Cartilage oligomeric matrix protein associates with a vulnerable plaque phenotype in human atherosclerotic plaques

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Abstract

Background and Purpose
Extracellular matrix proteins are important in atherosclerotic disease by influencing plaque stability and cellular behavior, but also by regulating inflammation. Cartilage oligomeric matrix protein (COMP) is present in healthy human arteries and expressed by smooth muscle cells. A recent study showed that transplantation of COMP-deficient bone marrow to apoE/−/− mice increased atherosclerotic plaque formation, indicating a role for COMP also in bone marrow derived cells. Despite the evidence of a role for COMP in murine atherosclerosis, knowledge is lacking regarding the role of COMP in human atherosclerotic disease.

Methods
In the present study, we investigated if COMP was associated with a stable or a vulnerable human atherosclerotic plaque phenotype by analyzing 211 carotid plaques for COMP expression using immunohistochemistry.

Results
Plaque area that stained positive for COMP was significantly larger in atherosclerotic plaques associated with symptoms (n=110) compared to asymptomatic plaques (n=101) (9.7% (4.7-14.3) vs 5.6% (2.8-9.8); p=0.0002). COMP was positively associated with plaque lipids (r=0.32, p=0.000002) and CD68 cells (r=0.15, p=0.036), but was negatively associated with collagen (r=−0.16, p=0.024), elastin (r=−0.14, p=0.041) and smooth muscle cells (r=−0.25, p=0.0002). COMP was positively associated with CD163 (r=0.37, p=0.00000006), a scavenger receptor for hemoglobin/haptoglobin and a marker of Mhem macrophages, and with intraplaque hemorrhage, measured as glycophorin A staining (r=0.28, p=0.00006).

Conclusions
The present study shows that COMP is associated to symptomatic carotid atherosclerosis, CD163 expressing cells and a vulnerable atherosclerotic plaque phenotype in humans.
Introduction

In atherosclerotic disease plaques are formed in the vessel wall. Atherosclerotic plaques that rupture, leading to thrombus formation, may cause arterial occlusion and result in myocardial infarction or stroke. Rupture-prone, so called vulnerable plaques, are characterized by a large necrotic core with high content of lipids, inflammatory cells and intraplaque hemorrhage covered by a thin fibrous cap, whereas stable plaques are characterized by thick fibrous caps with smooth muscle cells (SMCs) producing collagen\(^1\).

Extracellular matrix proteins are important in the atherosclerotic disease not only by influencing plaque stability and cellular behavior, but also by regulating inflammation\(^2\). Cartilage oligomeric matrix protein (COMP) interacts with several proteins, which could influence plaque stability, including collagen type I and growth factors\(^3\). Furthermore, COMP is a substrate for several proteases\(^3\). COMP is present in the medial layer of non-atherosclerotic arteries as well as in intimal SMCs of human primary atherosclerotic and restenotic lesions\(^4\), and regulates SMC differentiation and functions\(^5,6\). In murine atherosclerosis, COMP is present in both inflammatory and fibrous plaques\(^7\). Recent studies in mice revealed a protective role of COMP in atherosclerosis, where deficiency of COMP resulted in increased plaque size\(^7,8\) accompanied by increased plaque calcification\(^8\). Interestingly, bone marrow transplantation experiments revealed a significant role also for COMP in bone-marrow derived cells in plaque development\(^8\).

Despite the evidence of COMP playing a role in murine atherosclerosis, knowledge is lacking on COMP in human atherosclerotic plaques. Here, we analyzed COMP expression in 211 human carotid plaques, and its association to plaque vulnerability.
Methods

Because of the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to Isabel Goncalves, Lund University.

Detailed Materials and Methods are deposited in online-only Data Supplement.

Informed consent was given by each patient. The study was approved by the local ethical committee.

Immunohistochemical analysis

Transversal tissue sections from plaques were stained for COMP. The specificity of the COMP antibody was confirmed by staining of transfected cells overexpressing COMP (Supplemental figure I). Stained areas were quantified blindly, normalized to total plaque area and expressed as % of plaque area stained.
Results

COMP is associated with a vulnerable plaque phenotype

COMP levels were determined in tissue sections from a plaque biobank consisting of 110 plaques from patients with cerebrovascular symptoms and 101 plaques from asymptomatic patients. Baseline characteristics of the patients are shown in Supplemental table I. COMP staining was found in every plaque, and varied between 0.03% and 38.6% staining of total plaque area. COMP was significantly increased in atherosclerotic plaques from symptomatic patients compared to lesions from asymptomatic patients, and in plaques with a high vulnerability index (Figure 1). Furthermore, COMP was positively associated with plaque lipid area and with macrophages determined by CD68 staining, but was negatively associated with collagen and elastin levels as well as with SMCs areas (Table 1).

COMP was localized to areas rich in lipids and CD68-positive cells, and to some extent to areas with SMCs α-actin expression (Supplemental figure II). Co-staining of COMP together with either CD68 or SMC-α-actin revealed that COMP staining was present in both CD68 cells and SMCs (Supplemental figure IIIA-B). In the majority of the plaques, COMP was present in the shoulder regions, in the core, in the interface between the core and media, and between the core and cap, but was only detected in the cap region in 2% of the plaques (Supplemental figure IV).

COMP is associated with CD163-positive cells

Recent data show that COMP-deficient macrophages correlated inversely with the gene profile of M2c macrophages. CD163, a receptor for hemoglobin-haptoglobin, is a marker of M2c and Mhem macrophages. In accordance, COMP was positively correlated with CD163 in the lesions (Table 1). COMP and CD163 were localized to the same plaque areas, but COMP
often showed a more widespread localization (Figure 2). Furthermore, COMP was present in CD163-positive cells of human carotid plaques (Supplemental figure V). To investigate if COMP co-localization with CD163 reflects an upregulated COMP expression, we stimulated monocyte-derived macrophages with heme and measured CD163, COMP, and the Mhem associated cytokine IL-10 by real-time PCR. IL-10 expression peaked after 48 hours (p<0.05, ANOVA, Dunnett’s multiple comparison test), and was preceded by CD163 expression. COMP expression displayed a similar pattern as IL-10 with a transient but non-significant peak after 48 hours of stimulation (Supplemental figure VIA-C). CD163 macrophages are present at sites of intraplaque hemorrhage^10,11. In agreement, COMP correlated with the erythrocyte marker glycophorin A (Table 1). Intraplaque hemorrhage was increased in symptomatic plaques compared to asymptomatic plaques (score 2.1±0.9 versus 1.7±0.8, p=0.012).
Discussion

Previous studies suggest that COMP has a protective role in atherosclerosis. COMP is present in human vascular SMCs, maintains the contractile phenotype, and promotes collagen fibrillogenesis. COMP-deficiency in mice results in larger plaques, accompanied by increased collagen and thicker collagen fibrils. In the present study, we investigated the association of COMP with human plaque vulnerability. Unexpectedly, we found that COMP was increased in human plaques associated with symptoms and was positively correlated with lipid- and CD68 positive areas, but negatively with collagen, elastin and SMCs. Altogether, these data indicate that COMP is associated with a vulnerable carotid plaque phenotype in humans. Interestingly, COMP was associated and colocalized with CD163 cells, which could indicate that COMP modulates macrophage phenotype. This is supported by a previous study, where COMP-deficient macrophages correlated negatively with the gene profile of M2c macrophages and shifted macrophages to a more atherogenic and osteogenic phenotype. Although, lack of COMP in bone marrow-derived cells resulted in increased plaque area indicating a protective role of COMP in plaque progression, this does not exclude that COMP could have an opposite effect in advanced plaques, increasing plaque vulnerability. CD163 cells, previously considered as anti-inflammatory macrophages, were recently shown to be increased in ruptured/rupture-healed human plaques and associated with intraplaque angiogenesis, increased vascular permeability and inflammatory cell recruitment in mice. Thus, it is possible that COMP affects the polarization or function of CD163 macrophages, which in turn increases plaque vulnerability.

Limitations

The current study has some limitations, which should be noted. First, this is not a mechanistic study, and thus conclusions of the functional role of COMP in plaque vulnerability are not
possible. Second, we are only assessing COMP in the most stenotic part of the plaque, and
cannot rule out that the amount of COMP may be different in other parts of the lesions. Third,
in the present study only advanced plaques were used, and it is possible that the associations
of COMP to plaque components may be different in early lesions. On the other hand, the
purpose of the study was to assess COMP in relation to plaque vulnerability, and it thus
makes sense to analyze advanced lesions.

Future directions

Increased knowledge about molecules involved in plaque vulnerability is important for the
discovery of new targets aiming to stabilize the plaques. Future directions should include
mechanistic studies to determine the functional role of COMP in plaque vulnerability, aiming
for therapeutic interventions to stabilize the plaques possibly via macrophage phenotype
modulation.

Conclusion

In conclusion, the current study shows that COMP is increased in carotid atherosclerotic
plaques from symptomatic patients and associates with plaque vulnerability and CD163-
positive macrophages.
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Conflicts of interest

The authors report no conflicts of interest.
References


Figure legends

Figure 1.
COMP is increased in symptomatic and vulnerable human plaques. A, COMP is increased in carotid plaques associated with symptoms. B, COMP is increased in plaques with a high vulnerability index (above median). Vulnerability index was calculated based on the ratio between lipid-, CD68-, hemorrhage- and smooth muscle cell-, collagen-stainings.

Figure 2.
Human carotid plaques (A-C) stained for COMP and CD163. Boxed regions are magnified. Shoulder regions (sh), fibrous caps (fc), core (c), and lumen (l) are indicated.
**Table 1**
Correlation of COMP$^2$ to plaque components.

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<th>COMP$^2$</th>
<th>r</th>
<th>p</th>
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<tr>
<td>Collagen$^1$</td>
<td></td>
<td>-0.16</td>
<td>0.024</td>
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<tr>
<td>Elastin$^1$</td>
<td></td>
<td>-0.14</td>
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<tr>
<td>Lipids (Oil Red O) (%)$^2$</td>
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<tr>
<td>SMCs $\alpha$-actin (%)$^2$</td>
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<td>Macrophages CD68 (%)$^2$</td>
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<td>0.036</td>
</tr>
<tr>
<td>CD163 (%)$^2$</td>
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<td>0.37</td>
<td>0.00000006</td>
</tr>
<tr>
<td>Glycophorin A (%)$^2$</td>
<td></td>
<td>0.28</td>
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$^1$Milligram per gram plaque tissue. $^2$Percentage of total plaque area.
Asymptomatic | Symptomatic
---|---
0 | 10
20 | 30
40 | 50

COMP (% of plaque area stained)  

Vulnerability index

A  
P = 0.0002

B  
P = 0.0004