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(54) **ECDN PROTEIN AND DNA CODING FOR THE SAME**

ECDN - PROTEIN UND DAFUER KODEIERENDE DNA

PROTEINE ECDN ET ADN CODANT CETTE PROTEINE

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(73) Proprietors:
- **CANCER INSTITUTE**
  Toshima-ku,
  Tokyo 170-8455 (JP)
- **Eisai Co., Ltd.**
  Tokyo 112-88 (JP)

(72) Inventors:
- **NAKAMURA, Yusuke**
  Kanagawa 225 (JP)
- **SAITO, Hiroko**
  Tokyo 177 (JP)

(74) Representative: **Hansen, Bernd et al**
Hoffmann Eitle,
Patent- und Rechtsanwälte,
Arabellastrasse 4
81925 München (DE)

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EP 0 729 975 B9

Description

Background of the Invention

Field of the Invention

[0001] The present invention relates to a paucimolecular ECDN protein, which is a novel steroid hormone receptor-like protein, a DNA encoding the same and processes for the production and use of this protein.

Description of the Related Art

[0002] As steroid hormone receptor-like proteins, there have been found out and identified a group of proteins being homologous with each other in amino acid sequence. These proteins are respondent not only to steroid hormones but also to thyroid hormone and derivatives of fat-soluble vitamins such as vitamin D metabolites and natural and synthetic derivatives thereof, retinoic acid and natural and synthetic derivatives thereof and vitamin A metabolites. These ligand-dependent transcription regulating proteins constitute a superfamily and can regulate the expression of a specific gene by binding directly to a response element of a DNA specific for a stimulation by a hormone or a ligand. It has been revealed that these receptor proteins play very important roles in the development, differentiation and the maintenance of the homeostasis as transcription regulating factors which regulate the expression of specific genes with the use of hormones or vitamins as ligands [Evans, R. M., Science, 240, 889 (1988)]. Thus natural and synthetic derivatives which are capable of binding to these receptor proteins and acting as ligands have been widely studied and applied in the fields of medicine, pharmacology and agriculture.

[0003] A group of proteins seemingly belonging to the superfamily of steroid hormone receptor proteins involve a group of a number of proteins which have been produced by molecular clonings effected by hybridizations under the lowered stringency, paying attention to the homology of DNA binding domains common to the superfamily. The group of these proteins include a group of proteins called orphan receptors the ligands specific for which have been unidentified hitherto [Laudet, V. et. al., EMBO J., 11, 1003 (1992)]. It is considered that studies on the identification of the ligands specific for these orphan receptors and functions of these orphan receptors as transcription regulating factors are highly important, since novel medical and biological findings applicable to medicines and agriculture might be provided thereby. For example, it has been clarified that the ligand of retinoid X receptor (RXR), which was once regarded as an orphan receptor, is 9-cis-retinoic acid and that RXR is a receptor having an important role in transcriptional regulation [Levin, A., et. al., NATURE, 355, 359 (1992)]. It seems that in the proteins participating in the human transcriptional regulation mechanism depending on ligands, a number of receptors still remain unidentified. Therefore, it is highly meaningful to isolate and identify these receptors from the viewpoint of the application thereof to medicine and pharmacology too.

Disclosure of the Invention

Summary of the Invention

[0004] It is an object of the present invention to provide a novel receptor protein originating from human being which belongs to the steroid/thyroid hormone receptor superfamily, a gene encoding the protein, a process for analyzing the function of a receptor with the use of the protein and a process for searching a ligand with the use of the protein, thus broadening the application field thereof.

[0005] As the results of comparison of the structures of proteins in a group constituting the superfamily of steroid hormone receptor-like proteins and analyses of the functions with the use of mutated receptors constructed via gene modification, it has been clarified that these proteins in the group comprise functional domains. It has been clarified that a domain consisting of 66 to 68 amino acids and having 2 zinc fingers shows the highest homology among the proteins in the group belonging to the superfamily and binds to a DNA, while a domain consisting of about 250 amino acids on the C-terminal side participates in the ligand-dependency as a domain capable of binding to a ligand [Wahli, W. et. al., FASEB, J., 5, 2243 (1991)]. These steroid hormone receptor-like proteins have been detected and identified not only in mammals but also in insects. As a representative example thereof, a receptor to ecdysone, which is a steroid hormone controlling the metamorphoses of insects, is known as a member of the above-mentioned superfamily [Koelle, M. R. et. al., Cell, 67, 59 (1991)].

[0006] The present inventors randomly selected clones from a human fetal lung cDNA library and identified the nucleotide sequence on the 5'-terminal side of each clone. Then, the amino acid sequences deduced therefrom were subjected to a search. As a result, they found out a clone having an amino acid sequence highly homologous with that of the ecdysone receptor which had been isolated and identified from an insect fat body. Since this clone included no cDNA of the full length, they screened cDNA libraries again with the use of this clone as a probe. Thus they successfully

obtained a clone including a full-length cDNA from a human mammary gland library. The amino acid sequence of the protein encoded by this cDNA was highly homologous with those of the ecdysone receptor, human and mouse retinoic acid receptors and thyroid hormone receptor. In a site seemingly corresponding to the DNA binding domain, in particular, a region including 2 zinc fingers was conserved with a high homology. It was also confirmed that a site corresponding to the ligand binding domain showed a high homology with those of the above-mentioned ecdysone receptor and retinoic acid receptor and had a domain structure characteristic to those of steroid hormone receptor-like proteins. Thus, it was proved that this protein be a member of the group of the steroid hormone receptor-like proteins which had never been reported only by the invention of WO 9407916. The present inventors designated this protein as "ECDN protein".

[0007] By introducing the cDNA encoding this receptor protein into a host (Escherichia coli, a yeast, insect cells, mammalian cells, etc.) to thereby prepare a transformant, the receptor protein of the present invention can be isolated and purified and thus the structure of the ligand binding thereto can be determined. Moreover, a novel synthetic ligand or a response element capable of-binding to this receptor can be screened by a binding test with the use of the purified protein. When the cDNA encoding this receptor protein is subjected to cotransformation together with a plasmid including a response element and a reporter gene, it becomes possible to examine the functions of this receptor on the transcriptional activity in detail.

[0008] The present invention is highly meaningful, since it enables us to analyze the function of the above-mentioned protein as a transcription regulating factor by examining the interactions thereof with the DNA response element binding thereto and other transcription regulating factors and screening a ligand specific thereto, and to broaden the scope of investigations aiming at the development of novel pharmaceuticals and diagnostic and preventive drugs, thus bringing about a rapid progress in the findings in this field.

[0009] The present inventors prepared the anti-ECDN protein monoclonal antibody and analyzed the expression of this protein in various cells. As a result, it was surprisingly confirmed that, in addition to the ECDN protein of about 50 kDa, a paucimolecular protein of about 40 kDa was expressed specifically in cancer cells. Subsequently, the structure of this paucimolecular protein was identified. It was confirmed that this paucimolecular protein was a protein corresponding to the ECDN protein but lacking in 97 amino acids, as a result of the deletion of 291 bases from the DNA encoding the ECDN protein. Thus the present inventors designated this protein as "ECDN paucimolecular protein". From this fact, it is understood that the ECDN paucimolecular protein in accordance with the present invention is a unique protein which is widely applicable to, e.g., the diagnosis and treatment of cancers. (1) an ECDN paucimolecular protein having an amino acid sequence which comprises the whole or a part of an amino acid sequence encoded by the nucleotide sequence described in sequence ID NO: 2.

[0010] Furthermore, the present invention includes an ECDN paucimolecular protein which is substantially equivalent to the ECDN paucimolecular protein having an amino acid sequence described in sequence ID NO: 2 and obtained by the addition or insertion of one or more amino acid residues to(into) the protein, or the deletion of or substitution for one or more consecutive amino acid residues in the protein. Such equivalent substances are included in the present invention, so long as it exerts similar effects in the studies and diagnoses of ECDN paucimolecular proteins.

[0011] The present invention further provides and (3) a DNA encoding an ECDN paucimolecular protein which has a nucleotide sequence comprising the whole or a part of the nucleotide sequence described in sequence ID NO: 2.

[0012] Furthermore, similar to the case of the above-mentioned ECDN paucimolecular protein, DNAs which are substantially equivalent to a DNA encoding an ECDN paucimolecular protein having the amino acid sequence described in sequence ID NO: 2 and obtained by the addition or insertion of one or more nucleotides to(into) the DNA, or the deletion of or substitution for one or more consecutive nucleotides in the DNA, i.e., equivalent substances, are also included in the present invention.

[0013] The present invention furthermore provides (5) a vector comprising the above-mentioned DNA; (6) a transformant carrying this vector; and (7) a process for producing an ECDN protein or an ECDN paucimolecular protein which comprises culturing the transformant carrying the above-mentioned vector transfected thereinto and recovering an expression product thereof.

[0014] In addition, the present invention provides (8) a cotransformant carrying, transfected thereinto, a vector comprising a DNA encoding an ECDN protein and a reporter vector comprising a DNA respondent to the vector; (9) a cotransformant carrying, transfected thereinto, a vector which comprises a DNA encoding a chimeric ECDN protein obtained by replacing the DNA binding domain of an ECDN protein with the DNA binding domain of a known steroid hormone receptor-like protein other than an ECDN protein and a reporter vector comprising a DNA respondent to the vector; (10) a process for screening a compound capable of acting on the above-mentioned cotransformant which comprises using the cotransformant; and (11) a process for screening a compound capable of binding to the above-mentioned ECDN protein which comprises using the ECDN protein.

[0015] The present invention provides (12) a DNA probe which has a nucleotide sequence comprising the whole or a part of the nucleotide sequence described in sequence 2; (13) a DNA primer which has a nucleotide sequence comprising a part of the nucleotide sequence described in sequence ID No. 2; (14) a process for analyzing a gene of an ECDN protein or an ECDN paucimolecular protein which comprises hybridizing the above-mentioned DNA probe or

the above-mentioned DNA primer with a subject DNA; (15) a process for testing cells which comprises assaying a mRNA of an ECDN protein or an ECDN paucimolecular protein in a subject tissue or subject cells by using the above-mentioned DNA probe or the above-mentioned DNA primer; (16) a process for testing cells which comprises hybridizing the above-mentioned DNA probe with a subject DNA to thereby assay a gene of an ECDN protein or an ECDN paucimolecular protein; (17) a process for testing cells which comprises amplifying a subject mRNA by the RT-PCR method with the use of the above-mentioned DNA primer and assaying the expression of a gene of an ECDN protein or an ECDN paucimolecular protein; and (18) a process for testing cells which comprises amplifying a subject mRNA by the RT-PCR method with the use of the DNA primer described in sequence ID NO: 6 and the DNA primer described in sequence ID NO: 7 and assaying the expression of a gene of an ECDN protein or an ECDN paucimolecular protein.

[0016] The present invention further provides (19) a polyclonal antibody or a monoclonal antibody capable of specifically binding to an ECDN paucimolecular protein; (20) a process for immunochemically assaying an ECDN paucimolecular protein which comprises using the above-mentioned polyclonal antibody or monoclonal antibody; (21) a process for testing cells which comprises immunohistochemically staining a subject tissue or subject cells with the use of the above-mentioned polyclonal antibody or monoclonal antibody and determining the intracellular distribution of an ECDN paucimolecular protein; and (22) a process for testing cells which comprises determining the amount of an ECDN paucimolecular protein expressed in a subject tissue or subject cells by using the above-mentioned polyclonal antibody or monoclonal antibody.

[0017] The present invention furthermore provides (24) a peptide comprising at least eight consecutive amino acids in an amino acid sequence that an ECDN paucimolecular protein has.

[0018] In addition, the present invention provides (25) an antisense DNA or an antisense RNA hybridizable specifically with a mRNA of an ECDN paucimolecular protein; (26) a ribozyme capable of cleaving specifically a mRNA of an ECDN paucimolecular protein; and (27) a pharmaceutical for gene therapy which comprises, as the active component, a gene construction capable of expressing the above-mentioned antisense DNA, the above-mentioned antisense RNA or the above-mentioned ribozyme.

[0019] The present invention also includes (1) a protein comprising the one including the whole or a part of the protein represented by sequence ID NO: 2: (2) a DNA comprising the one including the whole or a part of the DNA represented by sequence ID NO: 2: (3) a plasmid including the whole or a part of the DNA described in sequence ID NO: 1, and a transformant carrying the same; (4) a process for producing the protein represented by sequence ID NO: 2: (5) an antibody which binds to the protein represented by sequence ID NO: 2; (6) a primer or a probe including a part of the DNA sequence represented by sequence ID NO: 2, and a process for analyzing a gene characterized by using the same; (7) a process for analyzing a binding ligand or a response DNA with the use of the whole or a part of the protein represented by sequence ID NO: 2: and (8) a process for analyzing a transcription regulating function with the use of a cotransformant transformed with both an expression vector including the whole or a part of the DNA sequence represented by sequence ID NO: 2 and a reporter plasmid which has been constructed in order to observe the promotion or suppression of the expression of a reporter gene in response to the protein described in sequence ID NO: 2. In this case as well, the above-mentioned protein and DNA also include those substantially equivalent thereto.

[0020] The DNA of the present invention and a DNA complementary to said DNA are applicable to an analysis of the gene of the ECDN protein or the ECDN paucimolecular protein, or an analysis of the expression of said gene, by using a part thereof as a primer or probe. The term "a part of the DNA" as used herein refers a sequence of consecutive at least eight nucleotides, preferably at least ten nucleotides, still more preferably at least fifteen to twenty-five nucleotides corresponding to (i.e., contained in or complementary to) the nucleotide sequence of the DNA according to the present invention. The primer or probe of the present invention which is an oligonucleotide or polynucleotide may contain also at least one nucleotide(s) not corresponding to the nucleotide sequence of the DNA encoding the ECDN protein or the ECDN paucimolecular protein.

[0021] The protein of the present invention is applicable to an antibody preparation and agents for study and diagnosis containing such an antibody, by the use of the whole or a part thereof as an epitope. The term "epitope" refers an antigenic determinant of a polypeptide. It is well known that the epitope is generally composed of at least six amino acid residues and that a polypeptide composed of six amino acid residues combines with an antibody [see WO of PCT Patent Applications No. 8403564, published on Sep. 13, 1984 (Applicant: COMMONWEALTH SERUM LABS AND GEYSEN, H.M.)]. The term "a part of the protein" as used herein refers to a polypeptide comprising at least six consecutive amino acid residues, preferably at least eight consecutive amino acid residues, still more preferably at least about ten consecutive amino acid residues, and particularly preferably at least about fifteen to twenty-five consecutive amino acid residues, on the basis of the amino acid sequence of the protein of the present invention. The above-mentioned polypeptide may contain also at least one amino acid residue(s) not corresponding to the amino acid sequence of the ECDN protein or the ECDN paucimolecular protein.

[0022] Now, the present invention will be described in detail.

Detailed Description of the Invention

(1) Isolation of cDNA clone SEQ ID1; only used for illustrative purposes and does not define the invention from this point

[0023] cDNAs are synthesized on the basis of human fetal lung mRNAs in a conventional manner and a cDNA library having cDNA inserts cloned in a definite orientation is prepared. Clones are randomly selected from this library and the nucleotide sequence of each clone is partially determined from the 5'-terminal side. Thus, a clone having a nucleotide sequence which is homologous with that of an ecdysone receptor cloned from a mosquito fat body tissue is selected. When the clone thus selected does not include the cDNA of the full length, various cDNA libraries are screened by using the cDNA of this clone as a probe to thereby give the target clone including the full-length cDNA.

(2) Identification of the full structure of the gene

[0024] It has been confirmed that the cDNA obtained by the above method is a novel DNA sequence described in sequence ID NO: 1. The present inventors designated the novel protein having an amino acid sequence encoded thereby as ECDN protein.

[0025] Similarly, the present inventors obtained a cDNA having a novel DNA sequence described in sequence ID NO: 2, and designated the novel protein having an amino acid sequence encoded thereby as ECDN paucimolecular protein.

(3) Recombinant expression vector and transformant thereof

[0026] Although illustration in accordance with the ECDN protein will be conducted herein, the same will be applicable to the ECDN paucimolecular protein.

[0027] The DNA encoding the human ECDN protein obtained by the above-mentioned method or a fragment thereof is inserted into an appropriate vector. Then this vector is transfected into suitable host cells. Thus a transformant can be obtained. When this transformant is cultured in a conventional manner, the human ECDN protein can be produced in a large amount from the culture. More particularly, the DNA encoding the human ECDN protein or a fragment thereof is religated to a vector suitable for the expression thereof downstream of a promoter according to the customary procedure with the use of restriction enzymes and DNA ligase. Thus a recombinant expression vector can be constructed. Examples of the vector usable include plasmids pBR322 and pUC18 derived from Escherichia coli, plasmid pUB110 derived from Bacillus subtilis, plasmid pRB15 derived from a yeast, bacteriophage vectors λgt10 and λtg11, and vector SV40. The vectors are not particularly limited as long as they can be replicated or amplified in the host. The promoter and terminator are also not particularly limited as long as they suit the host employed in the expression of the DNA sequence encoding the human ECDN protein. Appropriate members thereof can be used in combination in accordance with the host. The DNA to be employed is not limited to the one having the DNA sequence described in sequence ID NO: 1, as long as it encodes the human ECDN protein. Use may be made of a DNA having a DNA sequence resulting from intentional or unintentional substitution, deletion, insertion and/or addition conducted individually or in combination at a part of the DNA sequence of sequence ID NO: 1. Further, use may be made of one chemically synthesized.

[0028] The recombinant expression vector thus obtained is introduced into the host by, for example, the competent cell method [J. Mol. Biol., 53, 154 (1970)], the protoplast method [Proc. Natl. Acad. Sci. USA, 75, 1929 (1978)], the calcium phosphate method [Science, 221, 551 (1983)], the in vitro packaging method [Proc. Natl. Acad. Sci. USA, 72, 581 (1975)] or the virus vector method [Cell, 37, 1053 (1984)] to thereby give a transformant. As the host, use can be made of any of Escherichia coli, Bacillus subtilis, yeasts, insect cells, animal cells and the like. The transformant thus obtained is cultured in a medium appropriately selected depending on the host. The culturing is usually effected at a temperature of from 20 to 45°C within a pH of ranging from 5 to 8 optionally under aeration and/or stirring. The transformation to be effected is not restricted to the one effecting with only a recombinant expression vector of the ECDN protein according to the present invention. That is to say, it is possible to form a cotransformant by using a reporter plasmid including a DNA response element capable of binding to the ECDN protein, with the ECDN protein.

(4) Separation and purification of recombinant protein

[0029] The separation of the ECDN protein or the ECDN paucimolecular protein from the culture and its purification may be conducted by an appropriate combination of conventional separation and purification methods. As these conventional methods, for example, salting out, solvent precipitation, dialysis and gel filtration, electrophoresis, ion exchange chromatography, affinity chromatography, and reversed-phase high-performance liquid chromatography are cited.

(5) Preparation of antibody

[0030] An antibody can be prepared by the conventional method with the use of the whole or a part of the ECDN protein as an antigen. For example, a polyclonal antibody may be prepared by giving a plurality of subcutaneous, intramuscular, intraperitoneal or intravenous inoculations of the antigen to an animal such as a mouse, a guinea-pig and a rabbit to thereby satisfactorily immunize the same, collecting the blood specimen from the animal, and performing serum separation. In this procedure, commercially available adjuvants can be used.

[0031] A monoclonal antibody may be prepared by, for example, conducting the fusion of splenocytes of a mouse immunized with the ECDN protein with commercially available mouse myeloma cells to thereby prepare a hybridoma and either culturing the hybridoma followed by separation of the antibody from the resultant supernatant or administering the hybridoma to a mouse followed by separation of the antibody from the mouse ascites.

[0032] It is not always necessary that the ECDN protein to be used as the antigen has the whole amino acid structure. Namely, use may be made of a peptide having a partial structure of the protein, a variant or derivative of the protein, or a fusion peptide resulting from the fusion with another peptide. The method for preparing these is not critical and it may be biological or chemosynthetic.

[0033] According to the similar method to that described above, antibodies against the ECDN paucimolecular protein can also be prepared. When the ECDN protein and the ECDN paucimolecular protein are attempted to be analyzed individually, it is preferable to suitably select the portion to be used as an epitope from a portion lacking in the ECDN paucimolecular protein, a portion newly formed in the ECDN paucimolecular protein or the other portion.

[0034] The obtained antibody enables the identification and quantity determination of the ECDN protein and/or the ECDN paucimolecular protein in human biospecimens and can be used in, for example, various agents. The antibody of the present invention can be used also in the form of a fragment thereof, e.g., $F(ab')_2$, Fab' or Fab, or a derivative thereof, if necessary. Further, the antibody of the present invention is applicable also as a chimera antibody, a humanized antibody or a human antibody. All of these fragments and antibodies are included in the conception of the present invention.

[0035] The immunoassay of the ECDN protein or the ECDN paucimolecular protein may be conducted in accordance with the generally known procedure and can be executed by, for example, any of the fluorescent antibody technique, passive agglutination and enzyme antibody technique.

(6) Analysis of gene and expression thereof

[0036] Any mutation of a gene encoding the ECDN protein or the ECDN paucimolecular protein according to the present invention can be analyzed by the use of a probe comprising a restriction enzyme fragment of the DNA encoding the protein or by the use of, as a primer, an oligonucleotide obtained by appropriately selecting a suitably positioned nucleotide sequence from the DNA encoding the protein and synthesizing therewith.

[0037] Also, any abnormality such as insertion and deletion in the gene as a specimen can be detected by the above analysis.

[0038] Escherichia coli DH5α/pFATSR, which carries a plasmid including the DNA encoding this ECDN protein, has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry under the accession number FERM BP-4769 on August 4, 1994.

(7) Analyses of substance binding to ECDN protein and response DNA

[0039] Although illustration in accordance with the ECDN protein will be conducted herein, the same will be applicable to the ECDN paucimolecular protein.

[0040] The transformant prepared in the above (3) and capable of expressing the ECDN protein is cultured for a definite period of time. Then the ECDN protein expressed from the transformant cells is separated and recovered. The structure of the ligand binding to the fraction containing the ECDN protein under the culture conditions can be determined by combining customary procedures such as extraction, HPLC, mass spectrometry and nuclear magnetic resonance spectrometry. After clarifying the structure of the ligand, a receptor binding test is effected by using the crude extract obtained from the transformant or the ECDN protein, which has been isolated and purified, together with labeled ligands. Thus natural or synthetic derivatives capable of binding to the ECDN protein can be screened.

[0041] The response DNA to which the ECDN protein binds can be analyzed by a customary DNA binding test such as the gel shift method with the use of the crude extract obtained from the transformant or the ECDN protein, which has been isolated and purified, together with an oligonucleotide labeled with an isotope or a nonisotopic substance such as biotin.

(8) Analysis of transcription regulating function by using cotransformant with reporter plasmid

[0042] Although illustration in accordance with the ECDN protein will be conducted herein, the same will be applicable to the ECDN paucimolecular protein.

[0043] A DNA sequence to which the ECDN protein or known steroid hormone receptor-like protein analogous bind is inserted into, e.g., a promoter derived from SV40 or cytomegalovirus or an expression promoter of a yeast upstream, while a reporter gene of, for example, chloramphenicol acetyltransferase, placental alkaline phosphatase, luciferase or LacZ is inserted into the promoter downstream to thereby construct a reporter plasmid. An ECDN protein expression vector or an expression vector of a chimera ECDN protein obtained by substituting a DNA binding domain of a known steroid hormone receptor-like protein for the DNA binding domain of the ECDN protein is transfected into a yeast, insect cells or animal cells together with the reporter plasmid or an expression vector of another steroid hormone receptor-like protein to thereby give a cotransformant. In the case of the ECDN protein expressed in such a transformant, the ECDN protein per se or its complex interacts with another receptor protein expressed simultaneously or a protein in the cells and thus binds to the reporter plasmid, thus affecting the transcription of the reporter gene. The expression of the reporter gene product via the transcription of the reporter gene can be detected through a reaction specific for the reporter gene product. Such a method for analyzing the ability to regulate transcription makes it possible, for example, to screen a substance having a transcription regulating function via the ECDN protein.

(9) Expression of ECDN paucimolecular protein in cancer cells

[0044] As will be shown in Example 9 and thereafter, the present inventors analyze various cancer cell lines and cancer tissues by the Western blotting method and the RT-PCR method with the use of anti-ECDN antibodies and, as a result, find that an ECDN paucimolecular protein of about 40 kDa is cancer-specifically expressed in various cancer cell lines and cancer tissues. The nucleotide sequence of the DNA encoding this ECDN paucimolecular protein is identified and, as the result, it is revealed that this nucleotide sequence is one described in sequence ID NO: 1 but lacking in a part ranging from the base Nos. 387 to 677 thereof. Further, it is also confirmed that this ECDN paucimolecular protein is accumulated in the nucleoli of the cells by immunohistological staining.

[0045] These facts suggest that an extremely closely correlation is present between the progression of cancer and the formation of the ECDN paucimolecular proteines. Therefore, it becomes possible to distinguish normal cells from cancer cells by analyzing the genes of an ECDN protein and an ECDN paucimolecular protein and intracellular distribution thereof in subject cells or subject tissues.

[0046] The gene analysis is conducted by assaying a DNA or mRNA in a subject tissue with the use of a probe or a primer which has a DNA sequence comprising a part of the nucleotide sequence of the DNA encoding an ECDN protein or an ECDN paucimolecular protein. The DNA sequence of the probe or the primer can be appropriately selected depending on the purpose by considering the region which is deleted in the ECDN paucimolecular protein in the nucleotide sequence of the DNA encoding the ECDN protein. The assay of the DNA or mRNA in the subject tissue can be conducted in accordance with a known method.

[0047] It is also possible to use an antibody in the assay of the ECDN protein and the ECDN paucimolecular protein. An antigen (epitope) for the preparation of the antibody may be appropriately selected from among a part common to both proteins, a site deleted in the ECDN paucimolecular protein, a binding site of the ECDN paucimolecular protein after the deletion (a part including the neighborhood of the amino acid No. 61 described in sequence ID NO: 2), etc. It is also possible to select an antibody suitable for the occasion from among those prepared by using such sites each as an antigen.

(10) Application to medicine

[0048] It is expected to be found a substance, from among the substances acting on the regulation of the transcription via an ECDN protein, which stimulates the normal function of the ECDN protein to thereby introduce the differentiation of tumor cells wherein an ECDN paucimolecular protein is expressed into normal cells or which suppresses the abnormal multiplication of the tumor cells, as all-trans retinoic acid in the case of promyelocyte leukemia.

[0049] It is also expected that the abnormal multiplication of tumor cells could be suppressed by suppressing the expression of the ECDN paucimolecular protein in a tumor with the use of an antisense nucleic acid (DNA or RNA) hybridizable specifically with the mRNA of the ECDN paucimolecular protein or a ribozyme. It is furthermore expected that similar effects could be established by introducing a gene construction capable of expressing an antisense RNA hybridizable specifically with the mRNA of the ECDN paucimolecular protein or a ribozyme into a tumor. As the site of the mRNA of the ECDN paucimolecular protein at which another substance (antisense RNA or ribozyme) specifically hybridizes, a site at which bases are deleted in the nucleotide sequence of the ECDN protein followed by binding, namely, a part including the neighborhood of the base No. 387 in the nucleotide sequence encoding the ECDN paucimolecular

protein described in sequence ID NO: 2.

[0050] A substance suppressing the expression of the ECDN paucimolecular protein, which cannot be observed in any normal tissue, can be screened by detecting the expression of the mRNA of the ECDN protein or the ECDN paucimolecular protein with the use of an appropriate primer, probe or antibody.

[0051] It is expected that novel pharmaceuticals for treatment, diagnostic drugs and preventive drugs are invented by analyzing the function of the human ECDN protein of the present invention of regulating transcription and a gene per se encoding this protein with the use of the human ECDN protein or a DNA, which includes the whole or a part of the DNA encoding this protein, and thus analyzing natural and synthetic compounds capable of binding specifically to this receptor protein. Moreover, it is expected that the detection of the expression of the ECDN protein and the ECDN paucimolecular protein in a subject tissue and the analyses on the genes of the ECDN protein and the ECDN paucimolecular protein are applicable to the diagnosis and treatment of cancer.

Brief Description of the Drawings

[0052] Fig. 1 shows the results of the Western blotting on a colorectal cancer tissue and a breast cancer tissue (T) and normal tissues (N) corresponding respectively thereto with the use of the anti-ECDN protein monoclonal antibody.

[0053] Fig. 2 shows the results of the RT-PCR on mRNAs of a normal tissue and a cancer cell line, respectively. In the RT-PCR, use was made of primers having nucleotide sequences described in sequence ID NO: 6 and sequence ID NO: 7, respectively.

Examples

[0054] The following Examples will be given in detail and specifically to further illustrate the present invention, and not by way of limiting the present invnetion.

(Example 1) Preparation of human cDNA library

[0055] cDNAs were synthesized on the basis of human fetal lung and mammary gland mRNAs (purchased from Clontech) and cDNA libraries each having cDNA inserts, which had been cloned in a definite orientation, were prepared with the use of a Uni-ZAP XR vector kit (purchased from Stratagene).

(Example 2) Selection of clone

[0056] Clones were randomly selected from the human fetal lung cDNA library prepared in the above Example 1 and the nucleotide sequence of each clone was partially determined from the 5'-terminal side. When these nucleotide sequences were compared with known ones in a data base with the use of the FASTA algorithm, a clone L1-1793 having a nucleotide sequence which was homologous with that of the insect ecdysone receptor was found out.

(Example 3) Cloning of full-length cDNA

[0057] Since the clone L1-1793 was not a full-length clone, the cDNA insert of the clone L1-1793 was labeled with ^{32}P by the random prime labeling method [Feinberg et al., Anal. Biochem., 132, 6 (1983)] and used as a probe in the screening of various cDNA libraries by the hybridization method. As a result, a clone including the full-length cDNA was obtained from a human mammary gland cDNA library. As a result of the analysis of the structure of this clone, it was confirmed that this clone had a novel DNA nucleotide sequence consisting of 1,979 bp involving a 5'-noncoding region of 205 bp, a coding region of 1,386 bp and a 3'-noncoding region of 388 bp (see sequence ID NO: 1). The open reading frame involved in the sequence of this cDNA encoded a novel protein (ECDN protein, see sequence ID NO: 1) consisting of 461 amino acids.

(Example 4) Expression of gene encoding ECDN protein in various human tissues

[0058] The DNA insert of the cDNA clone (see sequence ID NO: 1) obtained in the above Example 3 was labeled with ^{32}P and employed as a probe in the Northern blot analyses of various human tissue mRNAs (the kit for Northern blot analysis was purchased from Clontech). As a result, expression was recognized in the form of a mRNA band having a size of about 2 kb in all the studied tissues, i.e., brain, heart, kidney, liver, lung, pancreas, placenta, skeletal muscle, large intestine, peripheral leukocyte, ovary, prostate, small intestine, spleen, testicle and thymus gland. (Example 5) Structural features of ECDN protein

[0059] The ECDN protein having the amino acid sequence described in sequence ID NO: 1, which has been deduced

from the open reading frame corresponding to the sequence ranging from the base No. 206 to the base No. 1,591 of the nucleotide sequence described in sequence ID NO: 1, is a novel protein consisting of 461 amino acids. The amino acid sequence ranging from the residue No. 87 to the residue No. 152 (i.e., 66 residues) conserves 2 zinc fingers characteristic to a group of steroid hormone receptor-like proteins and is highly homologous with the sequences of DNA binding domains of receptor proteins, such as ecdysone receptor, retinoic acid receptor and thyroid hormone receptor. In the amino acid sequence, 219 residues ranging from the amino acid No. 243 to the amino acid No. 461 at the C-terminus is highly homologous with the sequences of ligand binding domains located on the C-terminal side of ecdysone receptor and retinoic acid receptor, and corresponds to the ligand binding site of this protein.

(Example 6) Construction of recombinant ECDN protein expression vector

[0060] By using a cDNA including the nucleotide sequence encoding the ECDN protein described in sequence ID NO: 1 as a template, a partial sequence including its protein-coding region was amplified by the PCR method. The following sequences were selected for the primers.

[0061] Primer 1: 5'-GACGGATCCATGTCCTCTCCTACCACGAGTT-3' (a coding strand, corresponding to a sequence ranging from the base No. 206 to the base No. 227 in sequence ID NO: 1, described in sequence ID NO:3).

[0062] Primer 2: 5'-CTAGAATTCGGAGGGTGGTCAGGCAAGGC-3' (an antisense strand, corresponding to an antisense strand ranging from the base No. 1,634 to the base No. 1,615 in sequence ID NO: 1 in the reverse orientation, described in sequence ID NO: 4).

[0063] Primer 1 has a BamHI cleavage site, added thereto, at the 5'-terminus, while primer 2 has an EcoRI cleavage site, added thereto, at the 5'-terminus. The PCR product was digested with BamHI and EcoRI. The resultant fragment was inserted into expression vector pGEX-2T (purchased from Pharmacia) preliminaly digested with BamHI and EcoRI, thereby constructing expression plasmid pGST-FATSR. E. coli DH5α was transformed with the plasmid pGST-FATSR and resulting transformants were selected based on the ampicillin resistance, thereby obtaining a transformant capable of expressing a fusion protein of glutathione-S-transferase and ECDN protein.

(Example 7) Expression of recombinant ECDN protein and its purification

[0064] The transformant obtained in Example 6 was cultured, and the recombinant ECDN fusion protein was extracted from the resultant culture and purified.

[0065] Specifically, the transformant was cultured by shaking the same in 100 ml of LB medium (1% peptone, 0.5% yeast extract and 1% NaCl) at 37°C overnight. The resultant liquid culture was diluted 10-fold with LB medium preheated to 37°C and the resulting dilution was further cultured at 37°C for 30 to 90 minutes, thereby obtaining a culture of logarithmic growth phase. Isopropyl β-D-thiogalactopyranoside was added to 1 L of the culture so that the final concentration thereof became 1 mM, followed by culturing for 3 to 4 hours. The culture was centrifuged to thereby separate bacterial cells. 10 ml of a column buffer (150 mM NaCl, 16 mM Na_2HPO_4 and 4mM NaH_2PO_4, pH 7.3) was added to bacterial cells transformed with the expression vector pGST-FATSR, followed by sonication. A soluble fraction of a supernatant resulting from the cell disruption was applied to a glutathione-Sepharose 4B column (purchased from Pharmacia). The column was washed with the column buffer and then elution was conducted with an eluent containing 5 mM reduced glutathione. The eluted fraction was analyzed and fractionated by SDS polyacrylamide electrophoresis. As a result, a fraction in which the desired GST fusion protein of about 75 kDa was detected as a main band was obtained from the transformant constructed with the plasmid pGST-FATSR.

(Example 8) Preparation of monoclonal antibody and polyclonal antibody

[0066] The recombinant GST fused protein obtained in Example 7 was employed as an immune antigen, an antigen for purifying and screening antibodies and a standard antigen for assaying.

[0067] The anti-ECDN protein specific monoclonal antibody was prepared by immunizing a mouse with the GST fused protein. Namely, a solution of the GST fused protein in PBS (concentration: 500 - 1,000 μg/ml) was mixed with the complete adjuvant at a ratio of 1 : 1. The mixture thus obtained was intraperitoneally administered to mice in a dose of 100 μg/animal thrice at intervals of two weeks to thereby immunize the mice. After the completion of the immunization, hybridomas of P3U1 cells with the B cells of the mice were prepared by using PEG-15,000. These hybridomas were cultured and the antibody titer in the culture supernatant was monitored. Thus hybridomas capable of producing the anti-ECDN protein specific antibody were selected.

[0068] The determination of the antibody titer was conducted as follows: Specifically, first, the GST fused protein (1 pg/ml) obtained in Example 7 was immobilized on a polystyrene cup. Into this cup was introduced 100 μl of the culture supernatant to thereby effect the first reaction. The cup was washed and then antimouse IgG-HRP (horse-radish peroxidase) was introduced into the cup to thereby effect the second reaction. The cup was washed and then an enzyme

substrate solution [a mixture of an aqueous solution of hydrogen peroxide with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was introduced into the cup to thereby effect a color development reaction (the third reaction), which had been monitored.

[0069] The hybridomas selected were cultured in a 96-well multiplate, and then subjected to the HAT screening. About two weeks after the initiation of the culture, the antibody titer of the culture supernatant was determined to thereby select a clone capable of producing an antibody reacting specifically with the antigen. Cloning was further effected, and a clone (F1D5) was established as hybridomas capable of producing the antibody.

[0070] Each of 3×10^6 hybridomas thus obtained was intraperitoneally inoculated into a BALB/c mouse to which 0.5 ml of pristane had been intraperitoneally administered about 1 week before the inoculation. Eight to ten days after the inoculation, the ascites fluids were taken up. An antibody was purified from each ascites fluid by affinity chromatography with the use of a protein G column.

[0071] The preparation of a polyclonal antibody was conducted as follows: A sequence ranging from the amino acid No. 245 to the amino acid No. 260 was selected from the amino acid sequence of the ECDN protein described in sequence ID NO: 1. A peptide having the sequence thus selected was chemically synthesized and referred to as an antigen (sequence ID NO: 5). A complex of this antigen with Key Halle Limpet was prepared. Then, the complex was inoculated together with the complete adjuvant into a footpad of a rabbit four times at intervals of about two weeks to immunize the animal. Thus, an antibody was prepared. The antibody titer of the serum collected at each stage of the immunization was determined by the ELISA method. That is, the rabbit serum was introduced into a microplate coated with the antigen peptide and then reacted with the antigen peptide at room temperature for two hours. After washing the microplate, a goat anti-rabbit IgG antibody labeled with horse-radish peroxidase was added thereto. After reacting at room temperature for one hour and washing, an enzyme substrate solution was added to induce color development. Then the absorbance ($A_{405\text{-}490}$) was measured. As a result, five antiserum lots each showing an absorbance of 0.097 to 0.398 when used a 10,000-fold dilution of the serum were obtained .

(Example 9) Western blotting

[0072] By using the monoclonal antibody prepared in Example 8, the manner of existing of the ECDN protein in various tissues were analyzed by the Western blotting method in accordance with the conventional manner.

[0073] As a result, a normal ECDN protein of about 50 kilodalton (kDa) was observed in a normal tissue, while the over expression of an ECDN paucimolecular protein of about 40 kDa was observed in a colorectal cancer cell line, an esophageal cancer cell line and the HeLa cell line. Subsequently, the expressions of the ECDN protein and the ECDN paucimolecular protein were examined similarly by using breast cancer tissues, colorectal cancer tissues and normal tissues of the patients therewith. As a result, there were observed that the ECDN paucimolecular protein of about 40 kDa was expressed specific for cancer tissue in three colorectal cancer cases among seven cases and six breast cancer cases among nine cases, and that the expression of the normal ECDN protein lessened in these cancer tissues (see Fig. 1).

[0074] These results suggest that the ECDN paucimolecular protein with a low molecular weight is formed as the cancer proceeds, and therefore indicate that the detection of this abnormal protein (the ECDN paucimolecular protein) is applicable to the examination of cancer cells.

(Example 10) RT-PCR experiment

[0075] To study the mechanism of the formation of such the ECDN paucimolecular protein, mRNAs were isolated from five colorectal cancer cell lines, six esophageal cancer cell lines and normal tissues, and an RT-PCR experiment was conducted.

[0076] From mRNAs, single-strand cDNAs were prepared in a conventional manner. Three segments of which the coding regions overlapped one another were amplified by the PCR method with the use of three paris of primers (the sequences described in sequence ID NOs: 6 and 7, the sequences described in sequence ID NOs: 8 and 9, and the sequences described in sequence ID NOs: 10 and 11, respectively; when expressed in base Nos. as described in sequence ID NO: 1, sequence ID NO: 6 corresponds to a sense DNA of the base No. 206 to the base No. 227, sequence ID NO: 7 corresponds to an antisense DNA of the base No. 733 to the base No. 753, sequence ID NO: 8 corresponds to a sense DNA of the base No. 700 to the base No. 725, sequence ID NO: 9 corresponds to an antisense DNA of the base No. 1226 to the base No. 1244, sequence ID NO: 10 corresponds to a sense DNA of the base No. 1205 to the base No. 1226, and sequence ID NO: 11 corresponds to an antisense DNA of the base No. 1615 to the base No. 1634). As a result, none of the amplification products of the segments on the 5'-termial side and those on the 3'-terminal side originating from the cancer cell lines showed any difference in size, when compared with the amplification products originating from the normal tissues. However, when the center segments were amplified, in addition to the amplification product having a normal size, an amplification product having a shorter size which was never observed in amplification

products originating from normal tissues, was observed in all of the 11 cancer cell lines (see Fig. 2).

[0077] When the DNA sequence of this shorter amplification product was identified, it was proved that the 291 bases in the seventh exon (a portion corresponding to the base No. 387 to the base No. 677 in sequence ID NO: 1) in the DNA encoding the ECDN protein were deleted to be shortened. From this result, it was assumed that the variant mRNA specific for cancer cells would encode a protein of about 40 kDa in which 97 amino acids in the DNA binding domain were deleted. It is expected from such the fact that cancer cells can be detected by detecting the above-mentioned variant mRNA by using a manner such as the RT-PCR method and the hybridization method.

(Example 11) Immunohistochemical analysis

[0078] By comparing the result of the RT-PCR experiment with the result of the Western blotting, it was estimated that the ECDN paucimolecular protein lacking in the DNA binding domain would be accumulated in a caner cell line. To examine whether this abnormal protein existed in the cytoplasm or the nucleus, various cell lines were immunologically stained with the use of the monoclonal antibody.

[0079] As a result, staining was slightly observed over the whole of cells in normal tissues. In contrast, it was obtained such a result that the accumulation of a protein binding to the anti-ECDN antibody in a large amount in nucleoli was suggested. Since the results of the Western blotting in Example 9 clearly indicated that a large amount of the ECDN paucimolecular protein of about 40 kDa was expressed in cancer cell lines, it was assumed that the protein accumulated in the nucleoli might be the ECDN paucimolecular protein. Based on those described above, it is expected that the decision whether they are cancer cells or normal cells can be conducted by examining the accumulation of an abnormal protein (an ECDN paucimolecular protein) in nucleoli with the use of an antibody.

Sequence Listing

[0080]

Sequence ID NO: 1
Sequence length: 1979
Sequence type: nucleic acid
strandedness: double
Topology: linear
Molecule type: cDNA to mRNA
Original source

Organism: Homo sapiens

Immediate source

Library: human mammary gland cDNA library

Feature

Feature key: CDS

Location: 206..1591

Identification of the feature: experimental examination

Sequence description

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TTTTGAGGGT ATTTGAGTAG CGGCGGTGTG TCAGGGGCTA AAGAGGAGGA CGAAGAAAAG   60
CAGAGCAAGG GAACCCAGGG CAACAGGAGT AGTTCACTCC GCGAGAGGCC GTCCACGAGA  120
CCCCCGCGCG CAGCCATGAG CCCCGCCCCC CGCTGTTGCT TGGAGAGGGG CGGGACCTGG  180
AGAGAGGCTG CTCCGTGACC CCACC ATG TCC TCT CCT ACC ACG AGT TCC CTG     232
                           Met Ser Ser Pro Thr Thr Ser Ser Leu
                             1               5
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GAT ACC CCC CTG CCT GGA AAT GGC CCC CCT CAG CCT GGC GCC CCT TCT     280
Asp Thr Pro Leu Pro Gly Asn Gly Pro Pro Gln Pro Gly Ala Pro Ser
 10                  15                  20                  25
TCT TCA CCC ACT GTA AAG GAG GAG GGT CCG GAG CCG TGG CCC GGG GGT     328
Ser Ser Pro Thr Val Lys Glu Glu Gly Pro Glu Pro Trp Pro Gly Gly
                 30                  35                  40
CCG GAC CCT GAT GTC CCA GGC ACT GAT GAG GCC AGC TCA GCC TGC AGC     376
Pro Asp Pro Asp Val Pro Gly Thr Asp Glu Ala Ser Ser Ala Cys Ser
             45                  50                  55
ACA GAC TGG GTC ATC CCA GAT CCC GAA GAG GAA CCA GAG CGC AAG CGA     424
Thr Asp Trp Val Ile Pro Asp Pro Glu Glu Glu Pro Glu Arg Lys Arg
         60                  65                  70
AAG AAG GGC CCA GCC CCG AAG ATG CTG GGC CAC GAG CTT TGC CGT GTC     472
Lys Lys Gly Pro Ala Pro Lys Met Leu Gly His Glu Leu Cys Arg Val
     75                  80                  85
TGT GGG GAC AAG GCC TCC GGC TTC CAC TAC AAC GTG CTC AGC TGC GAA     520
Cys Gly Asp Lys Ala Ser Gly Phe His Tyr Asn Val Leu Ser Cys Glu
 90                  95                 100                 105
GGC TGC AAG GGC TTC TTC CGG CGC AGT GTG GTC CGT GGT GGG GCC AGG     568
Gly Cys Lys Gly Phe Phe Arg Arg Ser Val Val Arg Gly Gly Ala Arg
                110                 115                 120
CGC TAT GCC TGC CGG GGT GGC GGA ACC TGC CAG ATG GAC GCT TTC ATG     616
Arg Tyr Ala Cys Arg Gly Gly Gly Thr Cys Gln Met Asp Ala Phe Met
            125                 130                 135
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CGG CGC AAG TGC CAG CAG TGC CGG CTG CGC AAG TGC AAG GAG GCA GGG    664
Arg Arg Lys Cys Gln Gln Cys Arg Leu Arg Lys Cys Lys Glu Ala Gly
        140                 145                 150

ATG AGG GAG CAG TGC GTC CTT TCT GAA GAA CAG ATC CGG AAG AAG AAG    712
Met Arg Glu Gln Cys Val Leu Ser Glu Glu Gln Ile Arg Lys Lys Lys
    155                 160                 165

ATT CGG AAA CAG CAG CAG CAG GAG TCA CAG TCA CAG TCG CAG TCA CCT    760
Ile Arg Lys Gln Gln Gln Gln Glu Ser Gln Ser Gln Ser Gln Ser Pro
170                 175                 180                 185

GTG GGG CCG CAG GGC AGC AGC AGC TCA GCC TCT GGG CCT GGG GCT TCC    808
Val Gly Pro Gln Gly Ser Ser Ser Ser Ala Ser Gly Pro Gly Ala Ser
                190                 195                 200

CCT GGT GGA TCT GAG GCA GGC AGC CAG GGC TCC GGG GAA GGC GAG GGT    856
Pro Gly Gly Ser Glu Ala Gly Ser Gln Gly Ser Gly Glu Gly Glu Gly
            205                 210                 215

GTC CAG CTA ACA GCG GCT CAA GAA CTA ATG ATC CAG CAG TTG GTG GCG    904
Val Gln Leu Thr Ala Ala Gln Glu Leu Met Ile Gln Gln Leu Val Ala
        220                 225                 230

GCC CAA CTG CAG TGC AAC AAA CGC TCC TTC TCC GAC CAG CCC AAA GTC    952
Ala Gln Leu Gln Cys Asn Lys Arg Ser Phe Ser Asp Gln Pro Lys Val
    235                 240                 245

ACG CCC TGG CCC CTG GGC GCA GAC CCC CAG TCC CGA GAT GCC CGC CAG   1000
Thr Pro Trp Pro Leu Gly Ala Asp Pro Gln Ser Arg Asp Ala Arg Gln
250                 255                 260                 265
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CAA CGC TTT GCC CAC TTC ACG GAG CTG GCC ATC ATC TCA GTC CAG GAG   1048
Gln Arg Phe Ala His Phe Thr Glu Leu Ala Ile Ile Ser Val Gln Glu
                270                 275                 280

ATC GTG GAC TTC GCT AAG CAA GTG CCT GGT TTC CTG CAG CTG GGC CGG   1096
Ile Val Asp Phe Ala Lys Gln Val Pro Gly Phe Leu Gln Leu Gly Arg
            285                 290                 295

GAG GAC CAG ATC GCC CTC CTG AAG GCA TCC ACT ATC GAG ATC ATG CTG   1144
Glu Asp Gln Ile Ala Leu Leu Lys Ala Ser Thr Ile Glu Ile Met Leu
        300                 305                 310

CTA GAG ACA GCC AGG CGC TAC AAC CAC GAG ACA GAG TGT ATC ACC TTC   1192
Leu Glu Thr Ala Arg Arg Tyr Asn His Glu Thr Glu Cys Ile Thr Phe
    315                 320                 325

TTG AAG GAC TTC ACC TAC AGC AAG GAC GAC TTC CAC CGT GCA GGC CTG   1240
Leu Lys Asp Phe Thr Tyr Ser Lys Asp Asp Phe His Arg Ala Gly Leu
330                 335                 340                 345

CAG GTG GAG TTC ATC AAC CCC ATC TTC GAG TTC TCG CGG GCC ATG CGG   1288
Gln Val Glu Phe Ile Asn Pro Ile Phe Glu Phe Ser Arg Ala Met Arg
                350                 355                 360

CGG CTG GGC CTG GAC GAC GCT GAG TAC GCC CTG CTC ATC GCC ATC AAC   1336
Arg Leu Gly Leu Asp Asp Ala Glu Tyr Ala Leu Leu Ile Ala Ile Asn
            365                 370                 375

ATC TTC TCG GCC GAC CGG CCC AAC GTG CAG GAG CCG GGC CGC GTG GAG   1384
Ile Phe Ser Ala Asp Arg Pro Asn Val Gln Glu Pro Gly Arg Val Glu
        380                 385                 390
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GCG TTG CAG CAG CCC TAC GTG GAG GCG CTG CTG TCC TAC ACG CGC ATC   1432
Ala Leu Gln Gln Pro Tyr Val Glu Ala Leu Leu Ser Tyr Thr Arg Ile
    395                 400                 405

AAG AGG CCG CAG GAC CAG CTG CGC TTC CCG CGC ATG CTC ATG AAG CTG   1480
Lys Arg Pro Gln Asp Gln Leu Arg Phe Pro Arg Met Leu Met Lys Leu
410                 415                 420                 425

GTG AGC CTG CGC ACG CTG AGC TCT GTG CAC TCG GAG CAG GTC TTC GCC   1528
Val Ser Leu Arg Thr Leu Ser Ser Val His Ser Glu Gln Val Phe Ala
                430                 435                 440

TTG CGG CTC CAG GAC AAG AAG CTG CCG CCT CTG CTG TCG GAG ATC TGG   1576
Leu Arg Leu Gln Asp Lys Lys Leu Pro Pro Leu Leu Ser Glu Ile Trp
            445                 450                 455

GAC GTC CAC GAG TGAGGGGCTG GCCACCCAGC CCCACAGCCT TGCCTGACCA       1628
Asp Val His Glu
        460

CCCTCCAGCA GATAGACGCC GGCACCCCTT CCTCTTCCTA GGGTGGAAGG GGCCCTGGGC 1688
CGAGCCTGTA GACCTATCGG CTCTCATCCC TTGGGATAAG CCCCAGTCCA GGTCCAGGAG 1748
GCTCCCTCCC TGCCCAGCGA GTCTTCCAGA AGGGGTGAAA GGGTTGCAGG TCCCGACCAC 1808
TGACCCTTCC CGGCTGCCCT CCCTCCCCAG CTTACACCTC AAGCCCAGCA CGCAGTGCAC 1868
CTTGAACAGA GGGAGGGGAG GACCCATGGC TCTCCCCCCT AGCCCGGGAG ACCAGGGGCC 1928
TTCCTCTTCC TCTGCTTTTA TTTAATAAAA ACTAAAAACA GAAAAAAAAA A           1979
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Sequence ID NO: 2
Sequence length: 1688
Sequence type: nucleic acid
Strandedness: double
Topology: linear
Molecule type: cDNA to mRNA
Original source

Organism: omo sapiens

Sequence description

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TTTTGAGGGT ATTTGAGTAG CGGCGGTGTG TCAGGGGCTA AAGAGGAGGA CGAAGAAAAG   60
CAGAGCAAGG GAACCCAGGG CAACAGGAGT AGTTCACTCC GCGAGAGGCC GTCCACGAGA  120
CCCCCGCGCG CAGCCATGAG CCCCGCCCCC CGCTGTTGCT TGGAGAGGGG CGGGACCTGG  180
AGAGAGGCTG CTCCGTGACC CCACC ATG TCC TCT CCT ACC ACG AGT TCC CTG     232
                           Met Ser Ser Pro Thr Thr Ser Ser Leu
                             1               5
GAT ACC CCC CTG CCT GGA AAT GGC CCC CCT CAG CCT GGC GCC CCT TCT     280
Asp Thr Pro Leu Pro Gly Asn Gly Pro Pro Gln Pro Gly Ala Pro Ser
 10                  15                  20                  25
TCT TCA CCC ACT GTA AAG GAG GAG GGT CCG GAG CCG TGG CCC GGG GGT     328
Ser Ser Pro Thr Val Lys Glu Glu Gly Pro Glu Pro Trp Pro Gly Gly
                 30                  35                  40
CCG GAC CCT GAT GTC CCA GGC ACT GAT GAG GCC AGC TCA GCC TGC AGC     376
Pro Asp Pro Asp Val Pro Gly Thr Asp Glu Ala Ser Ser Ala Cys Ser
             45                  50                  55
ACA GAC TGG GGC GTC CTT TCT GAA GAA CAG ATC CGG AAG AAG AAG ATT     424
Thr Asp Trp Gly Val Leu Ser Glu Glu Gln Ile Arg Lys Lys Lys Ile
         60                  65                  70
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CGG AAA CAG CAG CAG CAG GAG TCA CAG TCA CAG TCG CAG TCA CCT GTG    472
Arg Lys Gln Gln Gln Gln Glu Ser Gln Ser Gln Ser Gln Ser Pro Val
     75                  80                  85

GGG CCG CAG GGC AGC AGC AGC TCA GCC TCT GGG CCT GGG GCT TCC CCT    520
Gly Pro Gln Gly Ser Ser Ser Ser Ala Ser Gly Pro Gly Ala Ser Pro
 90                  95                 100                 105

GGT GGA TCT GAG GCA GGC AGC CAG GGC TCC GGG GAA GGC GAG GGT GTC    568
Gly Gly Ser Glu Ala Gly Ser Gln Gly Ser Gly Glu Gly Glu Gly Val
                110                 115                 120

CAG CTA ACA GCG GCT CAA GAA CTA ATG ATC CAG CAG TTG GTG GCG GCC    616
Gln Leu Thr Ala Ala Gln Glu Leu Met Ile Gln Gln Leu Val Ala Ala
            125                 130                 135

CAA CTG CAG TGC AAC AAA CGC TCC TTC TCC GAC CAG CCC AAA GTC ACG    664
Gln Leu Gln Cys Asn Lys Arg Ser Phe Ser Asp Gln Pro Lys Val Thr
        140                 145                 150

CCC TGG CCC CTG GGC GCA GAC CCC CAG TCC CGA GAT GCC CGC CAG CAA    712
Pro Trp Pro Leu Gly Ala Asp Pro Gln Ser Arg Asp Ala Arg Gln Gln
    155                 160                 165

CGC TTT GCC CAC TTC ACG GAG CTG GCC ATC ATC TCA GTC CAG GAG ATC    760
Arg Phe Ala His Phe Thr Glu Leu Ala Ile Ile Ser Val Gln Glu Ile
170                 175                 180                 185

GTG GAC TTC GCT AAG CAA GTG CCT GGT TTC CTG CAG CTG GGC CGG GAG    808
Val Asp Phe Ala Lys Gln Val Pro Gly Phe Leu Gln Leu Gly Arg Glu
                190                 195                 200
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GAC CAG ATC GCC CTC CTG AAG GCA TCC ACT ATC GAG ATC ATG CTG CTA     856
Asp Gln Ile Ala Leu Leu Lys Ala Ser Thr Ile Glu Ile Met Leu Leu
            205                 210                 215

GAG ACA GCC AGG CGC TAC AAC CAC GAG ACA GAG TGT ATC ACC TTC TTG     904
Glu Thr Ala Arg Arg Tyr Asn His Glu Thr Glu Cys Ile Thr Phe Leu
        220                 225                 230

AAG GAC TTC ACC TAC AGC AAG GAC GAC TTC CAC CGT GCA GGC CTG CAG     952
Lys Asp Phe Thr Tyr Ser Lys Asp Asp Phe His Arg Ala Gly Leu Gln
    235                 240                 245

GTG GAG TTC ATC AAC CCC ATC TTC GAG TTC TCG CGG GCC ATG CGG CGG    1000
Val Glu Phe Ile Asn Pro Ile Phe Glu Phe Ser Arg Ala Met Arg Arg
250                 255                 260                 265

CTG GGC CTG GAC GAC GCT GAG TAC GCC CTG CTC ATC GCC ATC AAC ATC    1048
Leu Gly Leu Asp Asp Ala Glu Tyr Ala Leu Leu Ile Ala Ile Asn Ile
                270                 275                 280

TTC TCG GCC GAC CGG CCC AAC GTG CAG GAG CCG GGC CGC GTG GAG GCG    1096
Phe Ser Ala Asp Arg Pro Asn Val Gln Glu Pro Gly Arg Val Glu Ala
            285                 290                 295

TTG CAG CAG CCC TAC GTG GAG GCG CTG CTG TCC TAC ACG CGC ATC AAG    1144
Leu Gln Gln Pro Tyr Val Glu Ala Leu Leu Ser Tyr Thr Arg Ile Lys
        300                 305                 310

AGG CCG CAG GAC CAG CTG CGC TTC CCG CGC ATG CTC ATG AAG CTG GTG    1192
Arg Pro Gln Asp Gln Leu Arg Phe Pro Arg Met Leu Met Lys Leu Val
    315                 320                 325
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```
AGC CTG CGC ACG CTG AGC TCT GTG CAC TCG GAG CAG GTC TTC GCC TTG    1240
Ser Leu Arg Thr Leu Ser Ser Val His Ser Glu Gln Val Phe Ala Leu
330                 335                 340                 345
CGG CTC CAG GAC AAG AAG CTG CCG CCT CTG CTG TCG GAG ATC TGG GAC    1288
Arg Leu Gln Asp Lys Lys Leu Pro Pro Leu Leu Ser Glu Ile Trp Asp
                350                 355                 360
GTC CAC GAG TGAGGGGCTG GCCACCCAGC CCCACAGCCT TGCCTGACCA             1337
Val His Glu
        364
CCCTCCAGCA GATAGACGCC GGCACCCCTT CCTCTTCCTA GGGTGGAAGG GGCCCTGGGC 1397
CGAGCCTGTA GACCTATCGG CTCTCATCCC TTGGGATAAG CCCCAGTCCA GGTCCAGGAG 1457
GCTCCCTCCC TGCCCAGCGA GTCTTCCAGA AGGGGTGAAA GGGTTGCAGG TCCCGACCAC 1517
TGACCCTTCC CGGCTGCCCT CCCTCCCCAG CTTACACCTC AAGCCCAGCA CGCAGTGCAC 1577
CTTGAACAGA GGGAGGGGAG GACCCATGGC TCTCCCCCCT AGCCCGGGAG ACCAGGGGCC 1637
TTCCTCTTCC TCTGCTTTTA TTTAATAAAA ACTAAAAACA GAAAAAAAAA A           1688
```

Sequence ID NO: 3
Sequence length: 31
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
GACGGATCCA TGTCCTCTCC TACCACGAGT T 31

Sequence ID NO: 4
Sequence length: 29
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
CTAGAATTGG GAGGGTGGTC AGGAAGGC 29

Sequence ID NO: 5
Sequence length: 16
Sequence type: amino acid
Strandedness: single
Topology: linear
Molecule type: peptide
Sequence description

Asp Gln Pro Lys Val Thr Pro Trp Pro Leu Gly Ala Asp Pro Gln Ser

1 5 10 15

Sequence ID NO: 6
Sequence length: 22
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
ATGTCCTCTC CTACCACGAG TT 22

Sequence ID NO: 7
Sequence length: 21
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
CCTCAGTGTC AGTGTCAGCG T 21

Sequence ID NO: 8
Sequence length: 26
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
CCGGAAGAAG AAGATTCGGA AACAGC 26

equence ID NO: 9
Sequence length: 19
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
GTGGCACGTC CGGACGTCC 19

Sequence ID NO: 10
Sequence length: 22
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description
ACCTACAGCA AGGACGACTT CC 22

Sequence ID NO: 11
Sequence length: 20
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: another nucleic acid (synthetic DNA)
Sequence description

CGGAACGGAC TGGTGGGAGG 20

Claims

1. An ECDN paucimolecular protein that accumulates in the nucleoli of cancer cells, having an amino acid sequence which comprises the whole or a part of an amino acid sequence encoded by the nucleotide sequence described in sequence ID NO:2.

2. The ECDN paucimolecular protein according to claim 1 comprising the whole or a part of an amino acid sequence which is identical with the one encoded by the nucleotide sequence described in sequence ID NO:2, except that one or more amino acids are added to, deleted from or inserted into the amino acid sequence encoded by the nucleotide sequence described in sequence ID NO:2, or that one or more amino acids substitute for one or more amino acids in the amino acid sequence encoded by the nucleotide sequence described in sequence ID NO:2.

3. A DNA encoding an ECDN paucimolecular protein as described in claims 1 or 2 which has a nucleotide sequence comprising the whole or a part of the nucleotide sequence described in sequence ID NO:2.

4. The DNA according to claim 3 encoding an ECDN paucimolecular protein as described in claims 1 or 2 which has a nucleotide sequence comprising the whole or a part of a nucleotide sequence which is identical with the one described in sequence ID NO:2, except that one or more nucleotides are added to, deleted from or inserted into the nucleotide sequence described in sequence ID NO:2, or that one or more nucleotides substitute for one or more nucleotides in the nucleotide sequence described in sequence ID NO:2.

5. A vector comprising the DNA described in any of claims 3 or 4

6. A transformant carrying, transfected thereinto, a vector comprising the DNA described in any of claims 3 or 4.

7. A process for producing an ECDN paucimolecular which comprises culturing a transformant carrying, transfected thereinto, a vector comprising the DNA described in claims 3 or 4, and recovering an expression product thereof.

8. A cotransformant carrying, transfected thereinto, a vector comprising the DNA described in claims 3 or 4, and a reporter vector comprising a DNA respondent to the vector.

9. A cotransformant carrying, transfected thereinto, a vector which comprises a DNA encoding a chimeric ECDN paucimolecular protein obtained by replacing the DNA binding domain of the ECDN paucimolecular protein described in claims 1 or 2 with the DNA binding domain of a known steroid hormone receptor-like protein other than the ECDN protein, and a reporter vector comprising a DNA respondent to the vector.

10. A process for screening a compound capable of acting on the cotransformant which comprises using the cotransformant, the cotransformant carrying, transfected thereinto, a vector comprising the DNA described in claims 3 or 4, and a reporter vector comprising a DNA respondent to the vector.

11. A process for screening a compound capable of acting on the cotransformant which comprises using the cotransformant, the cotransformant carrying, transfected thereinto, a vector which comprises a DNA encoding a chimeric paucimolecular ECDN protein obtained by replacing the DNA binding domain of the ECDN protein described in claims 1 or 2 with the DNA binding domain of a known steroid hormone receptor-like protein other than the ECDN protein, and a reporter vector comprising a DNA respondent to the vector.

12. A process for screening a compound capable of binding to an ECDN paucimolecular protein which comprises using the ECDN paucimolecular protein described in claims 1 or 2.

13. A DNA probe which has a nucleotide sequence described in sequence ID NO:2.

14. Use of a DNA-Primer for the detection of an ECDN paucimolecular protein as described in the previous claims.

15. Use of a DNA-probe according to claim 13 for the analysis of the ECDN paucimolecular protein gene as described in the claims 3 or 4.

16. A process for analysing a gene of an ECDN paucimolecular protein as described in the claims 3 or 4 which comprises hybridizing the DNA probe or the DNA primers described in claims 13 and 14, respectively, with a subject DNA.

17. A process for testing cells which comprises assaying a mRNA of an ECDN paucimolecular protein as described in the previous claims in a subject tissue or subject cells by using the DNA-probe or the DNA-primers as described in claims 13 and 14, respectively.

18. A process for testing cells which comprises hybridizing the DNA probe described in claim 13 with a subject DNA to thereby assay the ECDN paucimolecular protein according to the previous claims.

19. A process for testing cells which comprises amplifying a subject mRNA by the RT-PCR method with the use of DNA primers as described in claim 14 for assaying the expression of a gene of an ECDN paucimolecular protein according to the previous claims.

20. A process for testing cells which comprises amplifying a subject mRNA by the RT-PCR method with the use of the DNA primer described in sequence ID NO:6 and the DNA primer described in sequence ID NO:7 and assaying the expression of a gene of an ECDN paucimolecular protein according to the previous claims.

21. A polyclonal antibody capable of binding site specifically to the ECDN paucimolecular protein described in claims 1 or 2.

22. A monoclonal antibody capable of binding site specifically to the ECDN protein described in claims 1 or 2.

23. A process for immunochemically assaying an ECDN paucimolecular protein which comprises using a polyclonal antibody or a monoclonal antibody which can bind site specifically to the ECDN paucimolecular protein described in claims 1 or 2.

24. A process for testing cells which comprises immunohistochemically staining a subject tissue or subject cells with the use of a polyclonal antibody or a monoclonal antibody which can bind site specifically to the ECDN paucimolecular protein described in claims 1 or 2, and determining the intracellular distribution of the ECDN paucimolecular protein.

25. A process for testing cells which comprises determining the amount of an ECDN paucimolecular protein expressed in a subject tissue or subject cells by using a polyclonal antibody or a monoclonal antibody which can bind site specifically to the ECDN paucimolecular protein described in claims 1 or 2.

26. An antisense DNA or an antisense RNA hybridisable specifically with a mRNA of an ECDN paucimolecular protein as described in the previous claims.

27. A ribozyme capable of cleaving specifically a mRNA of an ECDN paucimolecular protein as described in the previous claims.

28. A pharmaceutical for gene therapy which comprises, as the active component, a gene construction capable of expressing the antisense DNA or the antisense RNA described in claim 26, or the ribozyme described in claim 27.

Patentansprüche

1. Paucimolekulares ECDN-Protein, das in den Nukleoli von Krebszellen akkumuliert, mit einer Aminosäuresequenz, die die gesamte oder einen Teil der Aminosäuresequenz umfasst, die durch die Nukleotidsequenz codiert wird, die in SEQ ID NO: 2 beschrieben ist.

2. Paucimolekulares ECDN-Protein gemäss Anspruch 1, umfassend die gesamte oder einen Teil der Aminosäuresequenz, die identisch mit derjenigen ist, die durch die Nukleotidsequenz codiert wird, die in SEQ ID NO: 2 beschrieben ist, ausser dass eine oder mehrere Aminosäuren hinzugefügt, deletiert oder in die Aminosäuresequenz, die durch die Nukleotidsequenz, die durch SEQ ID NO: 2 beschrieben wird, inseriert sind, oder dass eine oder mehrere Aminosäuren eine oder mehrere Aminosäuren in der Aminosäuresequenz, die durch die Nukleotidsequenz, die in SEQ ID NO: 2 beschrieben wird, substituieren.

3. DNA, die ein paucimolekulares ECDN-Protein, wie in den Ansprüchen 1 oder 2 beschrieben, codiert, welche eine Nukleotidsequenz hat, die die gesamte oder einen Teil der Nukleotidsequenz, die in SEQ ID NO: 2 beschrieben ist, umfasst.

4. DNA gemäss Anspruch 3, codierend für ein paucimolekulares ECDN-Protein, wie in den Ansprüchen 1 oder 2 beschrieben, welche eine Nukleotidsequenz aufweist, die die gesamte oder einen Teil der Nukleotidsequenz umfasst, die mit derjenigen, die in SEQ ID NO: 2 beschrieben ist, identisch ist, ausser dass ein oder mehrere Nukleotide hinzugefügt, deletiert oder in die Nukleotidsequenz, die in SEQ ID NO: 2 beschrieben ist, inseriert sind, oder dass ein oder mehrere Nukleotide ein oder mehrere Nukleotide in der Nukleotidsequenz, die in SEQ ID NO: 2 beschrieben ist, substituieren.

5. Vektor, umfassend die DNA, die in einem der Ansprüche 3 oder 4 beschrieben ist.

6. Transformante, die einen darin transfizierten Vektor trägt, der die DNA umfasst, die in einem der Ansprüche 3 oder 4 beschrieben ist.

7. Verfahren zur Herstellung eines paucimolekularen ECDN-Proteins, welches das Kultivieren einer Transformante, die einen darin transfizierten Vektor trägt, der die DNA, die in den Ansprüchen 3 oder 4 beschrieben ist, umfasst, und Gewinnen des Expressionsprodukts daraus.

8. Cotransformante, die einen darin transfizierten Vektor, der die DNA, die in den Ansprüchen 3 oder 4 beschrieben ist, und einen Reporter, der eine DNA umfasst, die auf den Vektor anspricht, trägt.

9. Cotransformante, die einen darin transfizierten Vektor trägt, welcher eine DNA umfasst, die ein chimeres ECDN-paucimolekulares Protein codiert, das durch Ersetzen der DNA-bindenden Domäne des paucimolekularen ECDN-Proteins, das in den Ansprüchen 1 oder 2 beschrieben ist, durch eine DNA-bindende Domäne eines bekannten steroidhormonrezeptorartigen Proteins, das nicht ECDN-Protein ist, erhalten wird, und einen Reportervektor, der eine DNA umfasst, die auf diesen Vektor anspricht.

10. Verfahren zum Screenen einer Verbindung, die in der Lage ist, auf die Cotransformante zu wirken, welches die Verwendung der Cotransformante umfasst, wobei die Cotransformante einen darin transfizierten Vektor trägt, der die DNA umfasst, die in den Ansprüchen 3 oder 4 beschrieben ist, und einen Reportervektor, der eine DNA umfasst, die auf den Vektor anspricht.

11. Verfahren zum Screenen einer Verbindung, die in der Lage ist, auf die Cotransformante zu wirken, welches die Verwendung der Cotransformante umfasst, wobei die Cotransformante einen darin transfizierten Vektor trägt, welcher eine DNA umfasst, die ein chimeres paucimolekulares ECDN-Protein codiert, das durch Ersetzen der DNA-bindenden Domäne des ECDN-Proteins, das in den Ansprüchen 1 oder 2 beschrieben wurde, durch die DNA-bindende Domäne eines bekannten steroidhormonrezeptorartigen Proteins, das nicht das ECDN-Protein ist, erhalten wird, und ein Reportervektor, der eine DNA umfasst, die auf den Vektor anspricht.

12. Verfahren zum Screenen einer Verbindung, die in der Lage ist, an das paucimolekulare ECDN-Protein zu binden, welches die Verwendung des paucimolekularen ECDN-Proteins umfasst, das in den Ansprüchen 1 oder 2 beschrieben ist.

13. DNA-Sonde, die eine Nukleotidsequenz aufweist, die in SEQ ID NO: 2 beschrieben ist.

14. Verwendung eines DNA-Primers zur Detektion eines paucimolekularen ECDN-Proteins, das in den vorhergehenden Ansprüchen beschrieben wurde.

15. Verwendung einer DNA-Sonde gemäss Anspruch 13 zur Analyse des paucimolekularen ECDN-Proteingens, das in den Ansprüchen 3 oder 4 beschrieben wurde.

16. Verfahren zum Analysieren eines paucimolekularen ECDN-Proteingens, das in den Ansprüchen 3 oder 4 beschrieben wurde, welches die Hybridisierung der DNA-Sonde oder der DNA-Primer, die in den Ansprüchen 13 bzw. 14 beschrieben wurden, mit einer DNA umfasst.

17. Verfahren zum Testen von Zellen, das die Untersuchung der mRNA eines paucimolekularen ECDN-Proteins, das

in den vorhergehenden Ansprüchen beschrieben ist, in Geweben oder Zellen unter Verwendung der DNA-Sonde oder der DNA-Primer, die in den Ansprüchen 13 bzw. 14 beschrieben sind, umfasst.

18. Verfahren zum Testen von Zellen, welches die Hybridisierung der DNA-Sonde, die in Anspruch 13 beschrieben ist, mit einer DNA umfasst, um dadurch das paucimolekulare ECDN-Protein gemäss den vorhergehenden Ansprüchen zu testen.

19. Verfahren zum Testen von Zellen, das die Amplifizierung einer mRNA mit dem RT-PCR-Verfahren umfasst, unter Verwendung von DNA-Primern, die in Anspruch 14 beschrieben sind, zum Testen der Expression eines paucimolekularen ECDN-Proteingens gemäss den vorhergehenden Ansprüchen.

20. Verfahren zum Testen von Zellen, das die Amplifizierung einer mRNA mittels RT-PCR-Verfahren umfasst, unter Verwendung des DNA-Primers, der in SEQ ID NO: 6 beschrieben ist, und des DNA-Primers, der in SEQ ID NO: 7 beschrieben ist, und das Testen der Expression eines Gens des paucimolekularen ECDN-Proteins gemäss den vorhergehenden Ansprüchen.

21. Polyklonaler Antikörper, der in der Lage ist, locusspezifisch an das paucimolekulare ECDN-Protein, das in den Ansprüchen 1 oder 2 beschrieben ist, zu binden.

22. Monoklonaler Antikörper, der in der Lage ist, locusspezifisch an das ECDN-Protein, das in den Ansprüchen 1 oder 2 beschrieben ist, zu binden.

23. Verfahren zum immunochemischen Testen eines paucimolekularen ECDN-Proteins, das die Verwendung eines polyklonalen Antikörpers oder eines monoklonalen Antikörpers, der locusspezifisch an das paucimolekulare ECDN-Protein, das in den Ansprüchen 1 oder 2 beschrieben ist, binden kann, umfasst.

24. Verfahren zum Testen von Zellen, das das immunohistochemische Färben eines Gewebes oder von Zellen unter Verwendung eines polyklonalen Antikörpers oder eines monoklonalen Antikörpers, der locusspezifisch an das paucimolekulare ECDN-Protein, das in den Ansprüchen 1 oder 2 beschrieben ist, bindet, umfasst und Bestimmung der intrazellulären Verteilung des ECDN-paucimolekularen Proteins.

25. Verfahren zum Testen von Zellen, das die Bestimmung der Menge eines paucimolekularen ECDN-Proteins umfasst, das in einem Gewebe oder in Zellen exprimiert ist, unter Verwendung eines polyklonalen Antikörpers oder eines monoklonalen Antikörpers, die locusspezifisch an das in den Ansprüchen 1 oder 2 beschriebene paucimolekulare ECDN-Protein binden können.

26. Antisense-DNA oder Antisense-RNA, die spezifisch mit einer mRNA eines paucimolekularen ECDN-Proteins, das in den vorhergehenden Ansprüchen beschrieben ist, hybridisierbar sind.

27. Ribozym, das in der Lage ist, spezifisch die mRNA des paucimolekularen ECDN-Proteins, das in den vorhergehenden Ansprüchen beschrieben wurde, zu spalten.

28. Pharmazeutikum zur Gentherapie, welches als Wirkstoff eine Genkonstruktion umfasst, die in der Lage ist, die Antisense-DNA oder die Antisense-RNA, die in Anspruch 26 beschrieben ist, oder das Ribozym, das in Anspruch 27 beschrieben ist, zu exprimieren.

Revendications

1. Protéine paucimolécumaire ECDN qui s'accumule dans les nucléoles de cellules cancéreuses, ayant une séquence d'acides aminés qui comprend la totalité ou une partie d'une séquence d'acides aminés codée par la séquence nucléotidique décrite dans la séquence ID NO :2.

2. Protéine paucimolécumaire ECDN selon la revendication 1 comprenant la totalité ou une partie d'une séquence d'acides aminés qui est identique à celle codée par la séquence nucléotidique décrite dans la séquence ID NO :2, excepté que un ou plusieurs acides aminés sont ajoutés à, enlevés de, ou insérés dans la séquence d'acides aminés codée par la séquence nucléotidique décrite dans la séquence ID NO :2, ou que l'on substitue un ou plusieurs acides aminés à un ou plusieurs acides aminés dans la séquence d'acides aminés codée par la séquence nucléo-

tidique décrite dans la séquence ID NO :2.

3. ADN codant pour une protéine paucimolécumaire ECDN telle que décrite dans les revendications 1 ou 2, qui a une séquence nucléotidique comprenant la totalité ou une partie de la séquence nucléotidique décrite dans la séquence ID NO :2.

4. ADN selon la revendication 3, codant pour une protéine paucimolécumaire ECDN telle que décrite dans les revendications 1 ou 2, qui a une séquence nucléotidique comprenant la totalité ou une partie d'une séquence nucléotidique qui est identique à celle décrite dans la séquence ID NO :2, excepté que un ou plusieurs nucléotides sont ajoutés à, enlevés de, ou insérés dans la séquence nucléotidique décrite dans la séquence ID NO :2, ou que l'on substitue un ou plusieurs nucléotides à un ou plusieurs nucléotides dans la séquence nucléotidique décrite dans la séquence ID NO :2

5. Vecteur comprenant l'ADN décrit dans l'une quelconque des revendications 3 ou 4.

6. Transformant portant, transfecté à l'intérieur de celui-ci, un vecteur comprenant l'ADN décrit dans l'une quelconque des revendications 3 ou 4.

7. Procédé destiné à produire un ECDN paucimoléculaire, qui comprend la culture d'un transformant portant, transfecté à l'intérieur de celui-ci, un vecteur comprenant l'ADN décrit dans les revendications 3 ou 4, et la récupération d'un produit d'expression de celui-ci.

8. Cotransformant portant, transfecté à l'intérieur de celui-ci, un vecteur comprenant l'ADN décrit dans les revendications 3 ou 4, et un vecteur reporter comprenant un ADN répondant au vecteur.

9. Cotransformant portant, transfecté à l'intérieur de celui-ci, un vecteur comprenant un ADN codant pour une protéine paucimoléculaire ECDN chimère obtenue en remplaçant le domaine de liaison à l'ADN de la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2 par le domaine de liaison à l'ADN d'une protéine de type récepteur d'hormones stéroidiennes connue autre que la protéine ECDN, et un vecteur reporter comprenant un ADN répondant au vecteur.

10. Procédé destiné à cribler un composé capable d'agir sur le cotransformant, qui comprend l'utilisation du cotransformant, le cotransformant portant, transfecté à l'intérieur de celui-ci, un vecteur comprenant l'ADN décrit dans les revendications 3 ou 4, et un vecteur reporter comprenant un ADN répondant au vecteur.

11. Procédé destiné à cribler un composé capable d'agir sur le cotransformant qui comprend l'utilisation du cotransformant, l'agent cotransformant portant, transfecté à l'intérieur de celui-ci, un vecteur comprenant un ADN codant pour une protéine paucimoléculaire ECDN chimère obtenue en remplaçant le domaine de liaison à l'ADN de la protéine ECDN décrite dans les revendications 1 ou 2 avec le domaine de liaison à l'ADN d'une protéine de type récepteur d'hormones stéroidiennes connue autre que la protéine ECDN, et un vecteur reporter comprenant un ADN répondant au vecteur.

12. Procédé destiné à cribler un composé capable de se lier à une protéine paucimoléculaire ECDN, qui comprend l'utilisation de la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2.

13. Sonde ADN qui a une séquence nucléotidique décrite dans la séquence ID NO :2.

14. Utilisation d'une amorce ADN pour la détection d'une protéine paucimoléculaire ECDN comme décrite dans les revendications précédentes.

15. Utilisation d'une sonde ADN selon la revendication 13 pour l'analyse du gène d'une protéine paucimoléculaire ECDN comme décrit dans les revendications 3 ou 4.

16. Procédé destiné à analyser un gène d'une protéine paucimoléculaire ECDN comme décrit dans les revendications 3 ou 4, qui comprend l'hybridation de la sonde ADN ou des amorces ADN décrites dans les revendications 13 et 14, respectivement avec un ADN sujet.

17. Procédé destiné à tester des cellules, qui comprend le dosage d'un ARNm d'une protéine paucimoléculaire ECDN

comme décrite dans les revendications précédentes dans un tissu sujet ou des cellules sujettes en utilisant la sonde ADN ou les amorces ADN comme décrites dans les revendications 13 et 14, respectivement.

18. Procédé destiné à tester des cellules, qui comprend l'hybridation de la sonde ADN décrite dans la revendication 13 avec un ADN sujet afin de doser ainsi la protéine paucimoléculaire ECDN selon les revendications précédentes.

19. Procédé destiné à tester des cellules, qui comprend l'amplification d'un ARNm sujet par le procédé TI-ACP avec l'utilisation des amorces ADN comme décrites dans la revendication 14 afin de doser l'expression d'un gène d'une protéine paucimoléculaire ECDN selon les revendications précédentes.

20. Procédé destiné à tester des cellules, qui comprend l'amplification d'un ARNm sujet par le procédé TI-ACP avec l'utilisation de l'amorce ADN décrite dans la séquence ID NO :6 et de l'amorce ADN décrite dans la séquence ID NO :7 et le dosage de l'expression d'un gène d'une protéine paucimoléculaire ECDN selon les revendications précédentes

21. Anticorps polyclonal capable de se lier spécifiquement à un site, à la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2.

22. Anticorps monoclonal capable de se lier spécifiquement à un site, à la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2.

23. Procédé destiné à analyser par immunochimie une protéine paucimoléculaire ECDN, qui comprend l'utilisation d'un anticorps polyclonal ou d'un anticorps monoclonal qui peuvent se lier spécifiquement à un site, à la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2.

24. Procédé destiné à tester des cellules, qui comprend la coloration immunohistochimique d'un tissu sujet ou de cellules sujettes avec l'utilisation d'un anticorps polyclonal ou d'un anticorps monoclonal qui peuvent se lier spécifiquement à un site, à la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2, et la détermination de la distribution intracellulaire de la protéine paucimoléculaire ECDN.

25. Procédé destiné à tester des cellules, qui comprend la détermination de la quantité d'une protéine paucimoléculaire ECDN exprimée dans un tissu sujet ou cellules sujettes par l'utilisation d'un anticorps polyclonal ou d'un anticorps monoclonal qui peuvent se lier spécifiquement à un site, à la protéine paucimoléculaire ECDN décrite dans les revendications 1 ou 2.

26. ADN antisens ou ARN antisens que l'on peut hybrider spécifiquement avec un ARNm d'une protéine paucimoléculaire ECDN comme décrite dans les revendications précédentes.

27. Ribozyme capable de cliver spécifiquement un ARNm d'une protéine paucimoléculaire ECDN comme décrite dans les revendications précédentes.

28. Produit pharmaceutique pour thérapie génique, qui comprend, en tant que composé actif, une construction de gène capable d'exprimer l'ADN antisens ou l'ARN antisens décrits dans la revendication 26, ou le ribozyme décrit dans la revendication 27.

Drawings

FIG. 1

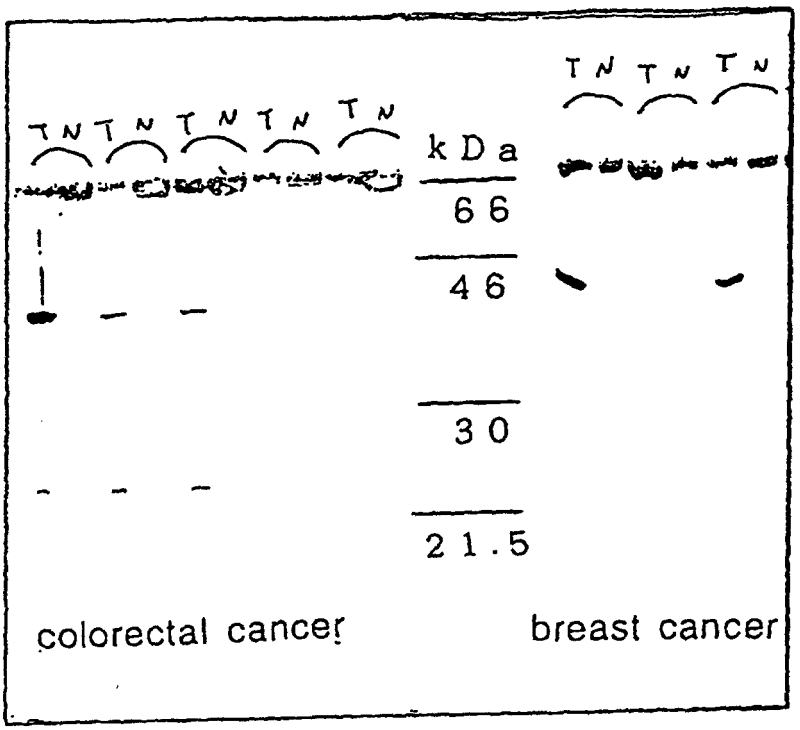

FIG. 2