FP-282

DEVELOPMENT OF AN IMMUNOASSAY THAT REVEALS ALTERED URINARY VANIN-1 IN HUMAN WITH KIDNEY DISEASE

The Antibody Lab

BIOMEDICA

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SUMMARY AND CONCLUSION

The sandwich immunoassay for the detection of VNN1 in human urine samples comprises well-characterized antibodies. The capture antibody is directed against one linear epitope and the detection antibody has several linear epitopes throughout the whole molecule. The ELISA covers a concentration range of 0 - 450 pmol/l with high specificity of generated sample signals. VNN1 concentrations in urine of dialysis patients in comparison with apparently healthy controls show a significant increase.
This human Vanin-1 ELISA provides a sensitive, well-characterized and highly specific tool for the detection of Vanin-1 in different matrices and may be used for further investigations of Vanin-1 as a renal disease biomarker.

INTRODUCTION

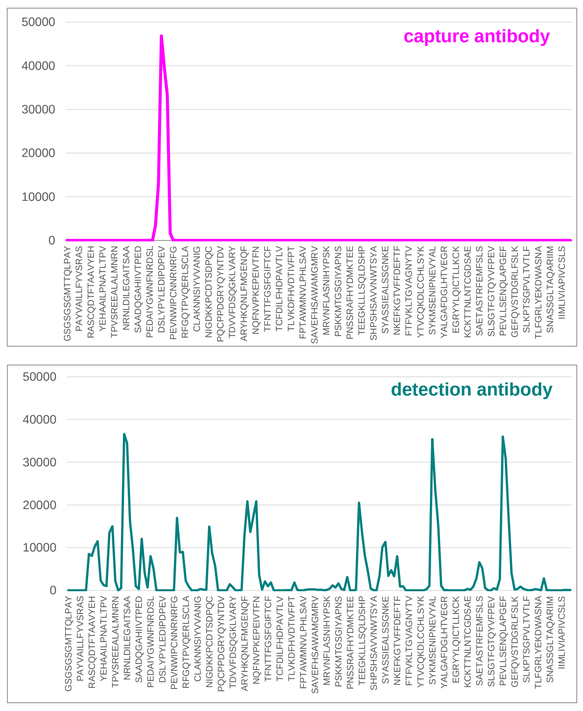
Vascular Non-Inflammatory Molecule 1 (Vanin-1), also known as pantetheinase, is a 60-80 kDa ubiquitously expressed enzyme. It is involved in the recycling of pantothenic acid (vitamin B5) and in the production of cysteamine, a potent anti-oxidant.
Vanin plays a role in oxidative stress, hepato-carcinogenesis, type 2 diabetes and gut inflammation. Recent studies indicate that urinary Vanin-1 may be an earlier biomarker for acute kidney injury, as well as for the detection of nephro-toxicant-induced renal injury, than established markers like NGAL and KIM-1.
Additionally, Vanin-1 could be also interesting for other renal diseases, therefore a highly-specific and well-characterized ELISA was developed and validated.

METHODS

We have developed a sandwich ELISA for the specific detection of human VNN1 in complex matrices like urine. Therefore an epitope mapping with overlapping peptides spotted to a microarray for a polyclonal sheep anti-human VNN1 antibody was performed.
An immune-affinity purified antibody against one single epitope was used as capture antibody, whereas the residual polyclonal antibody was horseradish peroxidase labelled and used as detection antibody. Association and dissociation of both antibodies was assessed with biolayer interferometry.
For the ELISA only 5 µl sample volume is needed and the overall incubation is less than 5 hours. Specificity of the signals was determined and different sample panels were measured.

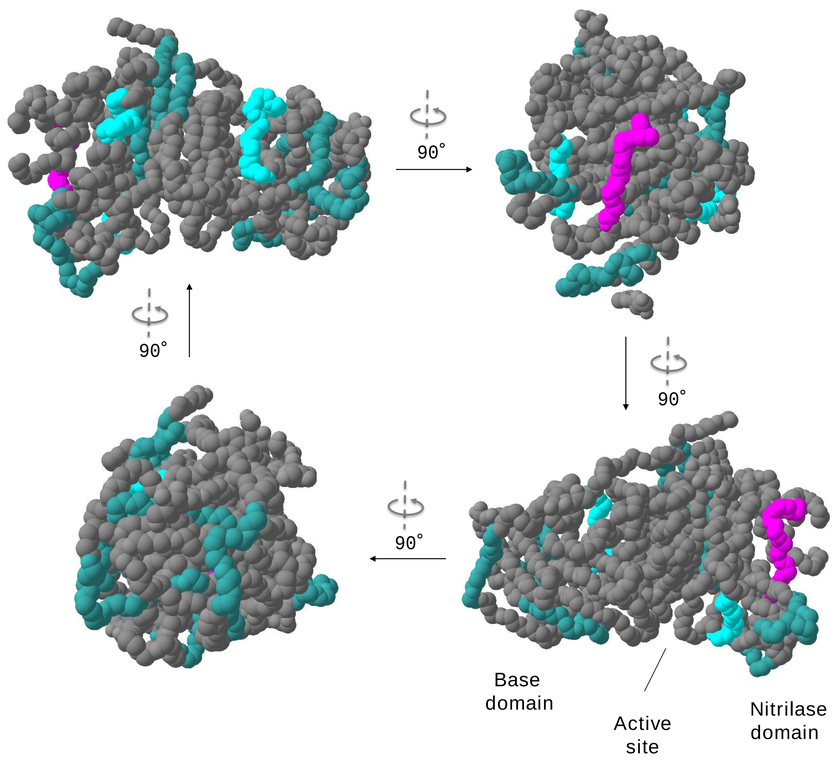
ANTIBODY CHARACTERISTICS

Epitope mapping of capture and detection antibody



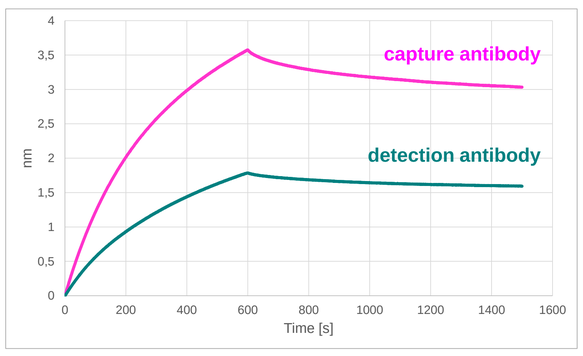
Epitope mapping of peptide purified capture antibody (upper panel) reveals the linear epitope at position 96-104 with the sequence LEDIPDPEV.
The detection antibody (lower panel) has 12 main epitopes throughout the whole sequence.

Distribution of linear epitopes of antibodies throughout the whole VNN1 molecule



3D structure of human VNN1 with designated binding sites of both antibodies. The epitope of capture antibody (pink) is located in the nitrilase domain, whereas the detection antibody can bind throughout the whole molecule. Displayed are 4 different orientations of the molecule.

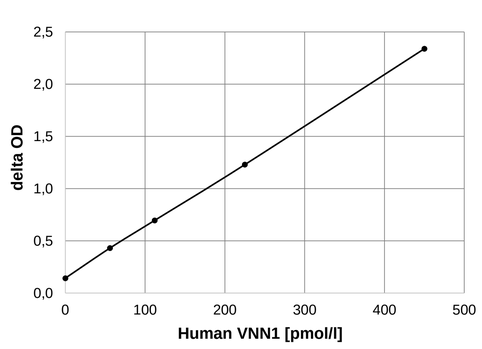
Association and dissociation to human VNN1 protein



Biolayer interferometry of capture (pink) and detection (turquoise) antibody. Association to human VNN1 was determined for 600s (x-axis) and dissociation for 900 s.

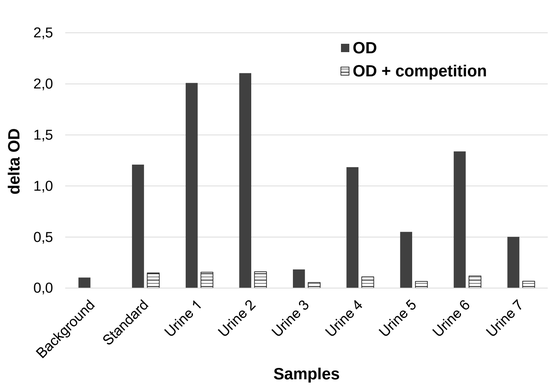
HUMAN VANIN-1 ELISA

Calibration range of 0 – 450 pmol/l



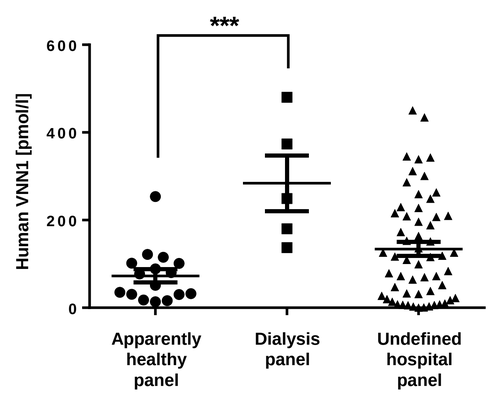
Typical standard curve of human VNN1 protein 1:2 step diluted in protein-based buffer. It covers a range from 0 – 450 pmol/l (x-axis) with a delta OD (y-axis) up to approximately 2.5.

Highly specific signals of VNN1 in human urine



Specificity of human VNN1 ELISA was determined for standard protein as well as for urine samples by adding at least a 5-fold excess of capture antibody to the samples. Mean competition was 99% (96-100%).

Significant increase of VNN1 in patients with impaired kidney function



Human VNN1 concentration is significantly higher in dialysis samples (284 ± 63 pmol/l) compared with controls (73 ± 15 pmol/l). The undefined hospital panel gives an impression that VNN1 might play a role in different diseases.

LITERATURE

1) Hosohata K., Ando H., Fujimura A. (2012): Urinary vanin-1 as a novel biomarker for early detection of drug-induced acute kidney injury. J Pharmacol Exp Ther 341(3):665-62
2) Hosohata K. (2017): Biomarkers for Chronic Kidney Disease Associated with High Salt Intake. Int J Mol Sci 18(10):

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We have no relevant financial relationship to disclose any COI for this research presentation within the period of 36 months.