Published in final edited form as:

J Mol Cell Cardiol. 2009 March ; 46(3): 289–291. doi:10.1016/j.yjmcc.2008.11.013.

Stable protein, unstable plaque?

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Introduction

The function of the Class II transactivator (CIITA) lies at the intersection of immune response and maintenance of the structural integrity of tissues. Accordingly, regulation of this interesting molecule has been studied intensively in the past 15 years, and a variety of mechanisms that control CIITA activity has been described. In this issue of the JMCC, Kong *et al.* add another dimension to the post-translational modifications that control CIITA, by providing evidence that stability of the protein is controlled by its acetylation status. Because inflammatory activity and structural integrity are increasingly recognized as essential aspects of the pathogenesis of atherosclerosis, this finding should be of considerable interest both to vascular biologists and to forward-looking clinical investigators aspiring to stabilize the unseen plaques lining their patients' arteries, or to prevent cardiac hypertrophy through the use of histone deacetylase (HDAC) inhibitors.

CIITA was first cloned using a complementation strategy to restore class II major histocompatibility complex (MHC) protein expression in cells derived from patients with bare lymphocyte syndrome, a hereditary immunodeficiency characterized by a complete absence of MHC class II antigens [1]. Subsequent studies established that CIITA functions as a non-DNA binding transactivator to control both constitutive and inducible class II MHC gene expression, and confirmed it as a master regulator of class II MHC gene activation [2]. CIITA is an important target of interferon (IFN)- γ signaling, and it is through the activity of CIITA that this pleiotropic cytokine mediates both induction of class II MHC gene expression and repression of type I collagen expression. While the precise mechanisms that allow these opposite effects on gene regulation remain a point of debate at present [3], it is clear that these CIITA functions serve respectively to promote immune activity (via T cell priming) and to decrease the production of a key structural component of the vascular wall.

IFN- γ , class II MHC proteins, and atherosclerosis

A number of studies over the past 20 years have characterized a significant role for IFN- γ and related adaptive immune function in the pathogenesis of atherosclerosis (see review [4]). In genetically modified hyperlipidemic mice, IFN- γ and its receptor promote atherosclerosis [5, 6]. IFN- γ is present in human atherosclerotic plaques [7], and is likely the prime stimulus for the expression of class II MHC proteins by plaque smooth muscle cells [8]. MHC class II molecules are cell surface glycoproteins that present foreign or autologous peptide antigens to T cells in atherosclerotic plaques and augment the proinflammatory Th1-type T cell response [7,9]. In inflammatory situations, IFN- γ can induce MHC class II gene expression in

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nonprofessional antigen-presenting cells such as fibroblasts, SMCs, and endothelial cells; this induction is mediated via the CIITA. Indeed, both class I and II MHC molecules are expressed abundantly on these cell types in atherosclerotic lesions [10].

Collagen and the vessel wall

The essential nature of type I collagen for maintenance of vascular wall structure is also well established. Type I collagen, the most abundant member of the collagen family, makes up over 80% of the collagen synthesized by fibroblasts and myofibroblasts. It has a triple helical protein structure that consists of two $\alpha 1$ chains ($\alpha 1(I)$) and one $\alpha 2$ chain ($\alpha 2(I)$). Disruption of type collagen I expression by insertional mutagenesis in the mouse leads to lethal vascular rupture between embryonic days 12 and 14 [11]. Loss of collagen due to increased degradation also precipitates vascular disease, and this phenomenon has been identified in vulnerable plaques in carotid arteries [12], and in vascular wall samples from patients suffering from abdominal aortic aneurysm [13]. CIITA is involved in the IFN- γ -mediated repression of Type I collagen as part of a regulatory complex that assembles on the *colla1* and *colla2* promoters [3,14].

CIITA structure

Based on its structural features, CIITA can be placed in the CATERPILLER family of proteins. These proteins, which are characterized by a variable but limited number of N-terminal domains, a central nucleotide-binding domain, and C-terminal leucine-rich repeats, are typically involved in regulation of inflammatory and apoptotic cell processes [15]. Like other CATERPILLER proteins, CIITA can act as a scaffold and has been identified as a participant in distinct multicomponent complexes. The CIITA amino terminus contains an important proline/serine/threonine (PST)-rich domain and an acidic activation domain that mediates interaction with factors involved in chromatin remodeling and modification as well as components of the general transcriptional machinery; this domain is essential for the ability of CIITA to downregulate type I collagen expression, and with over 20 potential phosphorylation sites, may be modified differentially to permit this regulated participation in different activator or repressor complexes [14,16]. For example, CIITA is recruited to the class II MHC promoter through interactions with an enhanceosome [17] that includes cyclic AMP responsive element binding protein (CREB), RFX5, NFY proteins, ATPase-dependent DNA remodeling molecules, and the preinitiation complex (see [3]). The GTP binding domain and a series of leucine-rich repeats in the carboxyl terminus of CIITA are involved in nuclear localization, self-association, and promoter transactivation. In addition, three separate nuclear localization signals are found throughout the length of the protein.

Regulation of CIITA activity

CIITA has been implicated directly as a key component in the opposing transcriptional effects of IFN- γ , which increases expression of MHC class II proteins to promote the adaptive immune response, and concurrently inhibits expression of type I collagen chains. Extensive studies over the past 15 years have shown that CIITA is regulated at multiple levels, including expression (primarily via differential transcription) and post-translational modification, as might be expected for a protein that occupies a central position in the control of the adaptive immune response.

Constitutive and inducible promoters

CIITA transcription is directed by three distinct promoters, CIITA α I, CIITA α II, and CIITA α IV, which show tissue-restricted patterns of expression and distinct cytokine responsiveness (summarized in [18]). The CIITA α IV promoter is of particular interest for the present discussion, since it conveys IFN- γ -responsive induction of CIITA expression in non-

professional antigen presenting cells such as VSMCs. The structure of the promoter is relatively simple and compact, as it consists of a STAT1-binding GAS element, an E-box that interacts with Upstream stimulating factor-1 (USF-1) or c-myc, and an Interferon Regulatory Element (IRF) binding site. The ATP-dependent SWI-SNF chromatin remodeling enzyme (brahma-related gene 1 (BRG1) is associated with the promoter both before and after initiation of IFN- γ signaling; the presence of BRG1 is necessary for recruitment of STAT1 to the GAS site. This event is followed by an increase in histone acetylation through recruitment of CREB-binding protein (CBP) and p300, and then binding of IRF1 and/or IRF2. Interestingly, this promoter is also subject to negative regulation by histone deacetylases (HDACs) 1 and 2 [19].

Ubiquitination

CIITA protein is normally expressed at very low levels, has a relatively short half-life of ~30 minutes, and is degraded via the ubiquitin-proteasome system, with instability mediated through N-terminal domains [20]. Monoubiquitination of CIITA, however, does not lead to its degradation, but rather to an enhancement in its function as a transcriptional activator [21].

Phosphorylation

CIITA modifications by phosphorylation affect its protein-protein interactions (including self-association), accumulation, and nuclear localization, and depending on the specific sites of phosphorylation, can either increase or decrease CIITA transcriptional activity [22-25].

Acetylation

CIITA associates physically with several histone acetyl transferases, including CBP, p300, p300/CBP associated factor (pCAF), and steroid receptor coactivator-1 (SRC-1), and CIITA is necessary for histone modifications at MHC class II promoters (see [18]). In addition, CBP and pCAF can acetylate CIITA at lysine residues within a nuclear localization signal, and this affects its shuttling between the nucleus and cytoplasm, with acetylation corresponding to increased nuclear accumulation [26].

GTP binding

Intracellular localization of CIITA is also controlled by its GTP binding domain, which mediates negative regulation of CIITA nuclear export [27].

CIITA regulates expression of critical target genes in the vasculature

As mentioned before, CIITA is a part of the IFN- γ -induced transcriptional regulatory complex that forms on both MHC class II and Type I collagen promoters. Despite the different effects of IFN- γ on promoter activity, the components of these complexes show a remarkable degree of overlap. Both promoters have binding sites for RFX5, NF-Y, and TFIID transcription factors, albeit in different linear relationships to one another. These DNA binding factors, notably RFX5, recruit CIITA to the respective promoters; in the case of class II MHC, CIITA associates with histone acetyl transferases (HATs) that increase promoter activity, while on the type I collagen promoter, CIITA recruits HDAC2 and the Sin3b corepressor, yielding inhibition of activity [3]. Although its recruitment initially stimulates class II MHC expression, CIITA has been identified subsequently in complexes containing Sin3A and HDAC1/HDAC2 that serve to down-regulate class II MHC expression [19]. The critical difference between the two regulatory events may lie in assembly and recruitment of distinct complexes: CIITA brings Sin3b and HDAC2 to the collagen promoter, while at class II MHC promoters, the dominant complex that is recruited includes HATs [3]. The distribution of CIITA between these different complexes may depend on its phosphorylation by GSK3, which has been linked to its

interaction with Sin3b [3]. Taken together, these findings support the idea that CIITA can act as a molecular switch by coordinating the functions of both histone acetylases and HDACs [19].

Acetylation and CIITA stability

The major contribution made by Kong *et al.* is the observation that CIITA stability is affected by its acetylation status, as regulated at least in part by HDAC2. Although the presence of HDAC2 in promoter bound complexes together with CIITA has been described previously, the work of Kong *et al.* advances understanding of the molecular mechanism underlying these effects by showing a new effect of HDAC2 on CIITA. The authors demonstrate a physical interaction between endogenous CIITA and HDAC2 in smooth muscle cells and macrophages; in the former, this interaction is shown to be IFN- γ dependent. Furthermore, they show that HDAC2 activity negatively affects the acetylation status of CIITA, supporting this finding with gain and loss of function strategies including pharmacologic treatment, kinase-dead mutants, and siRNA-mediated knockdown. The principle effect of acetylation on CIITA appears to lie in regulation of protein stability, with acetylated CIITA being degraded more rapidly than deacetylated CIITA. This degradation of CIITA is sensitive to the proteasome inhibitor MG132, indicating that it occurs via a proteasome-dependent pathway.

Interestingly, HDAC2 antagonizes both transcriptional activities attributed to CIITA — opposing its positive effects on the class II MHC promoter, and its negative effects on the Type I Collagen A2 promoter. It appears that these effects may be due to simple stoichiometry, since chromatin immunoprecipitation shows that gain of HDAC2 function results in less CIITA on both promoters, while loss of HDAC2 by siRNA-mediated knockdown leads to more CIITA on the promoters.

Implications

Despite its importance as a downstream mediator of IFN- γ effects, the role of CIITA in animal models of atherosclerosis has not been studied directly, and the understanding of CIITA function in atherosclerosis would benefit from further proof-of-concept studies in the mouse. This potential analysis is complicated somewhat by the recent observation that the CIITA gene targeted mouse first described over a decade ago [28] appears to be a hypomorph, in that it expresses a C-terminally truncated transcript that has lost the ability to induce MHC class II expression, but retains the ability to repress the type I collagen promoter [29]. In addition, the anticipated phenotype for CIITA loss of function (more stable plaques with less inflammation and less loss of collagen) speaks to the limitations of mouse models of atherosclerosis, which do not typically yield complex and mature plaques with vulnerable characteristics. Such considerations suggest that a transgenic gain-of-function strategy might yield a more compelling connection between CIITA activity and plaque destabilization.

Nevertheless, the findings of Kong *et al.* reinforce the notion that plaque stability might be favorably controlled through limiting CIITA activity, and point to HDAC2 function as a target through which this might be achieved. Interestingly, clinical studies of smokers suggest that loss of HDAC2 activity in lung tissue may be an important part of the pathogenesis of chronic obstructive pulmonary disease. In this setting, nitration of tyrosines in the HDAC2 active site interferes with its enzymatic activity [30]. Does a similar mechanism affect HDAC2 activity in the vascular wall of smokers, and following the mechanism mapped out by Kong *et al.*, lead to increases in CIITA? If so, this could account for some of the excessive vascular pathology associated with smoking. Remarkably, two pharmacologic agents widely used for pulmonary disease, corticosteroids and theophylline, may accrue some of their effectiveness through their ability to increase HDAC2 activities [30]. While the cumbersome side effect profiles of these agents argue strongly against their potential deployment for cardiovascular disease

prophylaxis, better understanding of the mechanisms through which they improve HDAC2 activity could inform new pharmaceutical strategies toward a similar end. Conceivably, novel small molecule strategies to increase HDAC2 activity with selectivity and less toxicity could be developed. It should be noted, however, that HDAC2 is involved in multiple aspects of gene regulation involving the CIITA, and the global effect of even selective HDAC2 inhibition may be difficult to predict.

It should be noted, however, that part of the remarkable effectiveness of statin therapy for prevention of cardiovascular events may be mediated through effects on CIITA. Statins can decrease CIITA expression by limiting its transcription [31]. This effect involves prenylation of Rho family GTPases, probably Rac1, and results in decreased recruitment of multiple components of the transcription complex, including STAT-1 α , IRF-1, USF-1, p300, Brg-1, and RNA polymerase II, to the type IV CIITA promoter [32]. Since statins are highly effective and well tolerated, these findings raise a legitimate question as to whether strategies to decrease CIITA activity at a post-translational level will be able to provide additional benefit. Preclinical studies that assess residual CIITA expression or activity in statin-treated animals with atherosclerosis could help in this evaluation.

Lastly, the findings of Kong *et al.* raise a question as to whether or not HDAC inhibition implemented for other conditions will unintentionally destabilize plaques. With HDAC-directed therapeutic agents on the horizon as possible remedies for cancers [33] and myocardial hypertrophy associated with heart failure [34–36], it is important to consider how these agents might impinge on CIITA-mediated gene regulation and atherosclerosis. From the preclinical standpoint, we need to learn more about the significance of the HDAC2-CIITA axis *in vivo*, especially how it affects the pathogenesis of atherosclerosis. From the pharmacologic standpoint, it becomes ever clearer that selectivity, in this case for methods to bolster HDAC2 effects on CIITA, is essential, but does not guarantee the desired pharmacologic effect to the exclusion of unintended side effects. As recent experience with cyclooxygenase-2 (COX-2) inhibitors demonstrates [37], there is little tolerance for drugs that precipitate myocardial infarction.

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