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Tumor Necrosis Factor-alpha Converting Enzyme: Implications for Ocular Inflammatory Diseases

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Abstract

Tumor necrosis factor-alpha (TNF- α) –converting enzyme (TACE), a member of the family of metalloproteinase disintegrin proteins, is responsible for the conversion of inactive TNF- α precursor from to active mature form. TNF- α is a pleiotropic cytokine that contributes to cellular immunity and inflammatory response in wide range of inflammatory pathologies. Although a large number of studies indicate the use of TACE inhibitors, which prevents processing of TNF- α as potential therapeutic drugs for the treatment of inflammatory diseases including rheumatoid arthritis, Crohn's disease and cancer, very few studies indicate its use in ocular pathologies. It is still not clearly understood how the TACE-mediated shedding of cytokines and growth factors in various ocular tissues plays a critical role in the cytotoxic signals causing tissue dysfunction and damage leading to blindness. Regulation of TACE activity is likely to have wide implications for ocular immunology and inflammatory diseases. Specifically, since anti-TNF- α therapies have been used to prevent ocular inflammatory complications, the use of TACE inhibitors could be a novel therapeutic approach for ocular inflammatory diseases especially uveitis.

Keywords

TNF-alpha; TACE; Uveitis; inflammation; Eye

1. Introduction

Augmented levels of inflammatory cytokines, specifically tumor necrosis factor- α (TNF- α) have been implicated in the pathogenesis of various inflammatory diseases including ocular inflammatory disease, uveitis. Uveitis is a major cause of severe visual impairment that accounts for 10–15% of all cases of total blindness in the US (Read, 2006; Reeves et al, 2006). Uveitis is even more common in developing nations with limited access to health care. The pathogenesis of ocular inflammation is believed to involve an abnormal T-cell response to ocular antigens, which leads to T-cell-mediated damage to the eye. Suppressing the immune response with steroids or with adjuvant immunotherapies such as cyclosporin A, azathioprine, or tacrolimus forms the mainstay of treatment (Lyon, et al., 2009). This achieves disease control and prevents vision-threatening complications in most patients, but a significant proportion of patients remain unresponsive to conventional immunosuppression and with diminished quality

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of life. A number of immunomodulatory agents, such as anti-TNF- α therapies which have a more targeted and sustained effect on the immune response, have proved successful in the treatment of autoimmune diseases, in particular rheumatoid arthritis and Crohn's disease (Taylor and Feldmann 2009; Haraoui and Krenbaum 2009). Recent studies have shown the efficacy and tolerability of TNF- α blockade in autoimmune ocular inflammatory disease (Jap and Chee, 2007). The use of TNF- α inhibitors such as soluble TNF- α receptor (etanercept) and monoclonal antibodies (infliximab) have been shown to improve the inflammatory conditions with limitations in uveitis and Behcet's disease (Cantarini et al, 2009; Ardoin et al, 2007). It has been shown that in autoimmune diseases the use of infliximab and etanercept could develop auto-antibodies, and in some cases, a clinical lupus-like syndrome (Jap and Chee, 2007). Therefore, a novel immunosuppressive therapy for ocular inflammatory disease is required. TNF- α converting enzyme (TACE) has been shown to be an efficient and therapeutic target in preventing a number of inflammatory diseases including cardiovascular and neurological degenerative diseases as well as cancer (Arribas and Esselens, 2009). In a number of clinical trials for autoimmune complications, inhibitors of TACE have been shown to be safe (Murumkar et al, 2010). However, activation of TACE that is responsible for shedding of inflammatory cytokines and growth factors in various ocular tissues has received little attention. This article highlights the potential for TACE as a therapeutic target in ocular inflammatory pathologies.

2. Structure

TACE is a major protease responsible for processing and release of a number of membrane bound inflammatory cytokines (Black et al, 1997). TACE with an apparent molecular mass of 85 kDa is also known as ADAM 17 and TNF- α sheddase (EC 3.4.24). It is a type I transmembrane protein belongs to a superfamily of Zn-dependent metalloproteases known as the metzincins, which also includes the matrix metalloproteinases (MMPs). The human TACE, an 824 amino acid polypeptide, is encoded by a gene of ~50 kb consisting 19 exons and 18 introns, and is localized at chromosome 2p25. The structure of TACE closely resembles that of ADAMs family of transmembrane proteins which belongs to the zinc protease superfamily. TACE is synthesized as a zymogen and it contains a pro-domain (1-213 amino acids), catalytic domain (214-472 amino acids), a disintegrin and cysteine-rich region (473-670 amino acids), a transmembrane segment (671-692 amino acids) and a cytoplasmic extension (693-824 amino acids). Coordination of free Cys residue in the pro-domain with zinc in the active site of TACE prevents enzymatic activity. Therefore, removal of pro-domain is a prerequisite for TACE catalytic activity. The potential furin cleavage site (RVKR) present between pro- and catalytic-domain is responsible for generating catalytically active TACE by separating it from pro-domain by a proprotein convertase, furin. The catalytic domain contains the Zn-binding motif which is involved in coordinating zinc with histidine residue and creating the active site of the enzyme. The crystal structure of the TACE catalytic domain has been solved (Maskos et al., 1998; Figure-1). The cysteine-rich domain is required for the substrate recognition and maturation. The cytoplasmic end of TACE contains potential sites which interact with Src-homology domains and potential tyrosine and MAPK phosphorylation sites important for signal transmission and cellular localization.

3. Expression, Activation and Turnover

Several reports suggest that activators of protein kinase C (PKC) such as phorbol esters increase the TACE expression as well as activity (Hahn et al, 2003; Wheeler et al, 2003). Inhibitors of Mitogen- Activated Protein Kinase (MAPK) have been shown to prevent shedding activity of TACE. Increased reactive oxygen species during oxidative stress could also activate TACE via PKC. Recently, we have also shown that inhibition of aldose reductase prevents high glucose-induced shedding of TNF- α by TACE in smooth muscle cells probably by inhibiting

PKC activation (Reddy et al, 2009). Most of the MMP inhibitors have been shown to have TACE inhibiting activity. For example, tissue inhibitor of metalloproteinase (TIMP-3) inhibits TACE activity by binding directly to the protein. Studies also suggest that TIMP-3 could be a physiological regulator of TACE (Federici et al, 2005). Down-regulation of TIMP-3 increases TACE activity while up-regulation of TIMP-3 inhibits TACE activity. Indeed, TIMP-3 deficient mice have shown increased levels of TNF- α and severity of inflammation (Gill et al, 2010). Recently the expression of TACE in corneal epithelial cells has been investigated (Sakimoto et al, 2008 and 2009). However the expression and regulation of TACE in other ocular tissues still need to be investigated.

4. Biological Function

TACE plays a key role in the regulation of the proteolytic release of some of the cytokines, chemokines, growth factors, and their receptors from cellular membranes by a process known as ectodomain shedding. However TACE is not the universal shedder of all the proteins. Various studies suggest that several TACE substrates are processed by other proteases. For example, interleukin-6 receptor (IL-6R) protein is found to be a substrate of TACE is also processed by other proteases in TACE knockout cells (Franchimont et al, 2005). Similar results were observed with L-selectin and pro-TGF- α . The substrate specificity of TACE is not still clear. Mutations at the cleavage sites of several substrate proteins such as pro-TNF- α , pro-TGF- α , L-selectin and TNF-receptor have not effected shedding activity of these proteins by TACE. However, a recent study indicates that amino acid residue Met435 serves to maintain the stability of the catalytic center of TACE for the hydrolysis of peptide bonds in substrates (Perez et al, 2007). Interestingly, most of the TACE substrates are cleaved within a short stretch of amino acids located in the juxtamembrane region of the extracellular domain (Hinkle et al, 2004). The major pro-inflammatory cytokine processed by TACE is TNF- α which is a pleiotropic inflammatory cytokine produced by a number of cell types including macrophages, monocytes, T-cells and plays a crucial role in the pathogenesis of inflammation. Inhibition of TNF- α by using monoclonal antibodies, soluble TNF- α receptors and TACE inhibitors has been shown to suppress Th1 cytokines, activation of infiltrating leucocytes, and tissue damage and destruction. TNF- α is synthesized as a membrane anchored 26 kDa precursor protein that is processed by cleavage of the Ala76–Val77 peptide bond to its mature 17 kDa by TACE (Black et al, 1997). Figure-2 shows bacterial endotoxin lipopolysacchide-induced TNF- α processing by TACE leading to ocular inflammation. The shedding of TNF by TACE has been well confirmed by in vitro as well as in vivo experiments. TACE deficient mice generated by eliminating Zn-binding domain, TACE Δ Zn/ Δ Zn, has shown developmental defects at birth such as the failure of eyelids to fuse and defects in skin and hair (Peschon et al, 1998). Further, monocytes and T-cells isolated from TACE Δ Zn/ Δ Zn transgenic mice are deficient in releasing TNF- α (Peschon et al, 1998). Moreover, most of the TACE deficient mice die at birth. These studies suggest that TACE is an important regulator that controls the inflammatory cytokine and growth factor levels in the body.

4. Disease Involvement and Future Perspective

It is well established that increased expression of cytokines, chemokines, growth factors and their receptors are involved in the pathophysiology of many inflammatory diseases; over 100,000 deaths in the U.S. each year can be attributed to an excessive inflammatory response alone. Hence understanding the role of molecules that regulate the expression of inflammatory cytokines and growth factors is most important for understanding multiple disease pathologies. Increased TACE mediated shedding of a number of inflammatory markers has been observed in a number of diseases such as ischemia, heart failure, arthritis, atherosclerosis, diabetes, cancer, neurological and immune diseases (DasGupta et al, 2009). Some of the TACE inhibitors are currently in the clinical trials for the prevention of rheumatoid arthritis and Cancer. Further,

recent studies have shown that TACE inhibition could be used for inhibition of pathogenic growth factor signaling in cancer and clinical studies are underway to investigate anticancer effect of TACE inhibitor (Murumkar et al, 2010). However, detailed studies on the involvement of TACE in ocular diseases such as uveitis, age-related macular degeneration (AMD), glaucoma, retinopathy and iritis are lacking. No reports are available in literature that delineate the mechanisms leading to TACE mediated shedding of cytokines and growth factors in uveitis and other ocular inflammatory diseases. Furthermore, regulation of TACE activity is likely to have wide implications to uveitis, AMD, posterior capsular opacification, and glaucoma where anti-TNF- α therapies have shown to be effective. Since cytokines such as TNF- α play a major role in the pathophysiology of number of eye diseases such as retinopathy, neovascularization, dry eye and intraocular tumors, TACE inhibition could be better therapeutic option in those diseases also. A recent study demonstrates that TACE mediated shedding of TNF- α and its receptors in corneal wound healing (Sakimoto et al, 2008 and 2009). Cook et al, 2008 have shown that TNFR1 is processed by TACE in human conjunctival epithelial cells. TNF and TNF receptors are highly expressed in many ocular diseases such as uveitis. A number of studies have shown that anti-TNF therapies such as antibodies to TNF- α and soluble receptors of TNF prevent complication of ocular inflammation leading to uveitis (Theodossiadis et al, 2007). However, the benefits of anti-TNF strategies are limited and their efficacy is not convincing. This could be due to involvement of more than one cytokine in uveitis. Therefore the use of TACE inhibitors that not only block TNF- α processing but also a number of other important cytokines and growth factors such as IL6, EGF and TGF- α could be a better therapeutic option for uveitis. Our unpublished studies indicate that inhibition of TACE prevents experimentally endotoxin-induced uveitis in rats, indicating potential novel use of TACE inhibitors for uveitis (Kalariya et al, 2010). The use of TACE inhibitors could also represent a substantial change in the current clinical approach in many other ocular diseases such as corneal and retinal inflammation, ulceration, conjunctiva and corneal wound healing. A better understanding of TACE mediated regulation of inflammatory cytokines and growth factors is of fundamental significance in increasing our understanding of ocular inflammation would be of major clinical importance, as it may identify possible therapeutic approaches that may be more successful and safer than existing treatments for a number of sight threatening ocular diseases.

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Figure 1.

Structure of catalytic domain of TACE. The chain starts and ends on the lower and upper left backside, respectively. The three disulfides are shown as green connections and the catalytic zinc is shown as a pink sphere. His-405, His-409, His-415, Met-435, Pro-437, and the inhibitor (white) are shown with their full structure. The figure was originally made using SETOR (Evans 1993). This figure was reproduced with permission from PNAS 1998; 95:3408-12 (Copyright (1998) National Academy of Sciences, U.S.A).

Figure 2.

TACE mediated ectodomain shedding of TNF- α . Gram negative bacterial endotoxins such as lipopolysaccharide (LPS) initiates oxidative stress-induced activation of protein kinases such as protein kinase C (PKC) or mitogen activated protein kinase (MAPK) which activates catalytically inactive zymogen of TACE to catalytically active TACE. The active TACE selectively processes membrane bound in active precursor form of TNF- α in to an active mature form of TNF- α . The soluble TNF- α (sTNF- α) binds to its cognate receptors (TNFR1 or TNFR2) and transduces the signals by autocrine and paracrine manner leading to cellular toxicity and inflammation.