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(54) Title: METHOD FOR THE DIAGNOSIS OF ALZHEIMER DISEASE

(57) Abstract: The invention relates to a method for the diagnosis of Alzheimer disease in a body fluid comprising determining the presence and/or amount of specific forms of neuregulin-,β.

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Method for the diagnosis of Alzheimer disease

Specification

The invention relates to a method for the diagnosis of Alzheimer disease in a body fluid comprising determining the presence and/or amount of specific forms of neuregulin-β.

Neuregulins (also ARIA, neurogenic differentiation factors, heregulins and DDF) belong to a family of wide-spread and known growth and differentiation factors. For example in publication Ozaka M. et al., Nature 1997, Dec 10-25, 390(6661): 691-4 the inducing influence of neuregulin-β on the expression of the NR2C subunit of the NMDA receptor is described.

PCT/EP01/01424 discloses a neuregulin-ß isoform having an isoelectric point of $\leq$ pH 7, particularly from pH 4.3 to pH 5.0 and more particularly from pH 4.5 to pH 4.7. This neuregulin-ß isoform was obtained from hippocampal primary cultures from pre- or neonatal rats and characterized as indicator or target, respectively, for neuronal processes. Further, neuregulin-ß could be identified as target in case of Morbus Alzheimer. Several novel neuregulin-β isoforms are disclosed in USSN 60/341,809.

A Western blot analysis of liquor (cerebrospinal fluid) samples from human patients suffering from Alzheimer disease with anti-neuregulin-β antibodies show the presence of four different neuregulin-β cleavage products and/or neuregulin-β isoforms having molecular weights (SDS-PAGE) of about 28 kDa, 55 kDa, 65 kDa and 80 kDa. Higher concentrations of these neuregulin-β forms are found in Alzheimer patients compared to non-Alzheimer patients. This is particularly true for the 65 kDa and 28 kDa

neuregulin forms. Particularly, in the most cases, the 65 kDa neuregulin form cannot be detected in non-Alzheimer patients.

Thus, a subject-matter of the present invention for the diagnosis of Alzheimer disease in a sample, particularly in a body fluid, more particularly in liquor (cerebrospinal fluid), comprising determining the presence and/or amount of at least one neuregulin-β form having a molecular weight (SDS-PAGE) of about 28 kDa, 55kDa, 65 kDa and 80 kDa.

Preferably, the method comprises determining at least two, e.g. two, three or four of said neuregulin-β forms. Determination of the 28 kDa form and/or the 65 kDa form is particularly preferred.

The sample may be obtained from body fluids or tissue, particularly neuronal fluids, more particularly liquor (cerebrospinal fluid) or neuronal tissue. The diagnostic method preferably comprises detection on the protein level, e.g. by immunological methods using neuregulin-β antibodies or antibody fragments. Such diagnostic methods are well known.

In order to differentiate between different neuregulin forms, the proteins in the sample are preferably subjected to a separation procedure according to their molecular weight and/or charge. The separation procedure is preferably a gel electrophoresis such as SDS-PAGE or an equivalent method. Furthermore, determination may also comprise chromatographic separation procedures such as HPLC and/or molecular weight exclusion chromatography.

In a preferred embodiment of the invention, a quantitative or semi-quantitative determination of the amount of the individual neuregulin-β forms is carried out. This quantitative or semi-quantitative determination may comprise the use of labelled detection reagents such as neuregulin-β antibodies and quantitatively or semi-quantitatively determining the amount

of label bound to a respective neuregulin-β form in a sample. In some cases, it is preferred that the method additionally comprises a determination of an internal standard, e.g. a reference protein, which allows quantification of the neuregulin-β forms to be determined. In a preferred embodiment, the internal standard is recognized by the same antibody which is used for determining the relevant neuregulin-β forms. Further, the reference protein exhibits characteristics, e.g. a molecular weight, which allows a differentiation from the neuregulin-β forms to be determined, particularly after electrophoretic separation of the sample. For example, the reference protein may be selected from biological extracts, which contain predetermined amounts of recombinant or native neuregulin-β, purified or partially purified native neuregulin-β proteins from biologic material, e.g. 42 kDa protein from rat hippocampus cell line HK3, purified recombinant protein, e.g. a full-length or truncated neuregulin-β protein or a hybrid protein containing relevant neuregulin-β epitopes as a "tag" coupled to a suitable carrier. A particularly preferred reference protein is a recombinant neuregulin-β protein from E.coli comprising the extracellular domain and having a molecular weight of about 30 kDa (Neomarkers).

Furthermore, the invention relates to the use of a region kit, comprising neuregulin-β antibodies for the diagnosis of Alzheimer disease, particularly in a method as described above. Further, the present invention shall be explained in more detail by the following Figure and Example.

Figure 1: Neuregulin-β Western blot from liquor (cerbrospinal fluid) samples obtained from Alzheimer patients (A) and non-Alzheimer patients (B).

Example

15 µg protein from liquor was separated by SDS-PAGE (9% polyacrylamide) and subsequently blotted on a nitrocellulose membrane (Optitran BA-S83, Reinforced NC, Schleicher & Schuell). The blot procedure was carried out in a semi-dry blotter for 120 min with 1 mA/cm^2

gel surface. As transfer buffers, the following were used: Anode buffer I (0.3 mol/l Tris pH 10.4, 10% methanol), anode buffer II (25 mmol/l Tris pH 10.4, 10% methanol) and cathode buffer (25 mmol/l Tris pH 9.4, 40 mmol/l 6-amino-n-caproic acid, 20% methanol).

After the blotting, the membranes were blocked in 0.1% TBS-T buffer (20 mmol/l Tris pH 7.4, 175 mmol/l NaCl, 3.5 mmol/l KCl, 0.1% Tween 20) and 4% Bovine Serum Albumin for 90 min. Then, the membrane was incubated overnight at 4°C with a primary antibody (neuregulin Ab-2, rabbit polyclonal antibody, Neomarkers; 1:5000 diluted in blocking buffer). Then, the membrane was washed 4 x 10 min in 0.05% TBS-T buffer (20 mmol/l Tris pH 7.4, 175 mmol/l NaCl, 3.5 mmol/l KCl, 0.05% Tween 20) incubated for 1 h with a secondary antibody (anti-rabbit IgG -Peroxidase-conjugate, Sigma; 1:10 000 diluted in blocking buffer). After this incubation, the membrane was washed 4 x 10 min in 0.05% TBS-T buffer. Then, an electro-chemiluminescence determination (Super Signal West Dura Kit, Pierce) was carried out according to the manufacturer's instructions. The DIANA-chemiluminescence detection system (Raytest) was used.

Claims

1. A method for the diagnosis of Alzheimer disease in a sample comprising determining the presence and/or amount of at least one neuregulin-β form having a molecular weight (SDS-PAGE) of about 28 kDa, 55 kDa, 65 kDa or 80 kDa.

2. The method of claim 1 comprising determining at least two of said neuregulin-β forms.

3. The method of claims 1 or 2 wherein the 28 kDa neuregulin-β form is determined.

4. The method of any one of claims 1-3 wherein the 65 kDa neuregulin-β form is determined.

5. The method of any one of claims 1-4 wherein the sample is liquor.

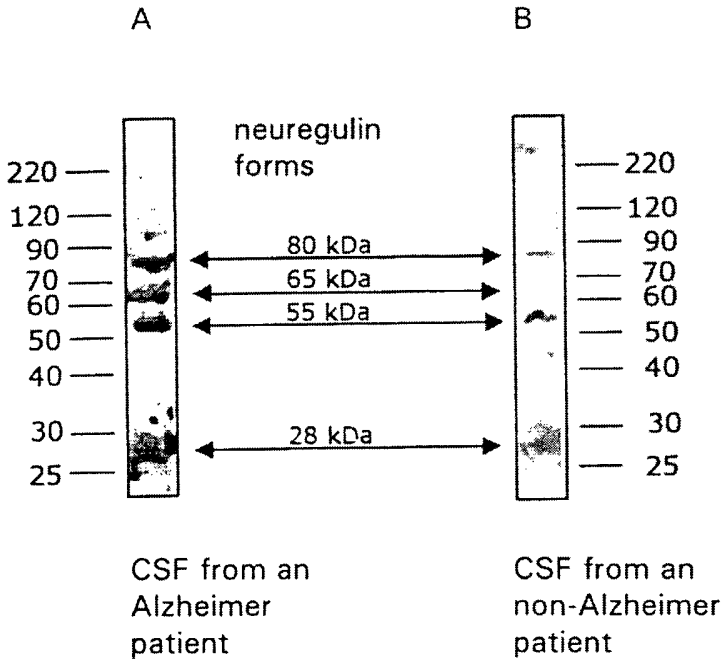
6. The method of any one of claims 1-5 wherein proteins in the sample are subjected to a separation procedure according to their molecular weight and/or charge.

7. The method of claim 6 wherein the separation procedure comprises SDS-PAGE.

8. The method of any one of claims 1-7 wherein the neuregulin-β forms are determined immunologically.

9. Use of a reagent kit comprising neuregulin-β antibodies for the diagnosis of Alzheimer disease in a method of any one of claims 1-8.

Figure 1


A
B
neuregulin forms
220
120
90
70
60
50
40
30
25
80 kDa
65 kDa
55 kDa
28 kDa
CSF from an Alzheimer patient
CSF from an non-Alzheimer patient