, due to the early and severe inflammatory pathologies affecting these animals (i.e., pulmonary hypertension, cardiac failure, neurodegeneration and arthritis).[6] Nevertheless, several animal experiments that manipulate levels of TNF-α within the tumour micro-environment have confirmed that an increase in TNF-α promotes cancer development and spread. Orosz and colleagues (1993) showed enhanced lung metastases of an experimental fibrosarcoma upon pre-treatment of the animals with TNF-α. Alternatively, lung metastases could be reduced by the addition of an anti-TNF-α antibody, neutralising endogenous levels of TNF-α.[57] Malik and colleagues (1990) described overexpression of TNF-α conferring invasive properties on xenograft tumours.[58] However, the effect is clearly dependent upon tumour type since the TNF-α transgene may either induce tumour rejection or decrease survival, for instance, by promoting hepatic metastases.[59] Again, TNF-α monoclonal antibodies reversed the hepatic metastases and prolonged survival. More recently, a vaccination strategy involving the generation of autologous auto-antibodies to TNF-α proved to be equally effective (as the administration of TNF-α antibodies) in reducing the size and number of metastases in the murine B16F10 melanoma model.[60] Further evidence for a tumour-promoting role of TNF-α has been provided by chemical carcinogenesis experiments with BALB/3T3 fibroblasts. Once initiated with a chemical carcinogen and exposed for 2 weeks to TNF-α, BALB/3T3 cells undergo transformation and yield tumours in nude mice.[61] Human xenograft studies of ovarian cancer in nude mice have also emphasised the paradoxical effects of TNF-α with conversion of ascites into peritoneal deposits.[62] In another ovarian cancer xenograft model, daily administration of thalidomide, an inhibitor of TNF-α production, was shown to reduce the volume of ascites, number of peritoneal and distant deposits and increased paclitaxel cytotoxicity.[63] These results further support a rationale for the use of anti-TNF-α based therapies for cancer treatment.

Murine models have confirmed that TNF-α is an important cytokine mediator of cancer cachexia, in addition to IL-1, IL-6 and IFN-γ.[64,64] A recent study using TNFRI$^{-/-}$ and TNFRII$^{-/-}$mice assessed the contribution of both tumour and host TNF-α to cancer cachexia. This revealed that tumour-derived TNF-α is more important than host TNF-α, since cachexia was not suppressed in the TNFR knockout mice.[65] Human xenograft data supports a role for TNF-α in mediating cachexia and other para-neoplastic features, such as, hypercalcaemia and leukocytosis.[66] These features could all be reversed in this model using anti-TNF-α antibodies.

Lastly, animal models have been employed to analyse NF-κB, a key transcription factor downstream of TNF-α that is involved in many aspects of inflammation. Constitutive activation of NF-κB in cancer cells is thought to stem partly from disordered cytokine production.[67,68] Moreover, a recent mouse model of colorectal cancer has provided compelling evidence that NF-κB activation mediated via IKKβ, the kinase responsible for targeting IκB (the endogenous inhibitor of NF-κB) for proteosomal degradation, is a major link between inflammation and cancer.[69] Significantly, this model also differentiated between the source of IKKβ and the effect on tumourigenesis. Thus, macrophage-derived IKKβ promoted colorectal cancer via the up-regulation of inflammatory mediators, including TNF-α, whereas enterocyte-derived IKKβ was important in preventing apoptosis rather than contributing to inflammation per se. Another study by Pikarsky and colleagues (2004) examined the role of NF-κB in liver carcinogenesis, showing the critical importance of stromal TNF-α in this process. This study showed that TNF-α production by adjacent inflammatory and endothelial cells stimulated the activation of NF-κB in hepatocytes triggering hepatocarcinogenesis. Blockade of NF-κB in early hepatitis and carcinogenesis (<7 months) was observed to have no effect on tumour initiation. However, inhibition of NF-κB at later stages (>7 months), by anti-TNF-α monoclonal antibodies or induction of IkB-super-repressor, resulted in increased apoptosis of initiated hepatocytes and thus, dramatically inhibited tumour growth and number.[70] The cell specificity of the NF-κB pathway is underscored by other studies showing that NF-κB has a tumour-protective function in skin and liver carcinogenesis models, lacking an inflammatory component.[71,72]

5. Conclusion

An increasing body of evidence implicates TNF-α as a critical mediator bridging inflammation and tumourigenesis. Hence, inhibition of tumour and/or stromal TNF-α may provide a novel therapeutic strategy for cancer, and this is currently the subject of several phase I/II clinical trials. Future studies that combine TNF-α antagonism with modulation of TNF-α-dependent pathways (e.g., EGFR or proteosome inhibition) may prove particularly instructive. Lastly, further studies addressing the dysregulated production of TNF-α in human cancer should lead to the development of more effective TNF-α antagonists for clinical testing in this patient population.

Conflict of interest statement

None declared.

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