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Immunohistochemical identification of PAPP-A in human atherosclerotic plaques

Solovyeva T.I.[1], Postnikov A.B.[1], Serebryanaya D.V.[2], Tamm N.N.[1], Paltseva E.M.[3], Katrukha A.G.[1]

1. HyTest Ltd, Turku, Finland, **2**. School of biology, Moscow State University, Moscow, Russia

3. Laboratory of molecular methods of diagnostics, I.M. Sechenov Moscow Medical Academy, Russia.

Introduction

Pregnancy Associated Plasma Protein-A (PAPP-A) is a metalloproteinase originally identified in the serum of pregnant women as a heterotetameric complex with proMBP. Recent studies indicated that homodimeric form of PAPP-A (dPAPP-A) is notebly expressed in unstable atherosclerotic plaques. It was shown that dPAPP-A is produced by activated cells of the immune system in unstable plaques and is released into the extracellular matrix. Also it was suggested that dPAPP-A could be involved in weakening of the fibrous cap.

However, there are certain methodological difficulties for investigation of plaque form of PAPP-A caused by its low concentration in tissue, high molecular weight of this protein and various posttranslational modifications. Moreover there is a lack of information about expression and localization of dPAPP-A in atherosclerotic plaque.

The aims of our study were:

1. to develop a method of PAPP-A immunodetection in atherosclerotic plaques of patients with atherosclerosis, to demonstrate distribution and localization of PAPP-A in the tissue;
2. to investigate the expression of dPAPP-A in vulnerable plaques.

Materials and Methods

Collection of tissue: The study group consisted of four patients with multifocal atherosclerosis: three patients were with aortic aneurysm, one with 50-60% carotid artery stenosis (all men) (Table 1).

Fragments of aorta walls and internal carotid artery with atherosclerosis were obtained at aorta aneurism resection from three patients, and carotid endarterectomy, consequently. The fragment of intact ("healthy") aorta wall was used as a control.

Immunohistochemistry: Samples were fixed by 4% formalin solution. Immunohistochemical staining was performed on 4-µm-thick paraffin sections by using peroxidase-labeled secondary antibodies.

In the study we have tested a set of mouse anti-PAPP-A monoclonal antibodies obtained with PAPP-A/proMBP complex as an immunogen **(HyTest Ltd)**. PAPP-A subunit–specific MAb **10A5** was selected for tissue staining. MAb 10A5 was used at a concentration of 10 µg/mL. Tissue sections were counterstained with hematoxylin and eosin.

Sandwich immunofluorescent assay (IFA): For the pairs design we used MAbs with different specificity:

MAb ***10A5, 7A6*** specific to PAPP-A subunit;

MAb ***5H9*** specific to proMBP.

Using these antibodies we have obtained two in-house assays:

first, specific to heterotetrameric complex PAPP-A/proMBP (5H9 – 7A6);

second, react with both heterotetrameric (PAPP-A/proMBP) and homodimeric (dPAPP-A) proteins (10A5 – 7A6).

Detection antibodies were labeled with Eu^{3+}-chelate. The fluorescence was measured by Victor 1420 multilabel counter (Wallac-Percin Elmer, Finland).

Results and discussion

We have investigated atherosclerotic plaques from four patients with multifocal atherosclerosis.

Microscopic analysis of histological sections, stained with hematoxylin and eosin revealed that all atherosclerotic plaques were **unstable**, on the stage of the thin-cap fibroatheroma. Moreover, the elements of calcification were detected in two of these samples.

Using MAb ***10A5*** for IHC analysis of atherosclerotic plaques' sections we have detected specific PAPP-A staining in all four patients, however it was not visualized in control tissue (Fig. 1).

We have developed a method of PAPP-A detection in atherosclerotic plaques using high affinity anti-PAPP-A MAb ***10A5*** (Fig. 2). This MAb 10A5 was specific to PAPP-A subunit of proteins, i.e. it recognized both heterotetrameric form (PAPP-A/proMBP) and homodimeric form (dPAPP-A).

To show that MAbs used in the study were specific to atherosclerotic tissue PAPP-A we have purified PAPP-A from atherosclerotic coronary vessels by affinity chromatography (see poster B-127) and then analyzed atherosclerotic tissue PAPP-A using two in-house sandwich immunoassays.

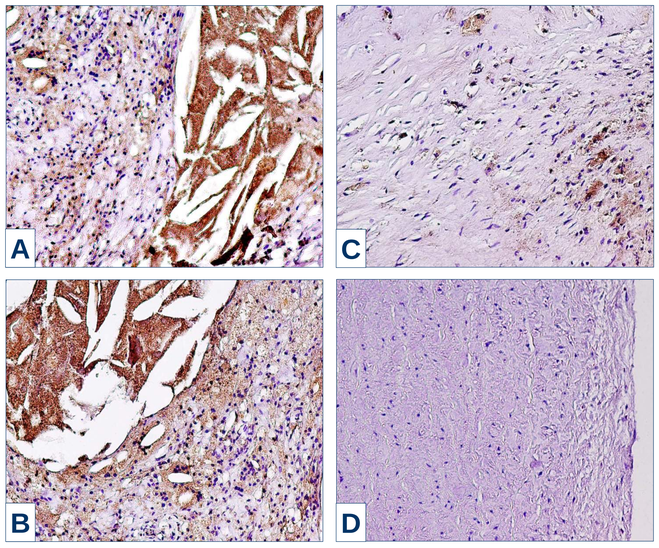

Figure 1.
Expression of Pregnancy-Associated Plasma Protein (PAPP-A) in unstable coronary atherosclerotic plaques. The expression of PAPP-A was determined by immunohistochemical staining with anti-human MAb ***10A5*** specific to PAPP-A and peroxidase detection. PAPP-A containing regions had reddish-brown staining.
Panel A, B – Intense PAPP-A staining was present in the area densely infiltrated by macrophages as well as in the area of lipid core with cholesterol crystals. The inflammatory infiltrate is present between the cholesterol core and luminal thrombus.
Panel C – Positive PAPP-A staining in area of lymph-macrophage infiltration.
Panel D – The absence of staining in fragment of intact aorta used as a control.

It was also shown that PAPP-A was co-localized with the macrophages infiltration in atherosclerotic plaques and expression of PAPP-A was higher in case of intensive infiltration. Study group characteristics is presented in Table 1.

Patient	Panel at Fig. 1	Diagnosis	Sample	Morphological characteristic	Intensity of PAPP-A staining
1	Data not shown	Multifocal atherosclerosis, aneurism infrarenal part of aorta	Part of abdominal aorta wall with atherosclerotic plaques	Fibroatheroma with elements of calcification	+
2	A	Multifocal atherosclerosis, stenosis of left internal carotid artery 50-60%	Part of left internal carotid artery	Fibroatheroma	+++
3	Data not shown	Multifocal atherosclerosis, aneurism infrarenal part of aorta	Part of abdominal aorta wall with atherosclerotic plaques	Fibroatheroma	++
4	B, C	False aneurism of descending part of aorta	Part of thoracic aorta wall with atherosclerotic plaques	Fibroatheroma	++
5	D		Part of thoracic aorta wall	Intact aorta	-

Table 1.
The table shows the information about study group: the number of tested samples, diagnoses, morphological analysis and the level of PAPP-A staining.

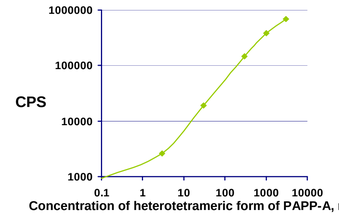

Figure 2. Calibration curve of PAPP-A assay. MAb 10A5 was used as a capture antibody. MAb 7A6 was used as detection and was labeled with Eu^{3+}-chelate. This assay recognize both heterotetrameric form of PAPP-A and dPAPP-A.

Two in-house sandwich immunoassays were developed for analysis of PAPP-A from atherosclerotic plaques. Assay ***5H9 – 7A6*** was used to detect PAPP-A/proMBP heterotetramer, whereas assay with MAbs ***10A5*** and ***7A6*** specific to PAPP-A was able to detect both – PAPP-A in PAPP-A/proMBP complex and homodimeric form of the protein.

We have demonstrated the absence of PAPP-A/proMBP complex in atherosclerotic plaques, whereas dPAPP-A content in the plaque-containing vessels was determined as 80 ng/g. However dPAPP-A content exactly in atherosclerotic plaques could be significantly higher because total preparation of coronary arteries was used for protein isolation.

Using specific monoclonal antibodies, we have shown that PAPP-A was abundantly expressed in unstable plaques but was not expressed in normal tissue. Elevated PAPP-A expression in atherosclerotic plaque could lead to its destabilization and increased serum levels of dPAPP-A are associated with development of acute coronary syndrome (1, 2). Moreover serum dPAPP-A was suggested as a marker of some cardiovascular diseases, such as stable and unstable angina, myocardial infarction (3).

Conclusions

We have developed method of IHC detection of PAPP in atherosclerotic plaques.

Our finding confirms the hypothesis suggesting dPAPP-A participation in inflammatory processes that could result in destabilization and rupture of atherosclerotic plaque.

References

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For further information:

Phone: +358-2-512-0900,
Fax: +358-2-512-0909
hytest@hytest.fi
www.hytest.fi

HyTest