Methods for diagnosis of pancreatic cancer and composition useful therein

Verfahren/Präparat zur Erkennung von Pankreaskrebs

Methodes et compositions pour le diagnostic du cancer du pancreas

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References cited:
EP-A- 0 279 669
WO-A-02/08288

The invention relates to the specific diagnosis of pancreatic cancer. Pancreatic cancer is a cancer type which is difficult to diagnose and to control and most of such cancers can grow very rapidly. Neoplasms of the exocrine pancreas may arise from ductal, acinar and stromal cells. 80% of pancreatic carcinomas are derived from ductal epithelium. 60% of these tumors are located in the head of the pancreas, 10% in the tail and 30% are located in the body of the pancreas or are diffuse (Warshaw, A.L., and Fernandez-del, C.C., N. Engl. J. Med. 326 (1992) 4555-4565). Histologically, these tumors are graded as well as differentiated, moderately differentiated and poorly differentiated. Some tumors are classified as adenosquamous, mucinous, undifferentiated or undifferentiated with osteoblast-like giant cells (Gibson, J.B., and Sobin, L.H., Histological typing of tumors, of the liver, biliary tract and pancreas, WHO, Geneva, 1978). Since the disease is asymptomatic in its early stages, because of the lack of any test for early diagnosis and due to its aggressive character with respect to metastasis locally and to visceral organs, this disease is associated with a dismal prognosis. Only 20% of the tumors are resectable and the survival benefit of approved chemotherapy regimens is rather poor (Kroep, J.R., et al., Ann. Oncol. 10, Suppl. 4 (1999) 234-238). Identification of new targets for early diagnosis of pancreatic tumors is therefore a challenge of paramount importance.

WO 02/08288 describes secreted and transmembrane polypeptides and nucleic acids encoding the same. Fig. 130 shows the polypeptide sequence of UKW.

It was surprisingly found that nucleic acids coding for polypeptide UKW are overexpressed specifically in pancreatic tumor cells, whereas expression is considerably lower in normal pancreatic cells or in tumor cells of other origin, e.g., breast cancer or colon cancer cells. Therefore, UKW is a valuable new target for specific diagnosis.

The present invention therefore provides a method for determining the presence or absence of pancreatic cancer in a patient comprising:

(i) obtaining a biological sample from a patient;
(ii) detecting in the sample an amount of nucleic acid encoding UKW or an amount of polypeptide UKW; and
(iii) comparing the amount of nucleic acid or polypeptide with a predetermined standard value indicating the decision line for tumor-induced or non-tumor-induced UKW expression or presence in the cell and therefrom determining the presence or absence of pancreatic cancer in the patient.

Preferably, the detection is performed by the use of a binding agent which binds to UKW nucleic acid or polypeptide. More preferably, the binding agent is a probe hybridizing under stringent conditions with UKW nucleic acid or an antibody, preferably a monoclonal antibody binding to UKW polypeptide, preferably to the extracellular domain.

Moreover, the present invention provides a process for determining whether or not a test sample of tissue or fluid of a patient contains pancreatic tumor cells or is derived from pancreatic tumor cells, wherein the test sample and a second sample originating from non-pancreatic-tumor cells from the same individual or a different individual of the same species are used, which process comprises the following steps:

(a) : incubating each respective sample under stringent hybridization conditions with a nucleic acid probe which is selected from the group consisting of:

(i) a nucleic acid sequence of SEQ ID NO: 1, or a fragment thereof;
(ii) a nucleic acid sequence which is complementary to any nucleic acid sequence of (i);
(iii) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (i); and
(iv) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (ii); and

(b) determining the approximate amount of hybridization of each respective sample with said probe, and

(c) comparing the approximate amount of hybridization of the test sample to an approximate amount of hybridization of said second sample to identify whether or not the test sample contains a greater amount of the specific nucleic acid or mixture of nucleic acids than does said second sample.

The invention also includes a method for the detection of pancreatic tumors, comprising:

a) incubating a sample of a patient suspected of suffering from pancreatic cancer, selected from the group of body fluid, of cells, or of a cell extract or cell culture supernatants of said cells, whereby said sample contains nucleic
acids with a nucleic acid probe which is selected from the group consisting of

(i) the nucleic acid shown in SEQ ID NO:1 or a nucleic acid which is complementary to said sequence, and
(ii) nucleic acids which hybridize with one of the nucleic acids from (i) and

b) detecting hybridization; preferably by means of a further binding partner of the nucleic acid of the sample and/or
the nucleic acid probe or by X-ray radiography.

[0010] Both methods can also be performed by the use of antibodies, whereby the UKW polypeptide is detected by
interaction between the antibody and the polypeptide.

[0011] The invention further provides a method for monitoring the progression of pancreatic cancer in the patient. In
such a method, the amount of UKW nucleic acid or polypeptide in a biological sample (e.g., body fluids such as blood,
cell lysates or a reverse transcript of an RNA sample) of a patient suffering from pancreatic cancer is determined at at
least two different points in time and compared. From the change of the amount, information on the progression of said
pancreatic cancer can be deduced.

[0012] The invention further comprises diagnostic kits comprising one or more oligonucleotide probes or primers for
hybridization with UKW nucleic acid in a diagnostic assay using a sample obtained from a patient suffering from, or
being suspected to have, pancreatic cancer.

[0013] The invention further comprises the use of antibodies against UKW polypeptide.

Detailed Description of the Invention:

[0014] As used herein, the term "UKW" means a nucleic acid encoding a polypeptide of SEQ ID NO:2, preferably
the DNA sequence and the related mRNA sequence of SEQ ID NO:1 as well as the encoded polypeptide of SEQ ID
NO:2. As UKW is a transmembrane receptor protein; the polypeptide is of outstanding interest for diagnosis and as an
epitope for antibody binding the extracellular domain of UKW polypeptides is preferred. Therefore, it is preferred to direct
the nucleic acid sample and probes to this region and especially to parts thereof which have low homology with other
genes and polypeptides.

[0015] The UKW receptor is a transmembrane protein composed of 373 amino acids with a signal sequence of 18
amino acids. The receptor consists of an extracellular domain of 215 amino acids, a transmembrane domain of 23 amino
acids and a cytoplasmic domain of 117 amino acids. The extracellular domains displays two immunoglobulin C2-type
folds composed of 93 amino acids and 72 amino acids.

[0016] The phrase "nucleic acid or polypeptide" as used throughout this application refers to a nucleic acid or polypep-
tide having a UKW activity which is substantially free of cellular material or culture medium when produced by recombinant
DNA techniques, or substantially free of chemical precursors or other chemicals when synthesized chemically. Such a
nucleic acid, or polypeptide is preferably free of sequences which naturally flank the nucleic acid (i.e. sequences located at the 5' and
the 3' ends of the nucleic acid) in the organism from which the nucleic acid is derived.

[0017] "Nucleic acid probes and primers for UKW" as used herein means nucleic acid fragments useful for the detection
of UKW nucleic acids by hybridization methods. Hybridization techniques and conditions are well-known to one skilled
in the art. Such hybridization conditions are, for example, moderate stringent conditions including washing with a solution
of 5 x SSC, 0.5% SDS, 1.0 mmol/l EDTA, pH 8.0, followed by hybridization at 50-60°C 5 x SSC overnight, washing at
room temperature for 40 minutes with 2 x SSC containing 0.1% SDS and afterwards washing with 0.1 x SSC, 0.1% SDS
at 50°C for 40 min with one change of fresh solution. It is also possible to use higher temperatures for hybridization (e.g.
65-70°C) as high stringent hybridization conditions. The nucleic acid probe and primers usually consist of a UKW nucleic
acid segment of at least about 50 contiguous positions most preferably of 200 to 300 nucleotides. The optimization of
the probes and primers can be performed according to the state of the art. There exists informatic software (http://www-
genome.wi.mit.edu/genome_software/other/prime3.html) which is generally used for such probe and primer design.

[0018] For high selectivity it is preferred to use relatively low salt and/or high temperature conditions, for example, a
salt concentration from about 0.02 mol/l to about 0.15 mol/l and temperatures of from about 50°C to about 70°C.

[0019] The UKW polypeptides can be identified in diagnostic assays using specific probes and primers. Usually such
methods include amplifying the target sequence in the sample by amplification methods such as the PCR method.
Quantitative detection can be performed by PCR techniques, preferably by the use of quantitative RT-PCR using, e.g.,
the LightCycler® of Roche Diagnostics GmbH, DE.

[0020] In a preferred embodiment of the invention the coding nucleic acid of the sample is amplified before the test,
for example by means of the known PCR technique. Usually a derivatized (labeled) nucleic acid probe is used within
the framework of nucleic acid diagnostics. This probe is contacted with a denatured DNA, RNA or RT-DNA from the
sample which is bound to a carrier and in this process the temperature, ionic strength, pH and other buffer conditions
are selected - depending on the length and composition of the nucleic acid probe and the resulting melting temperature
of the expected hybrid - such that the labeled DNA or RNA can bind to homologous DNA or RNA (hybridization see also Wahl, G.M., et al., Proc. Natl. Acad. Sci. USA 76 (1979) 3683-3687). Suitable carriers are membranes or carrier materials based on nitrocellulose (e.g., Schleicher and Schüll, BA 85, Amersham Hybond, C.), strengthened or bound nitrocellulose in powder form or nylon membranes derivatized with various functional groups (e.g., nitro groups) (e.g., Schleicher and Schüll, Nytran; NEN, Gene Screen; Amersham Hybond M.; Pall Biodyne).

To determine whether a test sample contains pancreatic tumor cells, the approximate amount of hybridization of the nucleic acid with the target nucleic acid or nucleic acids is determined. The approximate amount of hybridization need not be determined quantitatively although a quantitative determination is encompassed by the present invention. Typically, the approximate amount of hybridization is determined qualitatively, for example, by a sight inspection upon detecting hybridization. For example, if a gel is used to resolve labelled nucleic acid which hybridizes to target nucleic acid in the sample, the resulting band can be inspected visually. When performing a hybridization of isolated nucleic acid which is free from pancreatic tumor cells from an individual of the same species, the same protocol is followed.

One can compare the approximate amount of hybridization in the test sample to the approximate amount of hybridization in the sample free from pancreatic tumor cells, to identify whether or not the test sample contains a greater amount of the target nucleic acid or nucleic acids than does the sample which is free from pancreatic tumor cells.

In a further method according to the invention no second sample is used. For the detection whether the expression of UKW gene is upregulated, the level of mRNA of UKW is compared with the level of mRNA of a standard gene (housekeeping gene) (see, e.g., Shaper; N.L., et al., J. Mammary Gland Biol. Neoplasia 3 (1998) 315-324; Wu, Y.Y., and Rees, J.L., Acta Derm. Venereol. 80 (2000) 2-3) of the cell, preferably by RT-PCR.

As is shown in accordance with the present invention, the UKW nucleic acid is expressed in a greater amount in a pancreatic tumor sample than in a sample free from pancreatic tumor cells and/or in a greater amount than a housekeeping gene. A test sample containing pancreatic tumor cells will have a greater amount of the UKW nucleic acid than does a sample which is free from pancreatic tumor cells. To identify a test sample as containing upregulated UKW nucleic acid, i.e., wherein the cells are pancreatic tumor cells or are tumor cells of a mammary carcinoma, it is preferable that the test sample have an approximate amount of UKW nucleic acid which is appreciably greater than the approximate amount in a sample free of pancreatic tumor cells. For example, a test sample having an upregulated UKW gene may have approximately 15- to approximately 60-fold increased amount of UKW gene than a sample free of pancreatic tumor cells or an at least 3-fold greater amount of UKW mRNA than mRNA of a housekeeping gene like glycerolaldehyde-3-phosphate dehydrogenase (GAPDH) or porphobilinogen deaminase.


Hybridizing DNA or RNA is then detected by incubating the carrier with an antibody or antibody fragment after thorough washing and saturation to prevent unspecific binding. The antibody or the antibody fragment is directed towards the substance incorporated during hybridization to the nucleic acid probe. The antibody is in turn labeled. However, it is also possible to use a directly labeled DNA. After incubation with the antibodies it is washed again in order to only detect specifically bound antibody conjugates. The determination is then carried out according to known methods by means of the label on the antibody or the antibody fragment.

The detection of the expression can be carried out for example as:

- in situ hybridization with fixed whole cells, with fixed tissue smears,
- colony hybridization (cells) and plaque hybridization (phages and viruses),
- Southern hybridization (DNA detection),
- Northern hybridization (RNA detection),
- serum analysis (e.g., cell type analysis of cells in the serum by slot-blot analysis),
- after amplification (e.g., PCR technique).

The nucleic acids according to the invention are hence valuable markers in the diagnosis and characterization of pancreatic tumors.

According to the invention inhibitors for the expression of UKW (e.g., antibodies or antisense nucleotides) can be used to inhibit pancreatic tumor progression in vivo.

The invention further provides methods for the identification and isolation of antagonists of UKW or inhibitors for the expression of UKW (e.g., antibodies and antisense nucleotides). Such antagonists or inhibitors can be used to inhibit pancreatic tumor progression and cause massive apoptosis of pancreatic tumor cells in vivo.

According to the invention there are provided methods for identifying UKW antagonists which have utility in the treatment of cancer. These methods include methods for modulating the expression of the polypeptides according to the invention, methods for identifying UKW antagonists which can selectively bind to the protein according to the invention, and methods of identifying UKW antagonists which can modulate the activity of said polypeptides. The methods further include methods for modulating, preferably inhibiting, the transcription of UKW gene to mRNA. These
methods can be conducted in vitro or in vivo and may make use of and establish cell lines and transgenic animal models of the invention.

[0031] A UKW antagonist is defined as a substance or compound which decreases or inhibits the biological activity of UKW, a polypeptide and/or inhibits the transcription or translation of UKW gene. In general, screening procedures for UKW antagonists involve contacting candidate substances with host cells in which invasiveness is mediated by expression of UKW under conditions favorable for measuring UKW activity.

[0032] UKW activity may be measured in several ways. Typically, the activation is apparent by a change in cell physiology, such as increased mobility and invasiveness in vitro, or by a change in the differentiation state, or by a change in cell metabolism leading to an increase of proliferation.

[0033] The UKW polypeptides can be produced by recombinant means or synthetically. Non-glycosylated UKW polypeptide is obtained when it is produced recombinantly in prokaryotes. With the aid of the nucleic acid sequences provided by the invention it is possible to search for the UKW gene or its variants in genomes of any desired cells (e.g., apart from human cells, also in cells of other mammals), to identify these and to isolate the desired gene coding for the UKW proteins. Such processes and suitable hybridization conditions are known to a person skilled in the art and are described, for example, by Sambrook et al., Molecular Cloning: A Laboratory Manual (1989) Cold Spring Harbor Laboratory Press, New York, USA, and Hames, B.D., Higgins, S.G., Nucleic Acid Hybridisation - A Practical Approach (1985) IRL Press, Oxford, England. In this case the standard protocols described in these publications are usually used for the experiments.

[0034] With the aid of such nucleic acids coding for a UKW polypeptide, the polypeptide according to the invention can be obtained in a reproducible manner and in large amounts. For expression in prokaryotic or eukaryotic organisms, such as prokaryotic host cells or eukaryotic host cells, the nucleic acid is integrated into suitable expression vectors, according to methods familiar to a person skilled in the art. Such an expression vector preferably contains a regulatable/inducible promoter. These recombinant vectors are then introduced for the expression into suitable host cells such as, e.g., E. coli as a prokaryotic host cell or Saccharomyces cerevisiae, teratocarcinoma cell line PA-1, sc 9117 (Bittner, R., et al., Mol. Cell. Biol. 11 (1991) 3573-3583), insect cells, CHO or COS cells as eukaryotic host cells and the transformed or transduced host cells are cultured under conditions which allow expression of the heterologous gene. The isolation of the protein can be carried out according to known methods from the host cell or from the culture supernatant of the host cell. Such methods are described for example by Ausubel, I., Frederick M., Current Protocols in Mol. Biol. (1992), John Wiley and Sons, New York. Also in vitro reactivation of the protein may be necessary if it is not found in soluble form in the cell culture.

[0035] UKW polypeptide or fragments thereof can be purified after recombinant production by affinity chromatography using known protein purification techniques, including immunoprecipitation, gel filtration, ion exchange chromatography, chromatofocussing, isoelectric focussing, selective precipitation, electrophoresis, or the like and can be used for the generation of antibodies against UKW.

[0036] The invention further comprises recombinant expression vectors which are suitable for the expression of UKW, recombinant host cells transfected with such expression vectors, as well as a process for the recombinant production of a protein which is encoded by the UKW gene.

[0037] The invention further comprises a screening method for identifying a compound which inhibits the biological activity of the UKW polypeptide comprising:

- contacting said polypeptide with a compound or a plurality of compounds under conditions which allow interaction of said compound with said polypeptide; and
- detecting the interaction between said compound or plurality of compounds with said polypeptide.

[0038] The invention further comprises a screening method for identifying a compound which either inhibits the biological activity of the UKW polypeptide or inhibits the transcription or translation of the UKW gene, comprising:

- contacting said compound or plurality of said compounds with a host cell in which invasiveness is mediated by expression of UKW; and
- detecting the interaction between said compound or plurality of compounds with said host cell.

[0039] The invention further comprises a screening method according to the invention, wherein the detection of the interaction between said compound or plurality of compounds with said host cell is measured by at least one of a change in cell physiology, a change in the differentiation state, and a change in cell metabolism leading to an increase of proliferation.

[0040] The invention further comprises a screening method for identifying a compound which is an antagonist of a UKW polypeptide comprising:
contacting said UKW polypeptide with a compound under conditions which allow interaction of said compound with said polypeptide;

determining a first level of activity of said polypeptide;

determining a second level of activity of said polypeptide expressed in a host which has not been contacted with said compound; and

quantitatively relating the first level of activity with the second level of activity, wherein when said first level of activity is less than said second level of activity, said compound is identified as an antagonist of said polypeptide.

[0041] Antibodies against UKW can be produced according to the methods known in the state of the art. For example, monoclonal or polyclonal antibodies can be produced using a polypeptide comprising about 10 to 100 amino acids of the UKW polypeptide sequence. Suitable polypeptides derived from UKW include preferably amino acids from the extracellular domain, preferably amino acids 70-80, 99-113, 120-140 and 167-182.


[0043] Antibodies which are useful according to the invention can be identified by reducing the proliferation and invasive potential of pancreatic tumor cells. For this purpose, pancreatic tumor cells or a pancreatic tumor cell line, preferably cell line SUIT-2 007 are treated with an antibody against UKW and proliferation and invasive potential are measured by Cell Proliferation Reagent WST-1 (a tetrazolium salt reagent, Roche Diagnostics GmbH, DE) and Matrigel invasion assay (BDS Biosciences, www.bdbiosciences.com).

[0044] Anti-UKW antibodies can be derived from any animal species or are chimeric or humanized antibodies. Especially preferred are human antibodies. Human monoclonal antibodies are obtained, for example, from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green, L.L., et al., Nat. Genet. 7 (1994) 13-21; Lonberg, N., et al., Nature 368 (1994) 856-859; and Taylor, L.D., et al., Int. Immun. 6 (1994) 579-591.

[0045] Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines et al., "Purification of Immunoglobulin G (IgG)", In: Methods in Molecular Biology, Vol. 10, pages 79-104, The Humana Press, Inc., 1992).

[0046] The antibodies can be used for immunoassays according to the invention. Detection can be performed by contacting a biological sample with an antibody, and then contacting the biological sample with a detectably labeled molecule, which binds to the antibody. The antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art. Alternatively, an antibody can be conjugated with a detectable label to form an immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably labeled immunoconjugates are well-known to those of ordinary skill in the art and are described in more detail below.

[0047] The following examples, references, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims.

Description of the Figures

[0048] Figure 1  Differential expression of UKW mRNA in pancreatic tumor cell lines SUIT-2 007 and SUIT-2 028 analyzed by Northern blot analysis.

(a: pancreatic tumor cell line SUIT-2 007; b: pancreatic tumor cell line SUIT-2 028)

Figure 2  Multiple Tissue Expression (MTE™) Array (Clontech, Palo Alto, CA, USA). This array contains normalized
loadings of poly A⁺-RNA from 76 different tissues as well as control RNA’s and DNA’s and enables to screen for the presence and relative abundance of a target mRNA in a broad spectrum of fetal and adult tissues. H6 corresponds to 1 μg total RNA from pancreatic tumor cell line SUIT-2 007. The code is revealed below.

**Figure 3**  Relatives expression (Taqman®, PCR) of RNA in samples derived from adenocarcinomas of the pancreas (PaCa), chronic pancreatitis (ChronPa) and normal pancreatic tissues (NorPA).

**Figure 4**  Relative expression of UKW mRNA in invasive and non-invasive pancreatic tumor cell lines.

**Figure 5**  Relative expression of UKW mRNA in invasive and non-invasive mammary tumor cell lines.

**Pancreatic tumor cell lines:**
- Suit-2 007
- MiaPaca-2, ATCC CRL 1420
- AsPC-1, ATCC CRL 1682
- BxPC-3, ATCC CRL 1687
- Capan-1, ATCC HTB79
- IMIM-PC-1³
- IMIM-PC-2³
- Panc-1, ATCC CRL 1469
- Suit-2 028¹
- Capan-2, ATCC HTB80
- Patu 8902²
- Patu 8988s²
- Patu 8988t²

**Mammary tumor cell lines:**
- BT-549, ATCC HTB122
- Hs578T, ATCC HTB126
- MCF-10A, ATCC CRL 10317
- MCF-12A, ATCC CRL 10782
- MDA-MB-436⁴
- MDA-MB-231, ATCC 45518
- MDA-MB-435, ATCC 45526
- MDA-MB-157, ATCC HTB24
- BT-20, ATCC HTB19
- BT-483, ATCC HTB121
- CAMA-1, ATCC HTB22
- Du4475, DSM ACC427
- MCF-7, ATCC HTB22
- MDA-MB-175, ATCC 45516
- MDA-MB-361, ATCC HTB27
- MDA-MB-453, DSM ACC65
- SK-BR-3, ATCC 45520
- T47D, ATCC HTB133
- UCAA-812⁴
- ZR-75-1, ATCC CRL 1500
- ZR-75-30, ATCC CRL 1504

Example 1

Materials and Methods:

Northern blot:

[0049] 10 μg of total RNA from SUIT-2 007 and SUIT-2 028 cell lines were loaded side by side on a denaturing 1% agarose formaldehyde gel and size-separated by electrophoresis. Blotting to BrightStar-Plus™ positively charged nylon membrane was performed by capillary downward transfer. After UV-crosslinking (Stratagene UV Stratalinker 2400) the blot was hybridized. The RT-PCR product was labeled with α-[32P]dATP to a specific activity of 2x10^9 cpm/μg using the Strip-EZ™ DNA Kit (Ambion Inc., Austin, Texas). Pre-hybridization (30 min) and hybridization (over-night) with the radioactive probe was performed in ExpressHyb™ Hybridization Solution (Clontech, Palo Alto, CA, USA) at 68°C. The membrane was washed in solution 1 (2x SSC, 0.05% SDS) at room temperature for 30-40 min with continuous agitation and several replacements of the wash solution 1 followed by a washing step with solution 2 (0,1 x SSC, 0.1% SDS) at 50°C for 40 min with one change of fresh solution. The membrane was then exposed to Cronex, Medical X-Ray Films (Sterling Diagnostic Imaging Inc., USA) at -70°C for 2 h. Equal loading and transfer of mRNA to the membrane was assessed by rehybridizing the blot with α-[32P]dATP-labeled GAPDH cDNA probe.

Multiple tissue expression array (MTE™)

[0050] The blot was hybridized with an α-[32P]dATP labeled probe derived from UKW cDNA according to the instructions of the manufacturer and exposed to X-ray film at -70°C for 62 h.

Taqman®-PCR

[0051] Real-time quantitative PCRs were performed with the TaqMan® technology and the ABI PRISM 7700 apparatus (Applied Biosystems, Foster City, CA). 10 μg total RNA isolated from frozen adenocarcinomas of the pancreas, chronic pancreatitis and normal pancreatic tissues were used for reverse transcriptase reactions in a volume of 20 μl. The PCRs were then carried out by mixing 200 ng cDNA with 4μl of 10x SYBR-Green buffer, 3 mM MgCl_2, 1 mM dNTPs, 0.2 units Uracl-N Glycosylase, 1 unit AmpliTag Gold, 4μl primers mix (300 nM each primer: forward 5'TTCTCTTTGACAGTTCTAGC3' (SEQ ID NO:3); reverse 5'GGTTGGAAATGAGGCTCT3' (SEQ ID NO:4) in a final volume of 40 μl. PCR primers were designed to generate a DNA fragment of 50 bp using the Primer Express Software (PE Biosystems, CA, USA). The amplification cycles were as follows: 2 min at 50°C followed by 10 min at 95°C and 40 amplification cycles (95°C for 15 sec and 60°C for 60 sec). These experiments were performed twice. The results were calculated by subtraction of gene UKW and housekeeping gene RNA steady-state levels for each sample. Each value was divided by the averaged steady-state level of UKW mRNA of three healthy tissues. Ratios were squared and a reciprocal value was formed.

[0052] Primers:

xs13for: 5'AGATCCGATGTCCTCC3' (SEQ ID NO:5);
xs13rev: 5'CCTGCGATGTCCTCC3' (SEQ ID NO:6).

LightCycler®-PCR

[0053] LightCycler®-PCR was performed with a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) in LightCycler capillaries using a commercially available master mix containing Taq DNA polymerase, SYBR-Green I, deoxyribonucleoside triphosphates (LightCycler DNA master SYBR-Green I, Roche Molecular Biochemical). After addition of primers (forward 5'CCCCAGGAGGTATCGCTGGG3' (SEQ ID NO:7); reverse 5'GCTGCTGAGCACTCAGG3' (SEQ ID NO:8); final concentration: 0.5 μM), MgCl_2 (3 mM) and template DNA to the master mix, 37 cycles of denaturation (95°C for 1 sec, annealing (58°C for 5 sec) and extension (72°C for 8 sec) were performed. All temperature transition rates were set to 20°C per sec. After completion of PCR amplification, melting curve analysis was performed. For this procedure, PCR products were denatured at 95°C, annealed at 65°C, and gradually heated to 95°C. SYBR-Green I fluorescence was monitored stepwise every 0.1°C. The melting curve was analyzed by visual inspection, amplified UKW gene rearranged structures were melting at 85-88°C. To control for primer dimer formation a control without template DNA ('water control') was included in each experiment. A small peak was usually visible at 78°C, which could be differentiated from the peak caused by the specific amplification product at 85-88°C. Calibration curve for calnexin was generated using serial dilutions (1:10, 1:50 and 1:80) of cDNA from pancreatic cancer cell line SUIT-2 007. The relative amounts of UKW cDNA and calnexin were determined based on a calibration
curve. Relative expression of gene UKW was calculated as a normalized value generated by dividing the steady-state levels of gene UKW and calnexin for each sample. Calnexin forward primer 5'TTGTACATCGTATTGC3' (SEQ ID NO:9); reverse primer 5'ATGGAACAGGTAACCAGCAT3' (SEQ ID NO:10).

Example 2

Production of antibodies against UKW polypeptides

[0054] For immunization the peptides aa 167-182 and aa 306-320 (SEQ ID NO:2) were synthesized and coupled to a carrier molecule. Immunization of two rabbits with the mix of the peptides.

Standard immunization protocol (for rabbits):

[0055]
Day 0: Taking of pre-immune serum followed by the first immunization
Day 14: Second immunization
Day 28: Third immunization
Day 38: Blood sampling
Day 56: Fourth immunization
Day 66: Blood sampling
Day 80: Complete bleeping

[0056] The antiserum recovered was purified by affinity chromatography.

List of References

[0057]

Coligan et al. (eds.), Current Protocols in Immunology, pp. 9.3.1-9.3.5 and pp. 9.4.1-9.4.11 John Wiley & Sons, 1997
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EP-A 0 200 362
F. Hoffmann-La Roche AG

Methods for diagnosis and therapy of pancreatic cancer and composition useful therein

EP 02024539.5
2002-10-31

Patentin Ver. 2.1

DNA

Homo sapiens

CDS

(366)..(1484)
ggtaggaggg aaccatgtgg ttccagctga attttttttt tcctctcttc ttttttca 60
cctttttctt tccaacagg gaaaaagtgtt ccacgaagcg gtacgccctt tccgcctgc 120
gtttctctcc ctgacccctgg tccggctec cgtccggccg ccagctgggtg gggcgaagcc 180
cggagccca ctgcccceca gggcagccgg gcgcccggcc gcgtcggcgc cgccacatgg 240
cgcagcccac ctgcgcgcgcag cccgagggcg cggccgcceag ctcgcggcggag gtccgctgga 300
ggcggccggc cggcccggag ccaagcagca gtcgacgggg gaagcgcccg gtcgccggga 360
tcggg atg tcc ctc ctc ctc ctc ctc ttg cta gtt tcc tac tat gtt gga 410
   Met Ser Leu Leu Leu Leu Leu Leu Leu Val Ser Tyr Tyr Val Gly
1  5 10 15
acc ttg ggg act cac act gag atc aag aga gtg gca gaa aag gtc
Thr Leu Gly Thr His Thr Glu Ile Lys Arg Val Ala Glu Glu Lys Val
20 25 30
act ttg ccc tgc cac cat cca ctg ggg ctg cca gaa aaa gac act ctc
Thr Leu Pro Cys His His Gln Leu Leu Pro Gln Leu Leu Pro Asp Thr Leu
35 40 45
gat att gaa tgt ctc acc gat aat gaa ggg aac cca aaa gtt gtg
Asp Ile Glu Trp Leu Leu Thr Asp Asn Glu Glu Asn Glu Lys Val Val
50 55 60
ATC ACT TAC TCC AGT CGT CAT GTC TAC AAT AAC TTG ACT GAG GAA CAG
Ile Thr Tyr Ser Ser Arg His Val Tyr Asn Asn Leu Thr Glu Glu Gln 65 70 75

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Claims

1. Method for determining in vitro the presence or absence of pancreatic cancer in a patient comprising

(i) detecting in the biological sample from a patient an amount of a polypeptide of SEQ ID NO: 2; and
(ii) comparing the amount of polypeptide with a predetermined standard value indicating the decision line for tumor-induced or non-tumor-induced presence of polypeptide of SEQ ID NO: 2 in the cell and therefrom determining the presence or absence of pancreatic cancer in the patient, whereby overexpression is specifically for pancreatic tumor cells.

2. Process for determining in vitro whether or not a test sample of tissue or fluid of a patient contains pancreatic tumor cells or is derived from pancreatic tumor cells, wherein the test sample and a second sample originating from non-pancreatic-tumor cells from the same individual or a different individual of the same species are used, which process comprises the following steps:

(a) incubating each respective sample under stringent hybridization conditions with a nucleic acid probe which is selected from the group consisting of:

(i) a nucleic acid sequence of SEQ ID NO: 1, or a fragment thereof;
(ii) a nucleic acid sequence which is complementary to any nucleic acid sequence of (i);
(iii) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (i); and
(iv) a nucleic acid sequence which hybridizes under stringent conditions with the sequence of (ii); and
(b) determining the approximate amount of hybridization of each respective sample with said probe, and
(c) comparing the approximate amount of hybridization of the test sample to an approximate amount of hybrid-
ization of said second sample to identify whether or not the test sample contains a greater amount of the specific
nucleic acid or mixture of nucleic acids than does said second sample.

3. Method according to claim 2, characterized in that
   a) said sample is selected from the group of body fluid, of cells, or of a cell extract or cell culture supernatants
   of said cells,
   b) said determining of hybridization is by means of a further binding partner of the nucleic acid of the sample
   and/or the nucleic acid probe or by exposure to X-ray film.

4. A screening method for identifying in vitro a compound which inhibits the biological activity of the polypeptide of
   SEQ ID NO: 2 comprising:
   contacting said polypeptide with a compound or a plurality of compounds under conditions which allow interaction
   of said compound with said polypeptide; and detecting the interaction between said compound or plurality of
   compounds with said polypeptide.

5. A screening method for identifying in vitro a compound which either inhibits the biological activity of the polypeptide
   of SEQ ID NO: 2 or inhibits the transcription or translation of the nucleic acid encoding a polypeptide of SEQ ID NO:
   2, comprising:
   contacting said compound or plurality of said compounds with a host cell in which invasiveness is mediated by
   expression of a nucleic acid encoding a polypeptide of SEQ ID NO: 2; and detecting the interaction between said compound or plurality of
   compounds with said host cell.

6. The screening method according to claim 5, wherein the detection of the interaction between said compound or
   plurality of compounds with said host cell is measured by at least one of a change in cell physiology, a change in
   the differentiation state, and a change in cell metabolism leading to an increase of proliferation.

7. A screening method for identifying in vitro a compound which is an antagonist of a polypeptide of SEQ ID NO: 2
   comprising:
   contacting said polypeptide of SEQ ID NO: 2 with a compounds under conditions which allow interaction of said
   compound with said polypeptide;
   determining a first level of activity of said polypeptide;
   determining a second level of activity of said polypeptide expressed in a host which has not been contacted
   with said compounds; and
   quantitatively relating the first level of activity with the second level of activity, wherein when said first level of
   activity is less than said second level of activity, said compound is identified as an antagonist of said polypeptide.

8. An antibody against the aa 167-182 or aa 306-320 of the UKW polypeptide of SEQ ID NO:2.

9. A kit for the diagnosis of a pancreatic tumor comprising the antibody of claim 8.

Patentansprüche

1. Verfahren, zum Bestimmen des Vorhandenseins oder Nichtvorhandenseins von Bauchspeicheldrüsenkrebs in ei-
   nem Patienten in vitro, umfassend
   (i) das Nachweisen einer Menge eines Polypeptids der SEQ ID NO: 2 in der biologischen Probe eines Patienten
   und
   (ii) das Vergleichen der Polypeptidmenge mit einem vorher festgelegten Standardwert, der die Richtlinie für
tumorinduziertes oder nicht-tumorinduziertes Vorhandensein des Polypeptids der SEQ ID NO: 2 in der Zelle
   angibt, und dadurch Bestimmen des Vorhandenseins oder Nichtvorhandenseins von Bauchspeicheldrüsen-
   krebs in dem Patienten, wobei Überexpression für Zellen von Bauchspeicheldrüsentumoren spezifisch ist.
2. Verfahren, zum Bestimmen in vitro, ob eine zu untersuchende Probe aus dem Gewebe oder der Flüssigkeit eines Patienten Zellen von Bauchspeicheldrüsentumoren enthält oder aus Zellen von Bauchspeicheldrüsentumoren stammt oder nicht, wobei die zu untersuchende Probe und eine zweite Probe, die aus Nicht-Bauchspeicheldrüsentumorzellen des gleichen Individuums oder eines anderen Individuums der gleichen Spezies stammt, verwendet werden, wobei das Verfahren die folgenden Schritte umfasst:

(a) Inkubieren der jeweiligen Probe unter stringenten Hybridisierungsbedingungen mit einer Nukleinsäuresonde, die ausgewählt ist aus der Gruppe bestehend aus:

(i) einer Nukleinsäuresequenz der SEQ ID NO: 1 oder einem Fragment davon,
(ii) einer Nukleinsäuresequenz, die zu einer Nukleinsäuresequenz gemäß (i) komplementär ist,
(iii) einer Nukleinsäuresequenz, die unter stringenten Bedingungen mit der Sequenz gemäß (i) hybridisiert und
(iv) einer Nukleinsäuresequenz, die unter stringenten Bedingungen mit der Sequenz gemäß (ii) hybridisiert und

(b) Bestimmen des ungefähren Ausmaßes an Hybridisierung der jeweiligen Probe mit der Sonde und
(c) Vergleichen des ungefähren Ausmaßes an Hybridisierung der zu untersuchenden Probe mit dem ungefähren Ausmaß an Hybridisierung der zweiten Probe, um zu identifizieren, ob die zu untersuchende Probe eine größere Menge der spezifischen Nukleinsäure oder Mischung an Nukleinsäuren enthält als die zweite Probe oder nicht.

3. Verfahren nach Anspruch 2, dadurch gekennzeichnet, dass

a) die Probe aus der Gruppe bestehend aus Körperflüssigkeit, Zellen oder einem Zellextrakt oder Zellkulturüberständen der Zellen ausgewählt ist,
b) das Bestimmen der Hybridisierung mittels eines weiteren Bindepartners der Nukleinsäure der Probe und/ oder der Nukleinsäuresonde erfolgt oder indem man sie einem Röntgenfilm aussetzt.

4. Screening-Verfahren, zum in vitro Identifizieren einer Verbindung, die die biologische Aktivität des Polypeptids der SEQ ID NO: 2 inhibiert, umfassend:

   das Inkontaktbringen des Polypeptids mit einer Verbindung oder einer Vielzahl an Verbindungen unter Bedingungen, die eine Wechselwirkung der Verbindung mit dem Polypeptid erlauben; und Nachweisen der Wechselwirkung zwischen dieser Verbindung oder der Vielzahl an Verbindungen mit dem Polypeptid.

5. Screening-Verfahren, zum in vitro Identifizieren einer Verbindung, die entweder die biologische Aktivität des Polypeptids der SEQ ID NO: 2 inhibiert oder die Transkription oder Translation der Nukleinsäure, die ein Polypeptid der SEQ ID NO: 2 kodiert, inhibiert, umfassend:

   das Inkontaktbringen der Verbindung oder der Vielzahl an Verbindungen mit einer Wirtszelle, in der die Invasivität durch die Expression einer Nukleinsäure, die ein Polypeptid der SEQ ID NO: 2 kodiert, vermittelt wird und Bestimmen der Wechselwirkung zwischen der Verbindung oder der Vielzahl an Verbindungen und der Wirtszelle.


7. Screening-Verfahren, zum in vitro Identifizieren einer Verbindung, die ein Antagonist eines Polypeptids der SEQ ID NO: 2 ist, umfassend:

   Inkontaktbringen des Polypeptids der SEQ ID NO: 2 mit einer Verbindung unter Bedingungen, die die Wechselwirkung der Verbindung mit dem Polypeptid ermöglichen, Bestimmen eines ersten Aktivitätsniveaus des Polypeptids, Bestimmen eines zweiten Aktivitätsniveaus des Polypeptids, das in einem Wirt exprimiert wird, der nicht mit der Verbindung in Kontakt gebracht wurde und Herstellen eines quantitativen Bezugs zwischen dem ersten Aktivitätsniveau und dem zweiten Aktivitätsniveau, wobei die Verbindung als ein Antagonist des Polypeptids identifiziert wird, wenn das erste Aktivitätsniveau geringer ist als das zweite Aktivitätsniveau.


**Revendications**

1. Procédé de détermination *in vitro* de la présence ou de l’absence d’un cancer du pancréas chez un patient, comprenant:

   (i) la détection dans un échantillon biologique du patient d’une quantité d’un polypeptide de SEQ ID NO: 2; et
   (ii) la comparaison de la quantité de polypeptide avec une valeur de référence prédéterminée indiquant la limite décisive pour la présence induite par une tumeur ou non induite par une tumeur du polypeptide de SEQ ID NO: 2 dans la cellule et la détermination à partir de là de la présence ou de l’absence de cancer pancréatique chez le patient, la surexpression étant spécifique des cellules tumorales pancréatiques.

2. Procédé pour déterminer *in vitro* si un échantillon d’essai de tissu ou de fluide d’un patient contient des cellules tumorales pancréatiques ou provient de cellules tumorales pancréatiques, dans lequel on utilise l’échantillon d’essai et un deuxième échantillon provenant de cellules qui ne sont pas des cellules tumorales pancréatiques, du même individu ou d’un autre individu de la même espèce, ce procédé comprenant les étapes suivantes:

   (a) on met chacun des échantillons respectifs à incuber dans des conditions d’hybridation stringentes avec une sonde acide nucléique qui est choisie dans le groupe constitué par:

      (i) une séquence d’acide nucléique de SEQ ID NO: 1 ou un de ses fragments;
      (ii) une séquence d’acide nucléique qui est complémentaire d’une séquence d’acide nucléique de (i) quelconque;
      (iii) une séquence d’acide nucléique qui s’hybride dans des conditions stringentes avec la séquence de (i); et
      (iv) une séquence d’acide nucléique qui s’hybride dans des conditions stringentes avec la séquence de (ii); et

   (b) on détermine la quantité approximative d’hybridation de chacun des échantillons respectifs avec ladite sonde, et
   (c) on compare la quantité approximative d’hybridation de l’échantillon d’essai à la quantité approximative d’hybridation du deuxième échantillon pour identifier si l’échantillon d’essai contient ou non une quantité plus grande de l’acide nucléique ou du mélange d’acides nucléiques spécifiques que le dit deuxième échantillon.

3. Procédé selon la revendication 2, *caractérisé en ce que*

   a) ledit échantillon est choisi dans le groupe constitué par un fluide corporel, des cellules ou des extraits de cellules ou des surnageants de cultures cellulaires desdites cellules,
   b) ladite détermination de l’hybridation s’effectue à l’aide d’un autre partenaire de liaison de l’acide nucléique de l’échantillon et/ou de la sonde acide nucléique ou par exposition à un film de rayons X.

4. Procédé de criblage pour l’identification *in vitro* d’un composé qui inhibe l’activité biologique du polypeptide de SEQ ID NO: 2, comprenant:

   la mise en contact dudit polypeptide avec un composé ou une pluralité de composés dans des conditions qui permettent l’interaction dudit composé avec ledit polypeptide; et la détection de l’interaction entre ledit composé ou la pluralité de composés avec ledit polypeptide.

5. Procédé de criblage pour l’identification *in vitro* d’un composé qui, soit inhibe l’activité biologique du polypeptide de SEQ ID NO: 2, soit inhibe la transcription ou la traduction de l’acide nucléique codant pour un polypeptide de SEQ ID NO: 2, comprenant:

   la mise en contact dudit composé ou d’une pluralité desdits composés avec une cellule hôte dans laquelle le pouvoir envahissant est médié par l’expression d’un acide nucléique codant pour un polypeptide de SEQ ID NO: 2; et
   la détection de l’interaction entre ledit composé ou la pluralité de composés avec ladite cellule hôte.
6. Procédé de criblage selon la revendication 5, dans lequel la détection de l’interaction entre ledit composé ou la pluralité de composés avec ladite cellule hôte est mesurée par au moins un changement de la physiologie de la cellule, un changement de l’état de différenciation, et un changement du métabolisme cellulaire conduisant à une augmentation de la prolifération.

7. Procédé de criblage pour l’identification in vitro d’un composé qui est un antagoniste d’un polypeptide de SEQ ID NO: 2, comprenant:
   - la mise en contact dudit polypeptide de SEQ ID NO: 2 avec un composé dans des conditions qui permettent l’interaction dudit composé avec ledit polypeptide;
   - la détermination d’un premier niveau d’activité dudit polypeptide;
   - la détermination d’un deuxième niveau d’activité dudit polypeptide exprimé dans un hôte qui n’a pas été en contact avec ledit composé; et
   - l’établissement d’un rapport quantitatif entre le premier niveau d’activité et le deuxième niveau d’activité où, lorsque ledit premier niveau d’activité est inférieur audit deuxième niveau d’activité, ledit composé est identifié comme antagoniste dudit polypeptide.


**Fig. 2**

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<td>medulla</td>
<td>pith trial</td>
<td>atrium, right</td>
<td>jejunum</td>
<td>stomach</td>
<td>uterus</td>
<td>thyroid gland</td>
<td>leukemia, MOLT-4</td>
<td>fetal liver</td>
<td>E. coli DNA</td>
</tr>
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<td><strong>E</strong></td>
<td>occipital lobe</td>
<td>caudate nucleus</td>
<td>spinal cord</td>
<td>ventricle, left</td>
<td>thalamus</td>
<td>peripheral blood leukocyte</td>
<td>prostate</td>
<td>salivary gland</td>
<td>Burkitt's lymphoma, Raji</td>
<td>fetal spleen</td>
<td>Poly (A)</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>temporal lobe</td>
<td>hippocampus</td>
<td>medulla oblongata</td>
<td>interventricular septum</td>
<td>thalamus</td>
<td>lymph node</td>
<td>testis</td>
<td>mammary gland</td>
<td>Burkitt's lymphoma, Daudi</td>
<td>fetal thymus</td>
<td>human Cgh-1 DNA</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>p. g. of cerebral cortex</td>
<td>medulla oblongata</td>
<td>interventricular septum</td>
<td>appendix</td>
<td>bone marrow</td>
<td>ovary</td>
<td>colorectal adenocarcinoma SW483</td>
<td>fetal lung</td>
<td>human DNA 100 ng</td>
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<td><strong>H</strong></td>
<td>pons</td>
<td>putamen</td>
<td>apex of the heart</td>
<td>colon, ascending</td>
<td>trachea</td>
<td>lung carcinoma, A549</td>
<td>human DNA 500 ng</td>
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</table>

* paracentral gyrus
Fig. 3

- Pancreatic cancer
- Chronic pancreatitis
- Normal pancreas

Relative expression vs. Tissue samples.
REFERENCES CITED IN THE DESCRIPTION

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