METHOD FOR IDENTIFYING CELL-SPECIFIC PROTEINS

DESCRIPTION:

The present invention relates to a method for identifying cell-specific proteins.

The identification of cell-specific protein combination patterns is a crucial factor for elucidating cell-to-cell interactions which may cause countless effects within an organism. Especially the knowledge of disease-specific target structures is a decisive prerequisite for the development of effective medications which at the same time have only few side-effects.

It is known that immune cells (lymphocytes) will express specific protein combinations - also referred to as protein combination patterns or (abbreviated) PCP - which are responsible for a binding to endothelial cells of the blood vessels in the brain and in muscle tissue. Other protein combinations, by contrast, will not cause such binding to these endothelial cells. Surprisingly, these specific combinations are inter-individually constant, always exhibiting the same binding functions. The specific

protein combination patterns therefore seem to be an inter-individually constant lymphocyte binding code of the cell surface for organ-specific endothelial cell surfaces which constitutes a cell-specific target structure. Cell-specific target structures may thus include very specific protein combination patterns.

Invasive tumor cells also exhibit specific protein combination patterns on their cell surfaces which result in a specific, i.e. organ-selective, invasive behavior. Such protein combination patterns therefore constitute target structures for potential medications.

However, an absolute prerequisite for the development of such highly selective medications is the knowledge of the molecular composition of these target structures.

Methods for identifying target structures are known in the prior art, which are based on the analysis of gene expression profiles of diseased tissues or cells as compared to gene expression profiles of healthy tissues or cells, with protein expression profiles as well as expression profiles of the messenger ribonucleic acid (mRNA) being intended to provide information on the

appearance of new proteins, malregulated or abnormally modified proteins in diseased tissues or cells (e.g. in: F. Lottspeich/H. Zorbas; Bioanalytik; Spektrum Akademischer Verlag; Heidelberg, 1998).

However, these methods all use cell homogenates usually produced from thousands or millions of cells since only such vast amounts of cells will allow expression profiles of the abovementioned kind to be established. The cells contained in the cell homogenates have been solubilized so as to allow the extraction and separation of the proteins or mRNA molecules by means of biochemical processes.

A shortcoming of these prior art methods, however, is that they are not suitable for identifying protein combination patterns, since the individual protein components of such a protein combination pattern will be completely separated by the generation of cell homogenates and by the subsequent extraction processes, and the fundamental information relating to their cell- and tissue-topological location will be lost. Furthermore, owing to

the destruction of the cell compartments, no information can be gained any longer regarding the combinations of proteins within these cell compartments and their relative topological relation to each other.

Moreover, another disadvantage of the prior art processes is that no analyses can be performed at an individual level, making it impossible to detect differences of the individual cells with respect to their protein combination patterns. These disadvantages are overcome by the methods disclosed in DE 197 09 348 C2 for determining, identifying and mapping cell-specific target structures. DE 197 09 348 C2 discloses an automated method for determining molecule classes, molecule groups, molecule portions in a solid or liquid object, wherein in one and the same object at least two molecule classes, molecule groups or molecule portions are examined and measured by means of simultaneous or sequential application of any number of reagent solutions Yn (n = 1, 2, 3 ... N). The method includes incubating and/or bleaching cycles repeatable with any frequency, and after each procedural cycle the signal distribution pattern of one and the same object to be examined is registered. The signal distribution patterns obtained in each procedural cycle are

transferred into a complex total image of the object to be examined by computer-aided image superposition, namely into a highly complex molecular combination pattern of the object. The method is performed to automatically detect and map any number of molecule species or epitopes in a cell or a tissue sample. Hereby, the aim is the knowledge of the arrangement and correlative distribution of the molecule groups to obtain information about various diseases and the treatment thereof. It is possible to obtain any number of individual label patterns in the respectively examined sample, cell or tissue sample, respectively, as images by repetitive labelling - image taking - bleaching cycles, and to determine the complex combination patterns of individual or numerous molecule distributions resulting therefrom by subsequent computer-aided image superposition of these individual patterns, and to quasi project as combinatorial patterns onto the biological structure (e.g. of a cell) in any manner.

This method can be used for comparatively examining protein combination patterns of individual cells or cell membranes of different cell or tissue samples. In doing so, those marker molecules can be identified which will bind to e.g. a certain protein combination pattern or to a certain

area of such a protein combination pattern of a first object stemming from a first tissue or cell sample, and which will at the same time not bind to a second object stemming from a second tissue or cell sample. By means of these identified marker molecules, using a sample portion of the first tissue and/or cell sample, those molecular areas (molecules or molecular complexes) of the protein combination pattern may now be located and/or selected and subsequently characterized which are bound by the identified marker molecules. This will allow the detection and understanding of the molecular composition of a protein combination pattern, of the arrangement of the molecules within said protein combination pattern as well as of the arrangement of the protein combination pattern within a tissue or a cell.

The cell-specific protein combination patterns thus obtained, however, will still be highly complex - which will clearly aggravate the development of highly selective medications.

It is therefore the object of the present invention to further improve on and provide a method of the above mentioned type which will further process identified and

highly complex protein combination patterns, thus facilitating the development of highly selective medications.

This object is accomplished by a method of the invention for identifying cell-specific proteins comprising the following steps: (a) determining cell-specific protein combination patterns of n cells; (b) comparing the protein combination patterns of healthy and pathologically or physiologically modified cells of one cell type or comparing the protein combination patterns of cells of a different cell type affected by the same disease; (c1) subtracting the coincident parts of the protein combination patterns of healthy and pathologically or physiologically modified cells of one cell type compared in step (b) and determining a cell-specific protein resulting therefrom; or (c2) subtracting the non-coincident parts of the protein combination patterns of cells of a different cell type affected by the same disease compared in step (b) and determining a cell-specific protein resulting therefrom; and (d) identifying the resulting cell-specific protein and its spatial arrangement within the cell or tissue.

Reducing the highly complex protein combination patterns thus determined to a single, highly selective and cell-specific protein will clearly facilitate the development of likewise highly selective medications, at the same time significantly shortening the time required for such development. Thus it will no longer be required in the development of medications to investigate the highly complex and likewise cell-characteristic protein combination patterns.

The invention merely requires a selection of a substance for suppressing the activity of the identified cell-specific protein. Such suppression will cause the cell-specific protein network, and hence the cell functions, to collapse. For example, switching off the cell-specific protein of tumor cells will in turn suppress the migration of these cells since the protein identified in this process controls and regulates the migration behavior of the tumor cell. The suppressing substance in this case may be an antibody.

Particular advantages of the method according to the invention will be obtained if pathologically modified cells are of the invasive type.

The knowledge of these e.g. disease-specific proteins within a protein combination pattern will moreover allow the development of highly specific medications which will be almost without undesired side effects owing to this very specificity.

The cell-specific protein combination patterns to be processed according to the invention will be determined by means of the methods disclosed in DE 197 09 348 C2 for determining, identifying and mapping cell-specific target structures.

15 JAN 2007

CLAIMS:

1. A method for identifying cell-specific proteins comprising the following steps:

a) determining cell-specific protein combination patterns of n cells;

b) comparing the protein combination patterns of healthy and pathologically or physiologically modified cells of one cell type or comparing the protein combination patterns of cells of a different cell type affected by the same disease;

c1) subtracting the coincident parts of the protein combination patterns of healthy and pathologically or physiologically modified cells of one cell type compared in step b) and determining a cell-specific protein resulting therefrom; or

c2) subtracting the non-coincident parts of the protein combination patterns of cells of a different cell type affected by the same disease compared in step b) and determining a cell-specific protein resulting therefrom; and

d) identifying the resulting cell-specific protein and its spatial arrangement within the cell or tissue.

2. The method as claimed in claim 1 characterized in that said method comprises the selection of a substance for suppressing the activity of the identified cell-specific protein.

3. The method as claimed in claim 2 characterized in that said substance is an antibody.

4. The method as claimed in one of the preceding claims characterized in that pathologically modified cells are of the invasive type.