Metalloproteinases and Foam Cell Macrophage Phenotypes in Atherosclerotic Plaques

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No Conflict of Interests
Macrophage Phenotypes in Granulomatous Diseases e.g. Tuberculosis
• Identify FCM populations that associate with vulnerable plaques
• Identify by expression of MMPs and TIMPs
• Define activation pathways of these FCMs
Matrix Metalloproteinases (MMPs)

- Family of endopeptidases
- Broad specificity
  - Contain Zn and Ca$^{2+}$
  - Possess ability to degrade one or more components ECM
  - Inhibited by specific TIMPs
  - Share amino acid similarities with other MMPs
  - Secreted in latent pro-form and activated extracellularly or at cell surface
MMPs upregulated in human atherosclerotic compared to normal arteries

<table>
<thead>
<tr>
<th>MMP#</th>
<th>Cell type</th>
<th>Principal reference</th>
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<tbody>
<tr>
<td>MMP-7</td>
<td>Mø</td>
<td>Halpert et al, <em>PNAS</em>, 1996</td>
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<tr>
<td>MMP-8</td>
<td>Mø, SMC &amp; EC</td>
<td>Herman et al, <em>Circulation</em>, 2001</td>
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<td>MMP-12</td>
<td>Mø</td>
<td>Halpert et al, <em>PNAS</em>, 1996</td>
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<tr>
<td>MMP-13</td>
<td>Mø</td>
<td>Sukhova et al, <em>Circulation</em>, 1999</td>
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Membrane Type 1 Matrix Metalloproteinase Expression in Human Atherosclerotic Plaques

Evidence for Activation by Proinflammatory Mediators

Presented at the 47th annual scientific sessions of the American College of Cardiology, Atlanta, Ga, March 29–April 1, 1998; and published in abstract form (J Am Coll Cardiol. 1998;31:419A).

Tripathi B. Rajavashisth, PhD; Xiao-Ping Xu, MD; Stefan Jovinge, MD, PhD; Simcha Meisel, MD; Xiao-Ou Xu, MD; Ning-Ning Chai, MD; Michael C. Fishbein, MD; Sanjay Kaul, MD; Bojan Cercek, MD; Behrooz Sharifi, PhD; Prediman K. Shah, MD
Collagen accumulation in the aortic intima of Ldlr\(-/-\) mice receiving Mmp14\(+/+\) or Mmp14\(-/-\) bone marrow cells

Low Tissue Inhibitor of Metalloproteinases 3 and High Matrix Metalloproteinase 14 Levels Defines a Subpopulation of Highly Invasive Foam-Cell Macrophages

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A

Protein

Optical Density

NFM
FCM

TEM-1
600
800
1000
1200

TEM-2
TEM-3
MT1-MMP

B

Protein

TEM-1
TEM-2
TEM-3
MT1-MMP

NFM
FCM
NFM
FCM

C

mRNA

Gene expression (relative units)

NFM
FCM

TEM-3
MT1-MMP

*
New Zealand White Rabbits

- **N = 16**
  - 4 weeks high cholesterol diet
    - Fatty Streak

- **N = 8**
  - 8 weeks cholesterol high cholesterol diet
    - Advanced Plaque

- **N = 16**
  - 8 weeks high cholesterol diet
    - Regressing Plaque

- 8 weeks normal diet
Immunohistochemistry for MT1-MMP in FCMs of Rabbit Aortic Plaques
AtheroExpress Biobank

- 2002
- Prospective cohort study
- UMCU Utrecht/Sint Antonius Nieuwegein
- 2000 + patients
- Consecutive symptomatic/asymptomatic patients with carotid disease >70%
- CEA
- Follow up of cardiovascular events
MT1-MMP Immunostaining in Foam-Cell Macrophages of Human Carotid Plaques
• consecutive cadaveric human donors from 2008
• Patient demographics, drugs and risk factors
• LCA pressure fixed
• RCA en bloc
• H+E/EVG/CD68/α-actin
• AHA (Stary) typed
MT1-MMP Immunostaining in an AHA Type 2 Human Coronary Plaque
MT1-MMP Immunostaining in an AHA Type 6 Human Coronary Plaque
Percentages of MT1-MMP Positive Foam-Cell Macrophages in Human Coronary Plaques
COX2 Immunostaining in Human Carotid Plaques

Unstable

NC

Stable

L

FC

*CD68 x4*

D

*CD68 x4*

*CD68 x40*

B

E

*COX2 x40*

C

F

% COX2 Positive FCMD

Stable

Unstable

***
CCR2 Immunostaining in Human Carotid Plaques
Colocalisation of MT1-MMP with CCR2 in FCMs within Unstable Human Coronary Plaques
Dual Immunohistochemistry for MT1-MMP and NFkB in a Type 4 Human Coronary Plaque
TIMP3 Immunostaining in Human Carotid Plaques

Unstable

A. L, FC, NC

B. CD68 x40

C. TIMP3 x4

Stable

D. L, FC

E. CD68 x40

F. TIMP3 x40

% TIMP3 Positive FCMs

Stable

Unstable

***
CD206 Immunostaining in Human Carotid Plaques

Unstable

Stable

A CD68 x4

B CD68 x40

C CD206 x40

D CD68 x4

E CD68 x40

F CD206 x40

**

Graph showing % CD206 Positive Cells in Stable and Unstable plaques.

Stable

Unstable
Colocalisation of TIMP3 with CD206 in FCMs within a Stable (Type VIII) Human Coronary Plaque
MMP12 Immunostaining in Foam-Cell Macrophages of Human Carotid Plaques
Localisation of MT1-MMP and MMP12 Positive FCMs within Human Carotid Plaques
Conclusions

• $\text{MT1-MMP}^{\text{high}}, \text{MMP-12}^{\text{high}}$ and $\text{TIMP3}^{\text{low}}$ FCMs associate with vulnerable plaques
Conclusions

- $\text{MT1-MMP}^{\text{high}}$, $\text{MMP-12}^{\text{high}}$ and $\text{TIMP3}^{\text{low}}$ FCMs associate with vulnerable plaques.
- These FCMs also express markers of classical activation (COX2, CCR2 and NFκB).
Conclusions

• MT1-MMP$^{\text{high}}$, MMP-12$^{\text{high}}$ and TIMP3$^{\text{low}}$ FCMs associate with vulnerable plaques
• These FCMs also express markers of classical activation (COX2, CCR2 and NF$\kappa$B)
• TIMP3$^{\text{high}}$, MT1-MMP$^{\text{low}}$ and MMP12$^{\text{low}}$ FCMs associate with stable plaques
• These FCMs express markers of alternative activation (CD206)
Conclusions

• $\text{MT1-MMP}^{\text{high}}, \text{MMP-12}^{\text{high}}$ and $\text{TIMP3}^{\text{low}}$ FCMs associate with vulnerable plaques
• These FCMs also express markers of classical activation ($\text{COX2, CCR2}$ and $\text{NFkB}$)
• $\text{TIMP3}^{\text{high}}, \text{MT1-MMP}^{\text{low}}$ and $\text{MMP12}^{\text{low}}$ FCMs associate with stable plaques
• These FCMs express markers of alternative activation ($\text{CD206}$)
• Clinical potential in identifying vulnerable plaques
• Target for plaque stabilising therapy
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