

DPP3 as therapeutic target in all kinds of diseases, due to ubiquitous expression of DPP3 in or at surface of cell. EP1498480 mentions the potential diagnostic and therapeutic use of hydrolytic enzymes, including DPP3.

5 Not only has DPP3 been proposed as potential biomarker but also as potential therapeutic target due to its ability to cleave several bioactive peptides. Overexpression of DPP3 protects neuroblastoma cells from oxidative stress (Liu et al. 2007). Influenza A virus changes host DPP3 levels for own replication (cell culture studies, Meliopoulos et al. 2012). Enkephalin and/ or angiotensin degrading enzymes in general, including DPP3, have a therapeutic
10 potential as targets for treatment of pain, cardiovascular diseases (CVD) and cancer and the corresponding inhibitors as potential treatments of pain, mental illnesses and CVD (Khaket et al. 2012, Patel et al. 1993, Igic et al. 2007).

Although DPP3 is known as an intracellular protein, DPP3 activity was detected in some
15 bodily fluids: retroplacental serum (Shimamori et al. 1986), seminal plasma (Vanha-Perttula et al. 1988) and CSF (Aoyagi et al. 1993). In CSF there were elevated DPP3 activity levels measured in patients suffering from Alzheimer's disease (AD, Aoyagi et al. 1993). Wattiaux et al. (2007) proposed the release of intracellular DPP3 as a marker for dead and/ or dying cells in a cell culture system. It has also been proposed that the release of DPP3 from necrotic
20 cells influences immune response in a mouse model (Gamrekelashvili et al. 2013).

Summary

Disclosed herein is a method for diagnosing a disease or condition in a subject accompanied
25 by or related to necrotic processes comprising:

- determining the amount of total DPP3 and/or determining the amount of active DPP3 in a sample of bodily fluid of said subject,
- comparing said determined amount of total DPP3 or active DPP3 to a predetermined threshold,
- 30 • wherein said subject is diagnosed as having a disease or condition accompanied by or related to necrotic processes if said determined amount is above said predetermined threshold.
- wherein said sample is selected from the group comprising whole blood, serum and plasma.

Disclosed herein is the use of an inhibitor of the activity of DPP3 in the manufacture of a medicament for the prevention or therapy of a disease or condition in a subject accompanied by or related to necrotic processes, wherein said disease is selected from the group comprising heart failure, chronic heart failure, acute heart failure (AHF), myocardial infarction (MI), stroke, liver failure, burn injuries, traumatic injuries, severe infection (microbial, viral (e.g. AIDS), parasitic (e.g. Malaria)), SIRS or sepsis, cancer, acute kidney injury (AKI), CNS disorders (e.g. seizures, neurodegenerative diseases), autoimmune diseases and vascular diseases (e.g. Kawasaki syndrome) and hypotension.

Disclosed herein is the use of an inhibitor of the activity of DPP3 as defined herein in a method of extracorporeal removal of DPP3 from plasma comprising apheresis and affinity chromatography.

Disclosed herein is a method for determining active DPP3 in a bodily fluid sample of a subject comprising the steps:

- Contacting said sample with a capture-binder that binds specifically to full-length DPP3,
- Separating DPP3 bound to said capture binder,
- Adding substrate of DPP3 to said separated DPP3,
- Quantifying of said active DPP3 by measuring and quantifying the conversion of a substrate of DPP3.

Disclosed herein is an assay or kit for determining active DPP3 in a bodily fluid sample of a subject comprising:

- A capture-binder that binds specifically to full-length DPP3,
- A substrate of DPP3.

Detailed Description

Provided herein is a method for determining active DPP3 in a sample of bodily fluid specifically, i.e. that active DPP3 is determined but not any other aminopeptidase than DPP3. Disclosed herein are respective assays and kits.

Disclosed herein is a method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes and methods of treating said disease.

CLAIMS

1. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes comprising:
 - determining the amount of total DPP3 and/or determining the amount of active DPP3 in a sample of bodily fluid of said subject,
 - comparing said determined amount of total DPP3 or active DPP3 to a predetermined threshold,
 - wherein said subject is diagnosed as having a disease or condition accompanied by or related to necrotic processes if said determined amount is above said predetermined threshold.
 - wherein said sample is selected from the group comprising whole blood, serum and plasma.
2. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to claim 1 wherein the amount of total or active DPP3 is determined in the unit of concentration.
3. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to claims 1 or 2, wherein said disease is selected from the group comprising heart failure, chronic heart failure, acute heart failure (AHF), myocardial infarction (MI), stroke, liver failure, burn injuries, traumatic injuries, severe infection (microbial, viral (e.g. AIDS), parasitic (e.g. Malaria)), SIRS or sepsis, cancer, acute kidney injury (AKI), CNS disorders (e.g. seizures, neurodegenerative diseases), autoimmune diseases and vascular diseases (e.g. Kawasaki syndrome) and hypotension.
4. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to any of claims 1-3, wherein the amount of total DPP3 and/or the amount of active DPP3 is determined in a bodily fluid sample of said subject and comprises the steps:

- Contacting said sample with a capture-binder that binds specifically to full-length DPP3,
 - Separating DPP3 bound to said capture binder,
 - Adding substrate of DPP3 to said separated DPP3,
 - Quantifying of said active DPP3 by measuring and quantifying the conversion of a substrate of DPP3.
5. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to claim 4, wherein said capture-binder may be selected from the group of antibody, antibody fragment or non-IgG scaffold.
 6. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to claims 4 or 5, wherein said capture-binder is an antibody.
 7. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to any of claims 4-6, wherein said capture binder is immobilized on a surface.
 8. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to any of claims 4-7, wherein said separation step is a washing step that removes ingredients of the sample that are not bound to said capture-binder from the captured DPP3.
 9. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to any of claims 4-8, wherein DPP3 substrate conversion is detected by a method selected from the group comprising: fluorescence of fluorogenic substrates (e.g. Arg-Arg- β NA, Arg-Arg-AMC), color change of chromogenic substrates, luminescence of substrates coupled to aminoluciferin (Promega Protease-GloTM Assay), mass spectrometry, HPLC/ FPLC (reversed phase chromatography, size exclusion chromatography), thin layer chromatography, capillary zone electrophoresis, gel electrophoresis followed by activity staining (immobilized, active DPP3) or western blot (cleavage products).

10. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to any of claims 4-9, wherein said substrate may be selected from the group comprising: angiotensin II, III and IV, Leu-enkephalin, Met-enkephalin, endomorphin 1 and 2, valorphin, β -casomorphin, dynorphin, proctolin, ACTH and MSH, or di-peptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.
11. Method for diagnosing a disease or condition in a subject accompanied by or related to necrotic processes according to any of claims 4-10, wherein said substrate may be selected from the group comprising: a di-peptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.
12. Method for monitoring a disease or condition in a subject accompanied by or related to necrotic processes wherein the method of diagnosing according to any of claims 1 to 11 is conducted at least twice.
13. Use of an inhibitor of the activity of DPP3 in the manufacture of a medicament for the prevention or therapy of a disease or condition in a subject accompanied by or related to necrotic processes, wherein said disease is selected from the group comprising heart failure, chronic heart failure, acute heart failure (AHF), myocardial infarction (MI), stroke, liver failure, burn injuries, traumatic injuries, severe infection (microbial, viral (e.g. AIDS), parasitic (e.g. Malaria)), SIRS or sepsis, cancer, acute kidney injury (AKI), CNS disorders (e.g. seizures, neurodegenerative diseases), autoimmune diseases and vascular diseases (e.g. Kawasaki syndrome) and hypotension.
14. Use according to claim 13 wherein said inhibitor is selected from the group comprising an anti-DPP3 antibody or anti-DPP3 antibody fragment or anti-DPP3 non-Ig scaffold.
15. Use according to claim 14 wherein said inhibitor is an antibody that is mono-binding or at least two-binding.

16. Use according to claim 14 or 15 wherein said inhibitor is an anti-DPP3 antibody or anti-DPP3 antibody fragment or anti-DPP3 non-Ig scaffold that binds to SEQ ID No. 1, in particular that binds to SEQ ID No. 2.
17. Use according to any one of claims 14 to 16 wherein said inhibitor is an antibody or fragment or scaffold that exhibits a minimum binding affinity to DPP3 of equal or less than 10^{-7} M.
18. Use according to any one of claims 14 to 17 wherein said inhibitor is an antibody or fragment or scaffold that is monospecific.
19. Use according to any one of claims 14 to 18 wherein said inhibitor is an antibody or fragment or scaffold binds to full-length DPP3 and inhibits activity of DPP3 of at least 10%, or at least 50%, more preferred at least 60%, even more preferred more than 70 %, even more preferred more than 80 %, even more preferred more than 90 %, even more preferred more than 95 %.
20. Use according to any one of claims 14 to 19 wherein said inhibitor is selective and/or specific to DPP3 and not cross cell membranes and/or the blood brain barrier.
21. Use according to any one of claims 14 to 20 wherein said subject has an amount of total DPP3 and/or an amount of active DPP3 in a sample of bodily fluid of said subject that is above a predetermined threshold.
22. Use according to any one of claims 14 to 21 wherein the medicament is a pharmaceutical composition comprising an inhibitor of the activity of DPP3.
23. Use of an inhibitor of the activity of DPP3 as defined in any one of claims 14 to 21 in a method of extracorporeal removal of DPP3 from plasma comprising apheresis and affinity chromatography.
24. Method for determining active DPP3 in a bodily fluid sample of a subject comprising the steps:

- Contacting said sample with a capture-binder that binds specifically to full-length DPP3,
 - Separating DPP3 bound to said capture binder,
 - Adding substrate of DPP3 to said separated DPP3,
 - Quantifying of said active DPP3 by measuring and quantifying the conversion of a substrate of DPP3.
25. Method for determining active DPP3 in a bodily fluid sample of a subject according to claim 24 wherein said capture-binder may be selected from the group of antibody, antibody fragment or non-IgG scaffold.
 26. Method for determining active DPP3 in a bodily fluid sample of a subject according to claims 24 or 25 wherein said capture-binder is an antibody.
 27. Method for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 24 to 26 wherein said capture binder is immobilized on a surface.
 28. Method for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 24 to 27 wherein said separation step is a washing step that removes ingredients of the sample that are not bound to said capture-binder from the captured DPP3.
 29. Method for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 24 to 28 wherein DPP3 substrate conversion is detected by a method selected from the group comprising: fluorescence of fluorogenic substrates (e.g. Arg-Arg- β NA, Arg-Arg-AMC), color change of chromogenic substrates, luminescence of substrates coupled to aminoluciferin (Promega Protease-GloTM Assay), mass spectrometry, HPLC/ FPLC (reversed phase chromatography, size exclusion chromatography), thin layer chromatography, capillary zone electrophoresis, gel electrophoresis followed by activity staining (immobilized, active DPP3) or western blot (cleavage products).

30. Method for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 24 to 29, wherein said substrate may be selected from the group comprising: A di-peptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the di-peptide is Arg-Arg.
31. Method for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 24 to 30, wherein said sample is a blood sample selected from the group comprising whole blood, serum and plasma.
32. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject comprising:
 - A capture-binder that binds specifically to full-length DPP3,
 - A substrate of DPP3.
33. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject according to claim 32, wherein said capture-binder may be selected from the group of antibody, antibody fragment or non-IgG scaffold.
34. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject according to claim 32 or 33, wherein said capture-binder inhibits less than 50% DPP3 activity in a liquid phase assay, preferably less than 40%, more preferably less than 30 %.
35. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 32 to 34, wherein the binding region of DPP3 for the capture-binder is not within the region of amino acids 316 - 669 of SEQ Id No. 1.
36. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 32 to 35 wherein said binder is an antibody.
37. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 32 to 36 wherein said capture-binder is immobilized on a surface.

38. Assay or kit for determining active DPP3 in a bodily fluid sample of a subject according to any of claims 32 to 37 wherein said substrate may be selected from the group comprising: angiotensin II, III and IV, Leu-enkephalin, Met-enkephalin, endomorphin 1 and 2, valorphin, β -casomorphin, dynorphin, proctolin, ACTH and MSH, or a dipeptide coupled to a fluorophore, a chromophore or aminoluciferin wherein the dipeptide is Arg-Arg.