REVIEW

Role of tumour necrosis factor alpha converting enzyme (TACE/ADAM17) and associated proteins in coronary artery disease and cardiac events

Rôle de l’enzyme de conversion TNF (TACE/ADAM17) et des protéines associées dans la maladie coronaire et les événements cardiaques

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KEYWORDS
Atherosclerosis; Transmembrane proteins; TNFα

Summary Tumour necrosis factor alpha converting enzyme (TACE/ADAM17) is a member of the A disintegrin and metalloproteinase (ADAM) family of ectodomain shedding proteinases. It regulates many inflammatory processes by cleaving several transmembrane proteins, including tumour necrosis factor alpha (TNFα) and its receptors tumour necrosis factor alpha receptor 1 and tumour necrosis factor alpha receptor 2. There is evidence that TACE is involved in several

Abbreviations: ACS, Acute coronary syndrome; AMI, Acute myocardial infarction; CAD, Coronary artery disease; MACE, Major adverse cardiac events; MAPK, Mitogen-activated protein kinases; TACE (CD156b), Tumour necrosis factor alpha converting enzyme or A disintegrin and metalloproteinase 17 (ADAM17); TIMP3, Tissue inhibitor of metalloproteinase 3; TNFα, Tumour necrosis factor alpha; TNFR, TNF receptor.

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Biomarker; TACE

inflammatory diseases, such as ischaemia, heart failure, arthritis, atherosclerosis, diabetes and cancer as well as neurological and immune diseases. This review summarizes the latest discoveries regarding the mechanism of action and regulation of TACE. It also focuses on the role of TACE in atherosclerosis and coronary artery disease (CAD), highlighting clinical studies that have investigated its expression and protein activity. The multitude of substrates cleaved by TACE make this enzyme an attractive target for therapy and a candidate for biomarker research and development in CAD.

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MOTS CLÉS
Athérosclérose ; Protéines transmembrannaires ; TNFα ; Biomarqueur ; TACE

Résumé L'enzyme de conversion du facteur alpha de nécrose tumorale (TACE/ADAM17), membre de la famille des A désintégrées et métalloprotéinases (ADAM) qui sont des protéines clivant l'ectodomaine des protéines transmembranaires, régule différents processus inflammatoires en clivant des protéines transmembranaires, y compris le facteur alpha de nécrose tumorale (TNFα) et ses récepteurs 1 et 2. Différentes études ont montré l'association de TACE avec des maladies inflammatoires tel que l'ischémie, l'insuffisance cardiaque, l'arthrite, l'athérosclérose, le diabète, le cancer ainsi que des maladies neurologiques et immunologiques. Cette revue résume les dernières découvertes concernant le mécanisme d'action et de régulation de TACE ainsi que son rôle dans l'athérosclérose et les maladies coronariennes en mettant en évidence les études cliniques les plus récentes en relation avec son expression et son activité. La multitude des substrats clivés par TACE rendent cette enzyme une cible thérapeutique intéressante surtout dans le domaine du développement des biomarqueurs pour les maladies coronariennes.

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Background

Tumour necrosis factor alpha converting enzyme (TACE), also known as A disintegrin and metalloproteinase 17 (ADAM17), is a membrane-anchored protein responsible for the ectodomain shedding of a variety of transmembrane proteins, such as cytokines, chemokines, and growth factors and their receptors. Shedding results in the initiation or inhibition of downstream signalling and cellular responses, and is associated with several major acute and chronic inflammatory diseases. Recent studies have reported overexpression of TACE in patients with coronary artery disease (CAD) as well as after acute myocardial infarction (AMI), indicating that TACE may be a useful cardiac prognostic biomarker of cardiac events. This review summarizes recent findings on TACE activity and regulation, with an emphasis on the role of TACE in CAD.

Structure

Black et al. first described TACE in 1997 when working with mammalian THP1 cells, as the enzyme that cleaves tumour necrosis factor alpha (TNFα), and reported purification and cloning of the protein [1]. Subsequently, different forms of TACE have been described including the full-length protein (~110 KDa under non-reducing conditions), a mature form of TACE lacking the prodomain (80 KDa), and a third form detected in cell lysates, which lacks the cytoplasmic domain (60 KDa) [2] (Fig. 1).

Localization

Immunohistochemical studies suggest that most of the active form of TACE is localized in the cellular perinuclear region, with a small amount present on the plasma membrane surface [2]. Tellier et al. further reported that TACE is sequestered into lipid rafts (Fig. 2). This spatial distribution has a role in the regulation of TACE activity by keeping the enzyme separate from its substrates [3]. Lipid rafts are known to have high concentrations of cholesterol, and interestingly, the shedding of TACE substrates, such as CD30 [4], interleukin-6 receptor (IL-6R) [5] and L-selectin (CD62L) [6], can be increased by cholesterol-lowering drugs. The increase in TACE shedding was also observed with TNFα, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) after membrane cholesterol depletion [3]. Disruption of the lipid rafts may displace the mature form of TACE in the non-raft region of the membrane that contains the major part of TACE substrates, and increase their shedding. Therefore, under normal conditions, the sequestration of the mature form of TACE in lipid rafts can be considered as the rate-limiting process of its shedding activity [7].

Activation and regulation

Tissue inhibitor of metalloproteinase 3 (TIMP3) is the only known endogenous inhibitor of TACE [8].

**Figure 1.** TACE domains. Signal peptide (1-17 aa); pro-domain (18-214 aa) acting as an inactivator and a chaperone domain; extracellular domain (215-671 aa) comprising: 1. A metalloprotease domain/catalytic domain (215-473 aa) responsible for an ectodomain shedding; 2. A disintegrin domain (474-572 aa); 3. An EGF-like/cysteine rich domain (573-671 aa) responsible for substrate recognition and activation; transmembrane domain (672-694 aa) necessary for effective cleavage of substrates; cytoplasmic domain (695-824 aa) binding to many proteins that regulate TACE activity. aa: amino acid; EGF: endothelial growth factor; TACE: tumour necrosis factor alpha converting enzyme.

**TIMP3** is downregulated in circulating human monocytes in people at high risk of diabetes and atherosclerosis [9]. Stöhr et al. demonstrated that TIMP3 also regulates lipid metabolism as well as the oxidative stress response, maintaining metabolic flexibility in the heart, particularly during episodes of increased cardiac stress [10]. TIMP3 overexpression has been shown to improve post-myocardial infarction cardiac remodelling related to lower extracellular matrix disruption in animal models [11,12].

Regarding TIMP3 activation, Cesaro et al. showed that strong TACE expression was associated with early acute phase inflammation in Crohn’s disease, whereas TIMP3 was upregulated during the quiescent phase of the disease [13]. TIMP3 may therefore be involved in a delayed regulatory mechanism following an increased TACE expression, and its role in inflammation and heart-related diseases should be studied further.

Studies have shown that only the monomeric form of TACE is active and can effectively cleave its substrates. However, TACE appears to be predominantly present as dimers at the cell surface, which enables its efficient association with TIMP3 and silences its activity. Hence, TIMP3 inhibits TACE only when it is in its dimer form. Upon activation of the p38 mitogen-activated protein kinase pathway, the balance can shift from TACE dimers to monomers, and this shift is associated with an increase in cell surface presentation of TACE and a reduction in TIMP3 association [14].

**Substrates and shedding process**

TACE mediates cell-cell interactions with a wide range of identified substrates, although the mechanisms and consequences of this binding are not yet fully understood. As the sequences cleaved in various substrates are highly variable, there is no apparent consensus for the TACE cleavage sequence. New evidence suggests that TACE activity is regulated by its non-catalytic domains and the secondary structure of its substrates [15].

Cleavage of TACE substrates occurs at extracellular sites proximal to the cell membrane, thereby releasing the soluble ectodomain from the cell surface. The cleaved molecules can then bind to their receptor on the same cell (autocrine effect) or to receptors on neighbouring cells (paracrine effect) or even enter the bloodstream (endocrine effect). When the substrate is cleaved and bound to its receptor, it can initiate downstream signalling events. Alternatively, the receptor can also be cleaved from the cell surface; thus, ectodomain shedding can actually stop the ligand-initiated signalling.

Because of its many functional properties, TACE plays a major role in inflammation through its shedding of a variety of inflammatory substrates. Studies have shown that TACE is implicated in platelet function through its cleavage of the von Willebrand factor (CD42b) receptor [16]. TACE is also responsible for the cleavage of L-selectin (CD62L), intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1) [17]. Many TACE substrates have been extensively investigated in atherosclerosis, and all are known to participate in the inflammatory process accompanying the formation and progression of plaque (Fig. 3). The consequences of TACE shedding on these factors need to be closely investigated, as their soluble forms may hold different properties compared to their membrane forms. For example, the cleavage of TNFRI by TACE sheds a soluble form of TNFRI that binds to free TNFα, dampening the inflammatory response [18]. In addition, TACE activates ligands (such as neuregulin) that bind to the ErbB tyrosine kinase family of receptors. The resulting signalling pathways have been involved in the growth of many tumour types as well as the maintenance of cardiac function [19,20].

**The fate of TACE after its activation**

There appears to be contradiction in the understanding what happens to TACE after it has been activated, and many postulations have been made.
ADAM10, another member of the ADAM family, is known to undergo regulated intramembrane proteolysis by presenilin after its ectodomain is shed by ADAM-9 or ADAM-15 [21]. In the intramembrane proteolysis process, a membrane protein typically undergoes two consecutive cleavages. The first results in the shedding of its ectodomain; the second one occurs within its transmembrane domain, resulting in secretion of a small peptide and the release of the intracellular domain into the cytosol. After intramembrane proteolysis, the cytoplasmic domain of ADAM10 can translocate to the nucleus to bind to gene loci undergoing transcription. Since ADAM10 is the closest relative of TACE, there is a high possibility that the cytoplasmic domain of TACE itself can undergo intramembrane
proteolysis and participate in gene transcription regulation. A recent study reported that TACE can play a role in post-myocardial infarction recovery by regulating vascular endothelial growth factor receptor-2 transcription and angiogenesis in cardiomyocytes [22]. Whether TACE is internalized and downregulated after activation is still unclear. However, recent studies have reported that soluble TACE can be detected in the plasma [23,24], which is suggestive of a particular mechanism behind its own shedding, a process that remains unknown.

**TACE and cardiovascular disease**

**TACE in heart diseases**

Several studies have investigated the role of TACE in heart diseases. Investigators found that TACE expression was increased in endomyocardial tissues in myocarditis, as well as in the peripheral blood in advanced stages of heart failure. TACE was upregulated together with TNFα in myocarditis and negatively correlated with left ventricular systolic function [25]. Patients with advanced congestive heart failure also had an increased expression of TACE and TNFα compared to controls [26]. The role of TACE has been highlighted in aortic aneurysm [27] and in heart development [28]. Interestingly, Takayanagi et al. recently reported that TACE may be a novel therapeutic target for the prevention of hypertensive complications [29].

**TACE and CAD**

The underlying pathological process behind the development and progression of CAD is atherosclerosis, which results from an imbalance in lipid metabolism and a maladaptive immune response leading to chronic inflammation in the arterial wall. Major adverse cardiac events (MACE) often occur suddenly in patients with CAD after a revascularization strategy, resulting in high mortality and morbidity. These events include death, stroke, myocardial infarction, heart
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failure and repeat coronary revascularization of the target lesion [30]. Many studies are currently investigating the association between different cardiovascular markers and MACE with a recent interest in multiple marker models versus a single marker model [31]. However, the investigation of TACE and associated proteins as a multiple marker model for MACE prediction has not yet been evaluated.

Clinical studies of TACE and associated proteins in CAD and AMI

Several studies have demonstrated that TACE plays a role in CAD initiation and progression to an acute coronary syndrome (ACS). Clinical studies have shown elevated plasma levels of TNFα in patients with AMI, suggesting that TNFα maturation, which relies on TACE, may activate systemic inflammation and contribute to plaque rupture [32]. Subsequent studies have found a positive association between TACE in AMI, as summarized in Table 1. Increased levels of gene expression of TNFα and TACE were found in circulating leukocytes of patients with myocardial infarction, obtained within 24 hours of onset [33]. The Killip-Kamball classification system is used in patients with an ACS to stratify their risk of mortality regarding the development of heart failure. Expression of TACE and TNFα was significantly higher in patients with Killip-Kamball class III or IV AMI than in those with class I or II AMI or in controls [34]. This finding demonstrates that higher levels of TACE-associated inflammation correlate with an increased risk of developing severe heart failure after an ACS. Shimoda et al. [35] reported that TACE gene expression levels in peripheral blood cells were higher in patients with AMI compared to healthy subjects, and particularly in those who had complications, such as malignant recurrent ventricular arrhythmia or pump failure. Both spontaneous and phorbol 12-myristate 13-acetate (PMA)-stimulated levels of TACE gene expression as well as TNFα gene and protein expression levels were found to be higher in patients with AMI compared to healthy subjects. Sustained increases in TACE and TNFα levels were reported 14 days after the onset of AMI and levels correlated positively with peak creatinine kinase levels.

Systemic gene expression levels of TACE and TNFα were documented to be higher in patients with AMI compared to patients with stable angina [36]. Interestingly, TACE levels were higher in local samples, near areas of ruptured coronary plaques, than in systemic samples obtained from patients with AMI. TACE and TNFα immunostaining showed that they were localized in infiltrating macrophages in ruptured coronary plaque/thrombus materials occluding the culprit coronary artery. In addition, increased levels of TACE in culprit coronary samples were the strongest independent predictor of adverse cardiac events (6 months after the onset of AMI) after adjustment for various clinical variables. The authors suggested that local expression of TACE in the culprit coronary artery leads to arterial remodelling and rupture or erosion of weakened coronary plaque, leading to the exacerbation of cardiac events [36].

Rizza et al. [37] recently measured TACE activity by evaluating the levels of its main four substrates (soluble VCAM-1, soluble ICAM, soluble IL6R and soluble TNFR1) in subjects with established vascular atherosclerosis who were followed for secondary MACE. They identified three homogeneous subgroups of patients, in terms of event risk, and an increased risk for incident events was observed among individuals with a high TACE score. Looking closely at TNFα receptors, soluble TNFR2 levels were found to be increased in heart failure, and other studies have shown higher circulating levels of soluble TNFR1 and soluble TNFR2 associated with nephropathy, cardiovascular events, and total mortality in type 2 diabetes [38]. Canaualt et al. demonstrated that TACE-containing microparticles of atherosclerotic plaques are partly of endothelial origin and that TACE on the surface of microparticles was still active [39]. A more recent study showed TACE activity in the plasma of patients with anti-neutrophil cytoplasmic autoantibodies vasculitis and indicated that TACE was also present on plasma microparticles derived mainly from platelets but also from endothelial cells. The authors reported that it is the active form of TACE that is detectable in plasma samples and that it is found on the surface of microparticles originating from platelets as well as endothelial cells [24].

Other studies have investigated the role of TACE polymorphisms in relation to cardiovascular disease. In the Atherogene study, TNFα, soluble TNFR1, and soluble TNFR2 concentrations were all significantly elevated in patients with future cardiovascular death. Moreover, individuals carrying the 747Leu allele in TACE displayed a borderline increased risk of future cardiovascular death. This study also suggests a role of TACE in the regulation of TNFα plasma levels and identified the TACE gene as a candidate for CAD risk [40].

TACE activation in CAD

The association of TACE with CAD is due first, to its role in shedding a variety of inflammatory molecules (Fig. 3) and second, to some of its activators that have been shown to be associated with atherosclerosis and pathophysiological functions in CAD.

TACE has an increased shedding rate when exposed to cell activators, such as phorbol esters (e.g. PMA) [41], the p38 MAPK pathway and lipopolysaccharide, which is dependent on reactive oxygen species stimulation [42]. Oxidative stress generating reactive oxygen species is known to be involved in the progression of atherosclerosis, disturbed blood flow and arterial wall remodelling [43]. When reactive oxygen species are generated, TACE activation is increased as a result local and systemic inflammation. Moreover, it is known that nitric oxide can activate TACE [44]. Nitric oxide is involved in the physiological regulation of blood flow and has pathophysiological functions in CAD [44]. On the other hand, C-reactive protein is also known to activate TACE and the release of soluble lectin-like oxidized low-density lipoprotein receptor-1, which plays an important role in the development and progression of atherosclerosis [45]. Therefore, TACE is not one inflammatory factor among many, but is a key enzyme. Its activators and subsequently shed proteins are essential mediators in the development and progression of CAD (Fig. 3).

TACE, associated proteins and endothelial dysfunction in CAD

TNFα is a major contributor to inflammatory processes in CAD. On cleavage of transmembrane TNFα by TACE,
## Table 1 Summary of studies of TACE and associated proteins, and their clinical implications in cardiovascular disease.

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<td>Canault et al., 2006 [63]</td>
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<td>Satoh et al., 2000 [25]</td>
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<td>Satoh et al., 2004 [26]</td>
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<td>AMI within 24 h of onset</td>
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<td>qPCR</td>
<td>37 patients 8 controls</td>
<td>TACE. TNFα gene expression was higher in circulating leukocytes in AMI patients compared with controls ($P &lt; 0.01$) TACE and TNFα gene expression levels were higher in AMI patients than in healthy controls ($P &lt; 0.001$) Levels of TACE and TNFα decreased 14 days after the onset of AMI The percentage of TACE and TNFα cells with positive staining was higher in AMI patients compared with healthy controls ($P &lt; 0.001$) By the 6-month follow-up study, local TACE levels remained the only significant independent predictor of adverse cardiac events in AMI</td>
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<td>AMI (blood samples on day 1 and day 14 after onset of myocardial infarction)</td>
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<td>AMI</td>
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<td>TACE and TNFα gene expression and protein levels in both local and systemic samples obtained from AMI patients were higher than those levels in systemic samples obtained from stable angina patients ($P &lt; 0.001$) In AMI patients, these levels were higher in local samples than in systemic samples ($P &lt; 0.001$) By the 6-month follow-up study, local TACE levels remained the only significant independent predictor of adverse cardiac events in AMI</td>
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**Table 1 (Continued)**

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AAA: abdominal aortic aneurysm; AAV: active proteinase-3 (PR3)-positive ANCA-associated vasculitis; AD: Alzheimer’s disease; AMI: acute myocardial infarction; ANCA: antineutrophil cytoplasmic autoantibodies; CHF: congestive heart failure; ELISA: enzyme-linked immunosorbent assay; FC: flow cytometry; FRET: fluorescent resonance energy transfer; IF: immunofluorescence; IHC: immunohistochemistry assay; MIC: mild cognitive impairment; PBMC: peripheral blood mononuclear cells; qPCR: real time polymerase chain reaction; TIMP3: metalloproteinase inhibitor 3; TNFα: tumour necrosis factor alpha; TNFR1: TNFα receptor type 1; TNFR2: TNFα receptor type 2; sTNFR1: soluble TNFR1; sTNFR2: soluble TNFR2; WB: western blot.
upregulation of TNFα can promote premature endothelial senescence and can participate in the ageing process of coronary arteries [46]. Recently, endogenous transmembrane TNFα was shown to protect against premature senescence in endothelial colony forming cells [47]. This is supported by a murine study in which transgenic mice that only express an uncleavable version of transmembrane TNFα developed fewer inflammatory atherosclerotic plaques than the wild-type mice [48].

It has been also reported that TNFR1 activates multiple signalling pathways that have been linked to apoptosis, endothelial cell dysfunction and inflammation, whereas TNFR2 signalling has been proven to be beneficial to the cardiovascular system by activating angiogenic and survival pathways [49]. Transmembrane TNFα has a higher affinity for TNFR2, which confers a survival signal, mediating angiogenic and blood vessel repair activities [50]. This suggests that transmembrane TNFα cleavage by TACE can have a deleterious effect on the protective and repair function properties provided by transmembrane TNFα. The role of TACE in vascular dysfunction was also highlighted in patients and mice, where ageing and obesity cooperatively reduced caveolin-1 expression and increased vascular endothelial TACE activity and soluble TNFα release in adipose tissue. This was believed to contribute to the development of remote coronary microvascular dysfunction in older obese patients [46].

**TACE inhibition**

As TACE seems to be implicated in many physiological processes, it is important to consider its inhibition and the potential consequences. Interestingly, a patient with homozygous TACE deficiency was identified [52] who, despite repeated skin infections and episodes of bowel disease, led a relatively normal life, indicating that loss of TACE in humans might have less severe consequences than in rodents [53]. The most promising TACE inhibition (without any major physiological consequences) seems to lie in the inhibition of its regulators. iRhom1 and 2 are needed for TACE transport to the cell surface (Fig. 2) and it is well established that iRhom2 is predominantly expressed in immune cells, such as neutrophils and macrophages [54], whereas iRhom1 is mostly expressed on non-immune cells [55]. Therefore, it is tempting to speculate that inhibition of iRhom2 would lead to a selective deficiency of TACE in neutrophils and macrophages with no effects on keratinocytes or intestinal epithelial cells where, in these cell types, iRhom1 would compensate for the blockade of iRhom2. Recently, it was found that reducing the release of TNFα in cardiomyocytes by pharmacologically attenuating the phosphorylation of TACE, reduced TNFα shedding activity by TACE [56]. It was also proven possible to inhibit specifically TACE activity by using its natural inhibitory domain and consequently modulating TNFα secretion in cells [57]. Another strategy of inhibiting TACE could be an injection of its inhibitor TIMP3 in the heart, which has been shown to prevent heart failure post-myocardial infarction [58]. However, since TACE inhibition will reduce TNFα activity, it is important to consider the complex cardiac effects observed after TNFα inhibition as it is becoming increasingly clear that a minimum level of TNFα is important for the normal function of the heart [59,60].

**Future work and conclusions**

TACE plays a major role in controlling inflammatory processes and is involved in several chronic diseases. The number of known TACE substrates continues to increase, with mounting evidence that TACE is implicated in many cellular functions. Recent research indicates a particular role for TACE and the TNF family members in CAD and cardiovascular events, but none have really looked at this panel from a biomarker development point of view. Further investigations are, however, required to ascertain the exact role and mechanism of action of TACE in this disease area.

Future prospective studies in clinical cohorts at varying degrees of cardiovascular risk stratification are needed to fully assess TACE as a potential biomarker for CAD and MACE risk. This will be crucial for the future development of new personalized predictive tests and therapeutics that can improve patient clinical care pathways and prevent the high mortality rates associated with CAD.

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**Disclosure of interest**

The authors declare that they have no competing interest.

**References**


Role of tumour necrosis factor alpha converting enzyme in CAD


