Investigation of immunochemical and biochemical properties of circulating IGFBP-4 fragment—a novel biomarker for cardiac risk assessment

Konev A.A.1, Postnikov A.B.1, Smolyanova T.I.1, Tamm N.N.1, Kharitonov A.V.1, Serebryanova D.V.1, Kozlovsky S.V.2, Kara A.N.2, Katrukha A.G.1
1HyTest Ltd, Turku, Finland; 2School of Biology, Moscow State University, Moscow, Russia

Introducing

The dimeric form of the zinc metalloprotease Pregnancy Associated Plasma Protein A (dPAPP-A) is overexpressed in vulnerable atherosclerotic plaque and is associated with the plaque destabilisation [1]. Insulin-like growth factor-binding protein 4 (IGFBP-4) was described as one of dPAPP-A substrate molecules. It was shown that dPAPP-A is able to specifically cleave IGFBP-4, forming the N- and C-terminal fragments (NT- and CT-IGFBP-4; Fig. 1 and 2). Recent findings [2] show that NT- and CT-IGFBP-4 can be utilized as biomarkers for the prediction of major adverse cardiac events in patients with suspected acute coronary syndrome (ACS). It was suggested that increased NT- and CT-IGFBP-4 levels in patients’ blood is associated with increased expression and proteolytic activity of dPAPP-A and thus with atherosclerotic plaque destabilization. Novel NT- and CT-IGFBP-4 immunoassays utilizing monoclonal antibodies specific to proteolytic neo-epitopes (dPAPP-A dependent cleavage of IGFBP-4) were described in Fig. 2. However the level of truncation or degradation of NT- and CT-IGFBP-4 released into the circulation has not been investigated yet. The degraded or truncated forms of circulating IGFBP-4 fragments do not contain the native neo-epitopes. By this reason NT- and CT-IGFBP-4 cannot be detected by IGFBP-4 fragments immunoassays utilizing neo-epitope specific antibodies. The goal of our study was to investigate biochemical properties, the level of degradation and truncation of NT- and CT-IGFBP-4 in blood.

Results and discussion

In Western blotting (Fig. 5) NT- and CT-IGFBP-4 bands had the same apparent molecular masses as their recombinant analogues’ bands: 18 and 14 kDa, respectively. Endogenous NT- and CT-IGFBP-4 were detected using core-IBPs and neo-epitope-specific MAbs (IBP3 and IBP163, respectively). Thus we can suggest that the extracted endogenous NT- and CT-IGFBP-4 fragments contain the products of the proteolytic activity of dPAPP-A, and do not contain measurable amounts of degraded fragments.

Mass-spectrometry studies of endogenous NT- and CT-IGFBP-4 (Fig. 4) confirmed the data obtained by WB analysis revealing the only one peak for both proteins corresponding to the products of dPAPP-A dependent cleavage of IGFBP-4 (14615 and 11349 Da, respectively). Accuracy of mass measurements <0.05%. Peaks corresponding to the truncated forms of NT- and CT-IGFBP-4 were not detected.

Affinity purification of endogenous NT- and CT-IGFBP-4 from human ACS plasma. Mouse monoclonal antibodies IBP180 and IB185 (HyTest, Finland) were used for immunosorptions preparation. The extraction of endogenous NT- and CT-IGFBP-4 was realized utilizing pooled human plasma collected from 15 individual ACS patients. Immunochemical analysis of endogenous and recombinant NT- and CT-IGFBP-4 were compared using fragment-specific immunoassays IBP3-IBP180 and IB182-IBP163 (Fig. 2), respectively. MAbs IBP3 and IBP163 are specific to the neo-epitopes of NT- and CT-IGFBP-4, respectively, and do not cross-react with full-length IGFBP-4 as well as with truncated neo-epitopes [1]. The native state of the standard proteins’ neo-epitopes was demonstrated using MALDI mass-spectrometry analysis (Fig. 3).

Western blotting. Extracted proteins were analyzed by WB using MAbs IBP3, IBP180 (NT-IGFBP-4), IB183 and IBP165 (CT-IGFBP-4).

Size-exclusion chromatography on Superdex 75 column was utilized for characterization of IGFBP-4 fragments in ACS patients’ plasma.

Materials and methods

Affinity purification of endogenous NT- and CT-IGFBP-4 from human ACS plasma. Mouse monoclonal antibodies IBP180 and IB185 (HyTest, Finland) were used for immunosorptions preparation. The extraction of endogenous NT- and CT-IGFBP-4 was realized utilizing pooled human plasma collected from 15 individual ACS patients. Immunochemical analysis of endogenous and recombinant NT- and CT-IGFBP-4 were compared using fragment-specific immunoassays IBP3-IBP180 and IB182-IBP163 (Fig. 2), respectively. MAbs IBP3 and IBP163 are specific to the neo-epitopes of NT- and CT-IGFBP-4, respectively, and do not cross-react with full-length IGFBP-4 as well as with truncated neo-epitopes [1]. The native state of the standard proteins’ neo-epitopes was demonstrated using MALDI mass-spectrometry analysis (Fig. 3).

Western blotting. Extracted proteins were analyzed by WB using MAbs IBP3, IBP180 (NT-IGFBP-4), IB183 and IBP165 (CT-IGFBP-4).

Size-exclusion chromatography on Superdex 75 column was utilized for characterization of IGFBP-4 fragments in ACS patients’ plasma.

Conclusions

Here we for the first time describe the endogenous NT- and CT-IGFBP-4 from ACS patients’ blood. Both proteins display the biochemical and immunochemical features similar to the recombinant proteins expressed in mammalian cell lines.

References


For further information: www.hytest.fi Alexey.Katrukha@hytest.fi