The clinical course of anti-p200 pemphigoid is variable. In contrast to epidermolysis bullosa acquisita and bullous pemphigoid, what is concerned here is a relatively benign disease with a good therapeutic response. Therefore, it is of great clinical importance to differentiate between these diseases.

US6,682,911 discloses laminin 12 and preparations of isolated laminin 12 and of subunits thereof.

Against this background, there is a need for a detection procedure for anti-p200 pemphigoid that has higher diagnostic reliability, especially sensitivity. At the same time, there is the additional aim of improving the differentiability between the various blistering autoimmune diseases of the skin.

Summary

In one example, the object underlying the disclosure is achieved by a polypeptide comprising laminin beta-4 or a variant thereof, preferably in recombinant and/or isolated form.

In a preferred example, the polypeptide is immobilized.

In a second example, the object is achieved by a carrier comprising the polypeptide according to the disclosure, and further comprising at least one antigen, preferably all the antigens, from the group comprising laminin gamma-1, BP180, BP230, desmoglein 1, desmoglein 3, envoplakin, deamidated gliadin, laminin 332 and collagen type VII or variants thereof.

In a preferred example, the carrier is selected from the group comprising a glass slide, preferably slide for microscopy, a biochip, a microtiter plate, a lateral flow device, a test strip, a membrane, preferably a blot, more preferably a line blot, a chromatography column material and a bead, preferably a magnetic or fluorescent bead.

In one aspect, the present disclosure refers to a kit comprising a polypeptide comprising laminin beta-4 or a variant thereof, preferably in recombinant and/or isolated form, and an agent for the detection of an autoantibody against laminin beta-4, wherein the variant has the capability of binding to an autoantibody against laminin beta-4.

In another aspect, the present disclosure refers to a therapeutically useful carrier comprising laminin beta-4 or a variant thereof, wherein the therapeutically useful carrier allows the contacting of human blood with laminin beta-4 or the variant thereof followed by the recirculation of the blood into the body of a patient, the therapeutically useful carrier preferably being an apheresis apparatus, wherein the variant has the capability of binding to an autoantibody against laminin beta-4, as is found in a patient suffering from an autoimmune disease associated with such an autoantibody.

In another example, the object is achieved by a therapeutically useful carrier comprising the polypeptide according to the disclosure, wherein the carrier allows the contacting of human blood with the polypeptide followed by the recirculation of the blood into the body of a patient, the carrier preferably being an apheresis apparatus.

In another example, the object is achieved by an antibody, preferably autoantibody, against laminin beta-4, preferably in isolated form.

In another example, the object is achieved by the use of the polypeptide, carrier or autoantibody according to the disclosure for the diagnosis of a disease.

In another example, the object is achieved by a method for producing a diagnostically or therapeutically useful carrier, comprising the step of coating the carrier with the polypeptide according to the disclosure.

In yet another example, the object is achieved by a method for purifying an autoantibody, comprising the steps of a) contacting a liquid comprising the autoantibody with the polypeptide according to the disclosure under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide, b) isolating the complex from step a), and c) optionally detecting the complex from step a) or dissociating the complex isolated in step b) followed by removal of the autoantibody from the polypeptide.

In yet another example, the object is achieved by a method comprising the step of detecting an autoantibody against laminin beta-4 in a sample.

In a further example, the object is achieved by a pharmaceutical composition comprising the polypeptide according to the disclosure or a variant thereof, preferably together with a pharmaceutically acceptable carrier.

In a further example, the object is achieved by a kit comprising the polypeptide according to the disclosure or the carrier according to the disclosure, wherein the kit contains one reagent or more than one reagent, preferably all the reagents, from the group comprising a wash solution, a calibrator solution, an antibody against laminin beta-4, and an agent for the detection of an autoantibody against laminin beta-4, preferably a secondary antibody.

In an example, the object is achieved by the use of the polypeptide according to the disclosure, of the carrier according to the disclosure or of an antibody, preferably autoantibody, against laminin beta-4 for the production of a kit or diagnostically or therapeutically useful carrier.

In another aspect, the present disclosure refers to an *in vitro* use of the kit as described herein for the diagnosis of a disease.

In another aspect, the present disclosure refers to an *in vitro* method for purifying an autoantibody, comprising the steps of: a) contacting a liquid comprising the autoantibody with a polypeptide comprising laminin beta-4 or a variant thereof under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide, wherein the variant has the capability of binding to an autoantibody against laminin beta-4; b) isolating the complex from step a); and c) optionally detecting the complex from step a) or dissociating the complex isolated in step b) followed by removal of the autoantibody from the polypeptide.

In another aspect, the present disclosure refers to an *in vitro* method comprising the step of detecting an autoantibody against laminin beta-4 in a sample.

In another aspect, the present disclosure refers to an *in vitro* use of a polypeptide comprising laminin beta-4 or a variant thereof for the determination of the capability, preferably capacity, of a diagnostic or therapeutic device or of a diagnostic or therapeutic reagent to bind an autoantibody against laminin beta-4, wherein the variant has the capability of binding to an autoantibody against laminin beta-4.

In a preferred example, an autoantibody against laminin beta-4 is detected using a method selected from the group comprising immunodiffusion, immunoelectrophoresis, light scattering, agglutination, immunoassay with labeling such as that from the group comprising immunoassay with radioactive labeling, with enzymatic labeling, more preferably ELISA, with chemiluminescence labeling, more preferably electrochemiluminescence labeling, and with immunofluorescence labeling, preferably indirect immunofluorescence.

In another example, the object is achieved by the use of the polypeptide according to the disclosure or of the antibody according to the disclosure for the determination of the capability, preferably capacity, of a diagnostic or therapeutic device or of a diagnostic or therapeutic reagent to bind an antibody against laminin beta-4.

Detailed Description of the Disclosure

The present invention is based on the surprising finding by the inventors that an autoantibody against laminin beta-4 exists and can be specifically detected in patients who suffer from anti-p200 pemphigoid.

The inventors further found that the detection of said autoantibody increases the sensitivity of the serological diagnostic assay for anti-p200 pemphigoid. It can be detected in some patients in whom it is not possible to detect the autoantibody against laminin gamma-1, which autoantibody was hitherto considered to be of sole importance.

Laminins are trimeric basal membrane proteins which consist of alpha, beta and gamma chains. Laminin beta-4 is a beta chain. Laminins are involved in many physiological functions such as, for example, cell adhesion, migration, differentiation, neuronal development and in the regulation of gene expression.

Choi *et al*. (2015) observed a reduced expression of laminin beta-4 in cancer tissue of the stomach and intestine. They identified somatic laminin beta-4 mutations in cancer tissue that possibly contributed to a reduced expression of laminin beta-4 (Choi, M. R., An, C. H., Yoo, N. J., Lee, S. H. Laminin gene LAMB4 is somatically mutated and expressionally altered in gastric and colorectal cancers. APMIS 123: 65-71, 2015). Moreover, reduced levels of laminin beta-4 might also play a role in diverticulitis (Coble JL, Sheldon KE, Yue F, Salameh TJ, Harris LR III, Deiling S, Ruggiero FM, Eshelman MA, Yochum GS, Koltun WA, Gerhard GS, Broach JR. Identification of a rare LAMB4 variant associated with familial diverticulitis through exome sequencing. Hum Mol Genet. 2017; 26(16): 3212-3220).

Claims

1. A kit comprising a polypeptide comprising laminin beta-4 or a variant thereof, preferably in recombinant and/or isolated form, and an agent for the detection of an autoantibody against laminin beta-4, wherein the variant has the capability of binding to an autoantibody against laminin beta-4.

2. The kit as claimed in claim 1, wherein the polypeptide is immobilized on a carrier.

3. The kit as claimed in claim 2, wherein the carrier further comprises at least one antigen, preferably all the antigens, from the group comprising laminin gamma-1, laminin 332, BP180, BP230, desmoglein 1, desmoglein 3, envoplakin, gliadin and collagen type VII or variants thereof and split skin, wherein a variant comprises a polypeptide fragment of the respective antigen or an amino acid sequence having at least 80% identity to the fragment, that has the capability to bind to an autoantibody against the respective antigen.

4. The kit as claimed in claim 2 or claim 3, wherein the carrier is selected from the group comprising a glass slide, preferably slide for microscopy, even more preferably with a eukaryotic cell overexpressing laminin beta-4 or a variant thereof, a biochip, a microtiter plate, a lateral flow device, a test strip, a membrane, preferably a blot, more preferably a line blot, a chromatography column material and a bead, preferably a magnetic or fluorescent bead.

5. A therapeutically useful carrier comprising laminin beta-4 or a variant thereof, wherein the therapeutically useful carrier allows the contacting of human blood with laminin beta-4 or the variant thereof followed by the recirculation of the blood into the body of a patient, the therapeutically useful carrier preferably being an apheresis apparatus, wherein the variant has the capability of binding to an

autoantibody against laminin beta-4, as is found in a patient suffering from an autoimmune disease associated with such an autoantibody.

6. *In vitro* use of the kit as claimed in any of claims 1 to 4 for the diagnosis of a disease.

7. An *in vitro* method for purifying an autoantibody, comprising the steps of

a) contacting a liquid comprising the autoantibody with a polypeptide comprising laminin beta-4 or a variant thereof under conditions which allow the formation of a complex comprising the autoantibody and the polypeptide, wherein the variant has the capability of binding to an autoantibody against laminin beta-4,

b) isolating the complex from step a), and

c) optionally detecting the complex from step a) or dissociating the complex isolated in step b) followed by removal of the autoantibody from the polypeptide.

8. *An in vitro* method comprising the step of detecting an autoantibody against laminin beta-4 in a sample.

9. A kit as claimed in any of claims 1 to 4, wherein the kit further contains one reagent or more than one reagent from the group comprising a wash solution, a calibrator solution and an antibody against laminin beta-4.

10. The method, use or kit as claimed in any of claims 6 to 9 wherein the autoantibody against laminin beta-4 is detected using a method selected from the group comprising immunodiffusion, immunoelectrophoresis, light scattering, agglutination, immunoassay with labeling such as that from the group comprising immunoassay with radioactive labeling, with enzymatic labeling, more preferably ELISA, with chemiluminescence labeling, more preferably

electrochemiluminescence labeling, and with immunofluorescence labeling, preferably indirect immunofluorescence.

11. *In vitro* use of a polypeptide comprising laminin beta-4 or a variant thereof for the determination of the capability, preferably capacity, of a diagnostic or therapeutic device or of a diagnostic or therapeutic reagent to bind an autoantibody against laminin beta-4, wherein the variant has the capability of binding to an autoantibody against laminin beta-4.

12. The method as claimed in any of claims 6, 8 or 10, wherein the autoantibody is detected in a tissue sample from a patient or with a carrier comprising laminin beta-4 or a variant thereof, wherein the variant has the capability of binding to an autoantibody against laminin beta-4, as is found in a patient suffering from an autoimmune disease associated with such an autoantibody.

13. The method as claimed in any one of claims 8, 10 or 12, wherein the sample is cerebrospinal fluid, blood, blood serum, lymph, interstitial fluid or split skin.

14. The method or use of the kit as claimed in any of claims 6, 8, 10, 12 or 13, wherein it is a method or use for the detection of anti-p200 pemphigoid, for the diagnosis of a blistering autoimmune disease or for the differentiation between blistering autoimmune diseases of the skin.