

An Overview of Tumor Necrosis Factor- α on Pathophysiological Mechanisms, Relevant Therapeutic Status in Breast Cancer

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Abstract. TNF α is an essential pro-inflammatory cytokine that is prevalent in the tumor microenvironment and is involved in mediating or activating many significant signaling pathways which result in inflammation, apoptosis, and tumor cell proliferation, survival, and invasiveness. In breast cancer, TNF α is involved throughout all stages from occurrence, development, procession, and metastasis to recurrence. Researchers have pointed out that TNF α plays a major role in the estrogen biosynthesis pathway, especially in the process of adipose tissue switching to estrogen. In the breast tumor microenvironment, TNF α may participate in the mediation of estrone sulfatase expression and activity. In terms of therapeutics, methods to suppress TNF α signaling in breast cancer have been proposed. To neutralize the pro-tumor and inflammatory effects of TNF α , most research opts to use anti-TNF α antibodies. According to the research, the administration of TNF α antagonists can suppress the development of breast cancer cells and strengthen the chemotherapeutic response when used as adjuvant therapy with chemotherapy. Consequently, tumor drug resistance can be well controlled. However, some side effects like systemic toxicity, the typical skin lesion, and the increasing risk of developing new cancers are still major issues. More extensive clinical trials have to be carried out for deeper investigation. This paper gives an overview of the intrinsic features of TNF α as a cytokine and gets insight into the pathophysiological mechanisms mediated by TNF α in breast cancer. Furthermore, the current state of knowledge in terms of TNF-related therapeutic strategies was adequately summarized and discussed.

Keywords: TNF α , TNF Receptor, Breast Cancer, Estrogen Biosynthesis Pathway, TNF α Antagonists.

1. Introduction

Tumor Necrosis Factor- α (TNF α) is a significant pro-inflammatory cytokine that possesses pro-inflammatory characteristics and is involved in tumor cell proliferation, apoptosis, and survival. The human TNF α gene is localized on chromosome 6 and is approximately 3 kilobases in length with 4 exons. TNF α exists in two forms: the membrane-bound form (mTNF α) and the soluble form (sTNF α). During synthesis, when TNF α translocates to the cell membrane, it can be converted into sTNF α by the TNF α converting enzyme (TACE) there. Consequently, transmembrane TNF α (tmTNF α) is a precursor version of sTNF α that is formed as a homotrimer on the transmembrane TNF α -bearing cells (TNF α -producing cells). It is composed of 157 amino acids (aa) and a 76 aa leader sequence. The function of mTNF α can be as both a ligand and a receptor, that is, tmTNF α acts as a ligand by binding to TNF α receptors as well as functioning as a receptor that transmits reversed signals back into the TNF α -producing cells [1]. While sTNF α receptors can bind to the cytokines on the cell membrane and initiate reverse signaling [2]. Hence, the tmTNF α shows up to mediate therapeutic effects, while the sTNF α is associated with the pathologic effects [3].

Breast cancer is the most common malignancy in women and the fifth cause of cancer death worldwide. In the original studies, high concentrations of TNF α could be detected in a breast tumor environment, especially with stimulated estrogen production, i.e., the estrogen receptor (ER) -positive breast cancer. It is also worth noting that TNF α plays a vital role in every step of breast cancer growth, impacting cancer oncogenesis, survival, metastasis, recurrence, and therapeutic resistance [4, 5].

2. Intracellular TNF α signaling pathways

As a trimer, TNF α effects by binding to and clustering high-affinity receptors located on cell membranes. TNF α is known to interact with two distinct tumor necrosis factor receptors (TNFRs). TNFR1, which is presented on the surface of most body cells, can be activated when combined with TNF α , then recruits cytoplasmic adaptor proteins, and results in the activation of numerous signaling pathways [6], including the signaling of NF- κ B, mitogen-activated protein kinase (p38/MAPK), c-Jun N-terminal kinase (JNK), and so forth. The intracellular signaling pathways are shown in Figure 1. When TNFR1 is shed and blocked with other free ligands, the TNF α -induced signaling is about to be prevented by the TNFR1 complex [7].

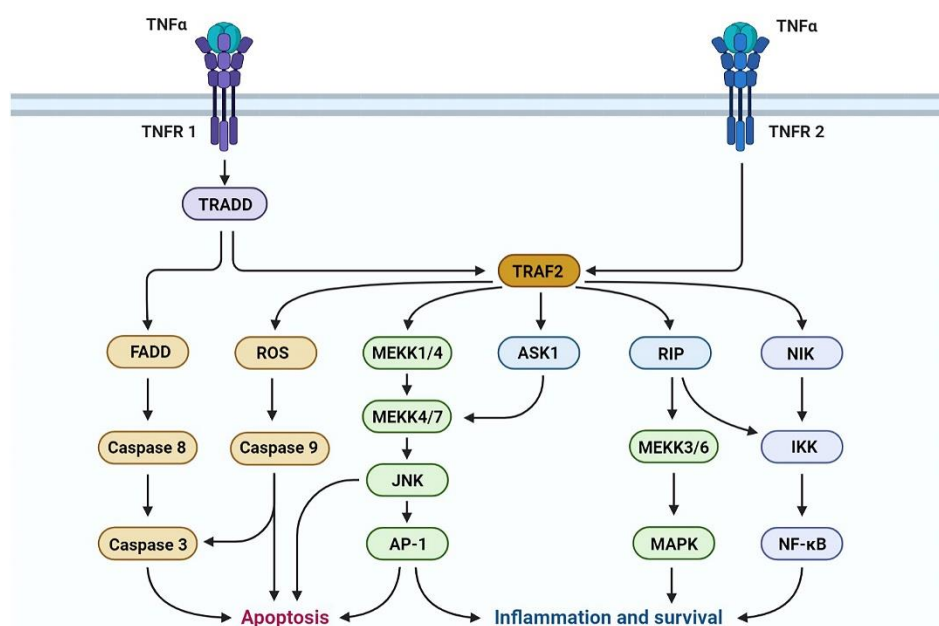


Figure 1. Intracellular TNF α signaling pathways

There are mainly two different results in TNFR1 activation. Inflammation and cell survival pathways are triggered as the basic pathway. A series of inflammatory cytokines and growth factors are released when ligands attach to TNFR1 and then promote the I κ B kinase (IKK) complex, which subsequently induces the nuclear factor- κ B(NF- κ B). It is crucial to highlight that the nonselective activation of NF- κ B via TNF α has significant effects on anti-apoptotic factors such as CylinD1, BCL-2, and superoxide dismutase [8].

When NF- κ B activation is insufficient, apoptosis develops as a late reply to TNF α activation. A protein termed TNFR associated death domain (TRADD) is recruited by TNF/TNFR1 activation and can be engaged in two different ways. One way is that TRADD can recruit the Fas-associated protein with death domain (FADD) that, in turn, induces caspase-8 and caspase-3 pathways [9]. TRADD can also stimulate the mitochondria to generate reactive oxygen species (ROS) and cause caspase-9 and caspase-3 pathways. Additionally, the aggregation of intracellular reactive oxygen and persistent activation of Jun amino-terminal kinase (JNK) can also contribute to apoptosis [10].

On the contrary, TNFR2 lacks a death domain and is expressed on hematopoietic cells or other specialized tissues, whereas the cell signaling activation is not fully understood. Several studies demonstrated that TNFR2 can directly bind to TNFR-associated factor 2 (TRAF2), and as well as activate the NF- κ B and MAPK signaling pathways [11].

3. Pathophysiological mechanism of TNF α in breast cancer

3.1. TNF α is involved in the estrogen biosynthesis pathway

Endogenous estrogens and their metabolites have long been linked to the development of breast cancer. And the estrogen receptor (ER) is seen as a vital biomarker to evaluate the risk, prognosis, and therapeutic effect of breast cancer [12]. Based on this, some studies found that TNF α played a role in the mediation of estrone biosynthesis and activity (shown in Figure 2).

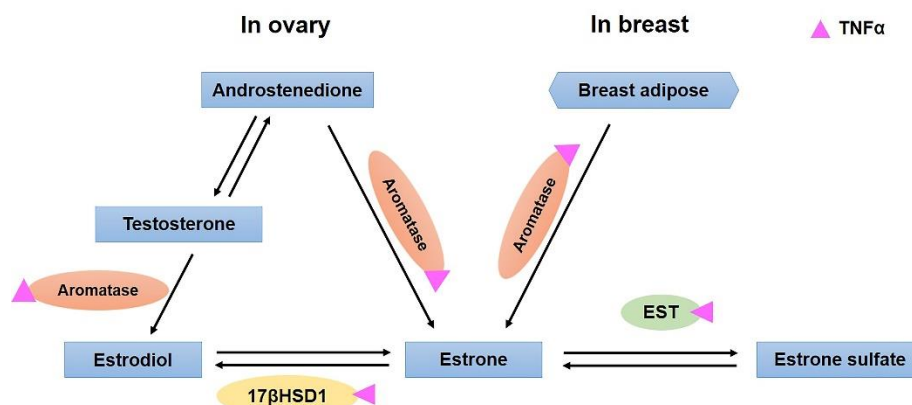


Figure 2. TNF α impacts the estrogen biosynthesis pathway

In adult females, estrogen is converted from androstenediones mainly in the ovaries by the key enzyme—aromatase. Researchers have pointed out that high expression of aromatase leads to high levels of estrogen and induces ER-positive breast cancer development [13]. However, estrogen biosynthesis switches from the ovary to the adipose tissue, especially in breast adipose, via the use of distal promoter I.4 (PI.4) during menopause or ovarian dysfunction. In vitro, cytokines such as TNF α , IL-6, or IL-11 can be used to enhance PI.4 activity in cooperation with the manmade glucocorticoid dexamethasone [14, 15].

In particular, the expression and efficiency of estrone sulfatase may also be regulated by TNF α in the breast tumor microenvironment. Estrone sulfatase converts estrone sulfate—a physiological inert form for storage—back to estrone. Most breast cancers produce high levels of estrone sulfatase mRNA compared to normal ones, and this has been linked to both increased tumor size and a worse prognosis [16, 17]. In the fibroblasts generated from normal and malignant breast tissue, TNF α enhances the activity of estrone sulfatase but not the level of its transcript [18].

Thus, the increase of tumor-derived TNF α has been taken as a more aggressive character, and it appears to respond strongly to the TNF α -induced proliferation signaling in menopausal ER-positive breast cancer [19].

3.2. TNF α participated in progression, metastasis, and therapeutic resistance of breast cancer

The NF- κ B pathway is an anti-apoptotic signaling pathway that induces the transcription of CyclinD1, Bcl-xL, TWIST, and SNAIL stabilization, resulting in tumor survival, invasiveness, and stress immune response. With the NF- κ B pathway being activated by TNF α accumulation, the chemokine CXCL1/2 also overexpressed in the breast TME to mediate cancer metastasis and chemoresistance. High expression of CXCL1/2 recruits specific myeloid cells into the tumor site, and specific myeloid cells, in turn, produce another kind of chemokine S100A8/9 which can stimulate cancer cell survival. On the contrary, several studies have demonstrated that when TNF α was silenced, the proliferation and motility of the triple-negative breast cancer cell were abolished and apoptosis was promoted [20].

Independently of NF- κ B, TNF α triggers stimulate the JNK, PI3K/AKT, and p38/MAPK pathways and activate the c-Jun signaling which is an alternative pathway to breast cancer proliferation and progression [21]. The c-Jun activation also induces the transcription of P53, MMP9/13, and other proteins, promoting tumor invasion and metastasis (shown in Figure 3).

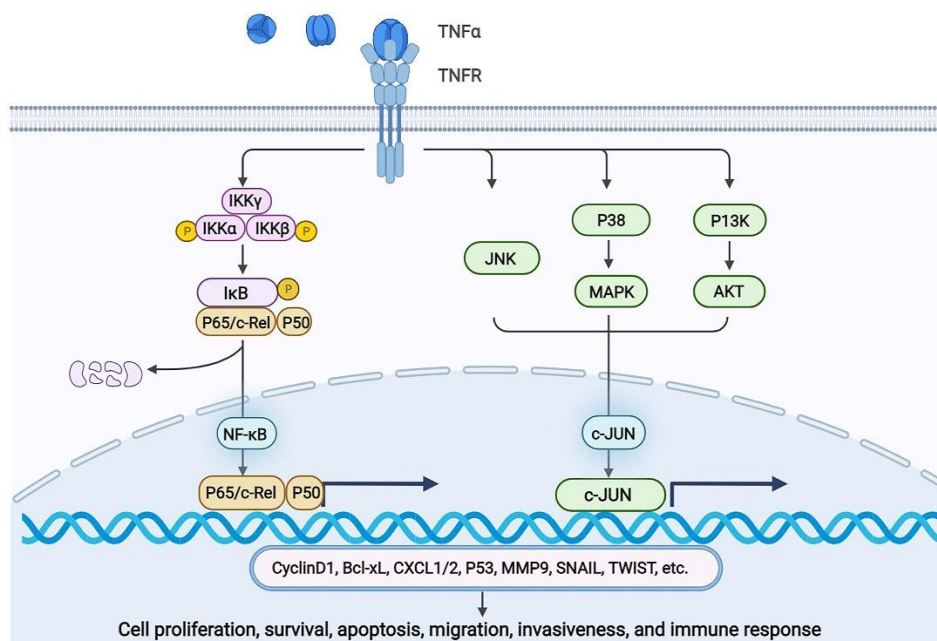


Figure 3. TNF α -induced pathogenic mechanisms in breast cancer

In addition, high levels of the ATP Binding Cassette Transporter 2 (ABCG2) can be found in the tumor tissues, that is noted to the cause of drug resistance. Researchers observed that ABCG2 is involved in poor responses to chemotherapy through estrogen signaling and NF- κ B cooperativity [22]. Hence, TNF α may have critical effects on the therapy resistance of breast cancer.

3.3. Serum concentration of TNF α in breast cancer patients

In recent decades, TNF α levels in serum have been demonstrated to be significantly associated with the activity and progression of disease and cancer, such as periodontal disease, ankylosing spondylitis, skin cancer, hematological tumor, etc [23-25]. In breast cancer patients, elevated levels of TNF α can be detected both in blood serum and in the tumor tissue [26]. The high serum concentration of TNF α is interconnected with the clinical disease stage, lymph node metastasis, and other clinicopathological characteristics of the patients. Thus, TNF α can be utilized as a predictive cancer biomarker for cancer treatments response. The finding, which is consistent with clinical observations and animal research, indicates that tumor endogenously released TNF α increases tumor proliferation and survival rather than regression [27].

4. Anti-TNF α therapeutics strategies

In the majority of cases, patients with breast cancer can generally show better responses when they are treated according to the standard treatments. However, cancer recurrence can be induced in some conditions by cancer cell survival after the first line of treatment. Even more, the fewer responses of cancer cells or the over-time response to drug exposure can lead to therapy resistance events. Hence, two main therapeutic strategies have been studied depending on TNF α -related treatment in breast cancer. One type of treatment is the administration of exogenous TNF α as a single agent or as adjuvant therapy. In the first two decades since TNF α was found in 1975 to promote hemorrhagic necrosis of tumors, the systemic administration of the recombinant human TNF α (rhTNF α) has been trailed as a single agent in many advanced cancers including breast cancer. All clinical trials revealed significant systemic toxicity and almost no effect on anti-cancer therapy. Another treatment strategy is to utilize the specific monoclonal TNF α antibodies to neutralize endogenous TNF α . The latter is more efficient and extensively used. However, most studies on TNF α antagonists administration are in vivo and animal models, and the available data from clinical trials is extremely limited.

4.1. Antibodies against TNF α

As mentioned above, TNF α antagonists can be carried out by monoclonal antibodies, fusion proteins, and dominant-negative substances. One is the antibodies against TNF α . That is anti-TNF α antibodies (Infliximab/Remicade, Adalimumab/Humira, and Golimumab). Research has presented that Infliximab, the chimeric murine monoclonal TNF α inhibitor, showed a high affinity to bind with TNF α . In BALB/c mice-bearing MDA-MB-231 breast cancer models, the infliximab treatment group demonstrated that not only the number of metastases but also the range of each metastasis lesion was significantly reduced compared with the control group [28].

In addition, a nanobody-based antibody was created to neutralize human TNF α selectively in *Pichia pastoris*. In vivo, the TNF α nanobody can suppress the spread and migration of the MCF-7 breast cancer cells, but also the aggression of MDA-MB-231 cells. Furthermore, the TNF α nanobody drug administration combined with paclitaxel dramatically improved the 4T-1 breast cancer sensibility to the chemotherapeutic response and the efficacy against tumor proliferation and lung metastasis in vivo [29].

4.2. Recombinant soluble receptors to inhibit TNF α

Another TNF α inhibitor is a recombinant soluble receptor (Fc fragments of human immunoglobulin 1 joined to extracellular domains of TNFR1/2) to prevent the interaction of TNF α with TNFRs. For instance, Etanercept/Enbrel is a recombinant human soluble p75 TNF α receptor that binds to TNF α and renders it biologically unavailable. Mercogliano M. F. et al. explored various types of experiments and demonstrated that administration of Etanercept combined with HER2-blockade (Trastuzumab) overcame de novo Trastuzumab resistance in HER2-positive breast cancer cells. Hence, the patients with MUC4-positive and HER2-positive breast cancer could be beneficiaries of a combination treatment of TNF α -blocking antibodies with Trastuzumab [30].

4.3. Combination of TNF α antagonists as adjuvant therapy

In recent studies, data showed better results when a combination of TNF α with the doxorubicin chemotherapy in vivo [31]. And in mouse-bearing breast cancer models, TNF α administration appeared to be efficient in improving standard chemotherapy outcomes, since the tumor size was reduced obviously [32, 33]. Nevertheless, these studies that TNF α was administered by intra-tumoral injection were all previous clinical trials, thereby the major problem of TNF α systemic toxicity is still left in the basket.

The only limited phase II clinical trial study in sixteen recruited patients with progressive metastatic breast cancer appeared to that Etanercept as a recombinant human soluble TNF α blocker is safe and well-tolerated. Etanercept demonstration can interfere with TNF α signaling and reduce the IL-6 and CCL2 levels in the serum. However, no partial or complete treatment response was shown in the patients, which may be due to the advanced stage of breast cancer [34].

Therefore, based on these therapeutic strategies of TNF α antagonists administration or as adjuvant therapy with chemotherapies in breast cancer, TNF α is still an attractive target potentially useful and is worthy of further investigation.

4.4. Side effects of TNF α antagonist

At present, most TNF α blockers are highly expensive. Moreover, some side effects produced by these TNF α antagonists are still a major issue. For instance, as immunosuppressant drugs, Infliximab, Etanercept, and Adalimumab may not only cause serious infections like tuberculosis or fungal infections but also increase the risk of secondary tumors.

European Crohn's and Colitis Organization (ECCO) recommended that patients with obstructive symptoms should tread cautiously when receiving anti-TNF α medication for active inflammatory CD. Particular care should be given to avoid the opportunistic infections as a side effect of anti-TNF α medication [35]. Additionally, two studies have revealed that rheumatoid arthritis patients with anti-TNF α therapies may have a high probability of secondary skin cancer or hematological malignancies

[36]. But the investigators also noted that there was no direct evidence of the TNF α inhibitors or the advanced disease itself as having caused the acquired lymphoma [37]. Furthermore, one study on patients who had ever been diagnosed with cancer showed that the anti-TNF α therapy may have a low risk of recurrent cancer when treating inflammatory bowel disease at this time [38]. Contrarily, another study found that TNF α blockers did not increase the recurrence of breast cancer in patients with rheumatoid arthritis [39].

These results are confusing since individuals with prior tumors are more likely to acquire new cancers, therefore the recurrent cancer cannot be totally referred to the anti-TNF α medications. Thus, how to make the TNF α -blocked agents more effective and affordable is still undergoing research and exploration.

5. Conclusion

As a pleiotropic cytokine, TNF α plays a vital role in affecting breast cancer oncogenesis, progression, recurrence, and therapeutic resistance. In breast cancer patients, elevated levels of TNF α can be detected both in blood serum and in tumor tissue. Patients with a high level of this cytokine are more likely to have had an advanced clinicopathological malignant sign. With the accumulation of TNF α , the NF- κ B pathway was activated and cooperated with the ER signaling, resulting in the overexpression of the ABCG2 gene and the chemokine CXCL1/2. As a result, TNF α may further mediate breast cancer metastasis and chemoresistance. TNF α antagonists contain anti-TNF α antibodies (Infliximab/Remicade, Adalimumab/Humira, and Golimumab) and TNF α -blocked agents (Denosumab, Etanercept/Enbrel). Several preclinical investigations have shown that TNF-based treatments work synergistically with both conventional chemotherapy and radiation to significantly enhance outcomes. In particular, the administration of TNF α antagonists as adjuvant therapy combined with standard chemotherapy was more investigated in the preclinical experiments currently and appeared to be effective to reduce the tumor size, increase the chemotherapeutic response, and be well controlled of the tumor acquired drug resistance. In the limited phase II clinical trial, Etanercept as a recombinant human soluble TNF α blocker was found to be safe and well-tolerated; nevertheless, hardly a partial or complete response to treatment was seen in the advanced breast cancer patients. However, some side effects like systemic toxicity, the typical skin lesion, and an increased risk of developing new cancers are still major issues. Research with detailed data is also extremely limited. Therefore, more extensive clinical trials have to be carried out for deeper investigation, but how to make the TNF α -blocked agents more safe, effective, and affordable is also undergoing research and exploration.

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