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(54) **LABELLED LIGANDS OF ANTI-MULLERIAN HORMONE FOR THE DIAGNOSIS OF ENDOMETRIOSIS**

MARKIERTE LIGANDEN DES ANTI-MÜLLER-HORMONS ZUR DIAGNOSE VON ENDOMETRIOSE

LIGANDS MARQUÉS DE L'HORMONE ANTI-MÜLLÉRIENNE POUR LE DIAGNOSTIC DE L'ENDOMÉTRIOSE

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- **GRIFFIN Y ET AL: "Radiology of Benign Disorders of Menstruation", SEMINARS IN ULTRASOUND, CT, AND MR, GRUNE AND STRATTON, ORLANDO, FL, US, vol. 31, no. 5, 1 October 2010 (2010-10-01), pages 414-432, XP027421415, ISSN: 0887-2171, DOI: 10.1053/J.SULT.2010.08.001 [retrieved on 2010-10-01]**

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Description

[0001] The present invention relates to isolated ligands of anti-Mullerian hormone marked so as to be directly detectable by means of imaging techniques in the endometriosis lesions. In particular, such ligands can be used in a method for the *in vivo* diagnosis of endometriosis wherein said method comprises a passage of localizing and/or evaluating the entity of the endometriosis lesions in a patient.

STATE OF PRIOR ART

[0002] Endometriosis is defined as a recurrent and benign gynaecological disorder characterized by the presence of endometrial tissue (glands and stroma) outside the cavity of uterus. It is one of the most common diseases in the gynaecological field, affecting about 10% of the female population in reproductive age, whereas its frequency rises up to 20-50% in women with fertility problems (Baldi A. et al. Endometriosis: pathogenesis, diagnosis, therapy and association with cancer. Oncology Reports 2008;19:843-846).

[0003] The endometriotic neoformations mainly are localized on the pelvic peritoneum and ovaries, but they can be commonly found in the sub-peritoneal areas and, more rarely, in any anatomic district, such as for example pericardium, pleurae, pulmonary parenchyma and even brain (Giudice LC , and Kao LC: Endometriosis. The Lancet, 364: 1789-1799, 2004; Signorile PG et al.. Rectovaginal septum endometriosis: an immunohistochemical analysis of 62 cases. In Vivo 2009;23,459-464).

[0004] The pathogenesis of such disease is still unacknowledged; the most reliable hypotheses are retrograde menstruation and coelomic metaplasia (Gazvani R. & Templeton A. New considerations for the pathogenesis of endometriosis. Int. J. Gynaecol. Obstet. 2002; 76:117-126.; Slater M. et al. Endometriotic cells exhibit metaplastic change and oxidative DNA damage as well as decreased function, compared to normal endometrium. J. Mol. Histol. 2005; 36:257-263;. Starzinski-Powitz A. et al. In search of pathogenic mechanisms in endometriosis: the challenge for molecular cell biology. Curr. Mol. Med. 2001; 1:655-664.).

[0005] Recently the presence of endometriosis lesions in the female foetus has been described and this represents the first demonstration of a different pathogenetic theory based upon defects of embryogenesis (Signorile PG, Baldi A: Endometriosis: new concepts in the pathogenesis. Int J Biochem Cell Biol 2010; 42:778-780).

[0006] The anti-Mullerian hormone (AMH) is a glycoprotein belonging to the superfamily of the "Transforming Growth Factor-beta" (TGF-beta). The AMH is produced by the cells of the Sertoli in the male foetus and it is responsible for the regression of the Mullerian ducts (La Marca A et al.: Anti-Mullerian hormone (AMH): what do we still need to know? Hum Reproduct, 24: 2264-2275, 2009). The AMH expression in the ovarian follicles starts in the female foetus, around the 32th week of gestation and keeps for the woman's whole fertile life. The AMH expression levels are considered good indicators of a woman's ovarian reserve; they decay with menopause (Lee MM et al.: Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab, 81: 571-576, 1996). Furthermore, an anti-cancer action for AMH has been proposed in the ovarian epithelial tumours and different experimental proofs seem supporting the cytotoxic effect on tumour cells (La Marca A., Volpe A: The anti-Mullerian Hormone and ovarian cancer. Hum Reproduct., 13: 265-273, 2007). Recent studies have demonstrated that AMH, as well as a receptor thereof (MISRII), are expressed in the adult woman even at the level of endometrium, wherein probably they perform a function of paracrine type.

WO 2009/052119 discloses the treatment of endometriosis with a Mullerian Inhibiting Substance (MIS), but not the diagnosis of endometriosis with an anti-Mullerian hormone ligand (*see also* Namkung J. et al.: "Mullerian inhibiting substance induces apoptosis of human endometrial stromal cells in endometriosis", J Clin Endocrinol Metab. 2012 Sep; 97(9)). Moreover, the detection of endometriotic lesions by MRI has been disclosed in the literature (Griffin Y. et al.: "Radiology of Benign Disorders of Menstruation", Seminars in ultrasound, CT and MR 2010 Oct; 31(5):414-32), but not with an anti-Mullerian hormone ligand.

[0007] Up to now no marker has been described allowing localizing exactly *in vivo* the endometriosis lesions, both cystic and connective solid ones. In particular, several of the endometriotic neoformations can even have very reduced sizes (smaller than 1 cm), which makes practically impossible, with the currently available analysis methods, to highlight *in vivo* the localization both of cystic endometriosis lesions smaller than two millimetres and of the connective solid lesions smaller than one centimetre.

[0008] Still nowadays endometriosis is a disease therefor the one and only effective therapeutic strategy is the surgical removal of the endometriosis lesions: there is no resolving pharmacological therapy and the only pharmacological treatments used by the medical-scientific community are able only to act on symptoms, by relieving them. However, the success of the surgical procedure is substantially based upon the possibility of displaying *in vivo* the endometriosis lesion, which display is strictly connected even to the size of the lesion itself. It follows that, the effectiveness of the surgical treatment is limited by the fact that, as the disease is multicentric and often microscopic, the surgeon not always succeeds in eliminating all disease foci.

[0009] Therefore, in the state of art it is highly felt the need of detecting procedures allowing to obtain a precise picture regarding the localization and the sizes of the disease's different foci endometriotic formations) in the patient, so as to be able to diagnose and intervene in the most effective way in patients with endometriosis even

in the states wherein the lesions have very reduced sizes.

[0010] The scope of the present invention is to overcome the problems associated to the detection of the endometriotic formations and, in particular, of the endometriotic neoformations, so as to develop alternative methods for diagnosing and/or treating endometriosis.

SUMMARY OF THE INVENTION

[0011] The present invention is defined in the claims and relates to isolated ligands of anti-Mullerian hormone marked so as to be directly detected in the endometriosis lesions by means of magnetic resonance imaging. In particular, such ligands can be used in an *in vivo* method for diagnosing endometriosis including a passage of localizing and/or evaluating the entity of the endometriosis lesions in a patient.

[0012] The invention subject of the present description is based upon the scientific observation, made by the inventors themselves, that the anti-Mullerian hormone (AMH) is over-expressed in the endometriosis lesions as shown in figure 1. From such observation derives the intuition of the inventors of being able to use AMH as target to detect foci (formations and/or neoformations) of the endometriosis disease.

[0013] In particular, as highlighted in the section "Examples", it was demonstrated that AMH can be used in an effective way as cellular target to allow to detect *in vivo* the exact localization of the endometriosis lesions. In fact, as shown in example 2 herebelow, a xenotransplant of human endometriotic tissue in nude mice can be subsequently displayed by means of using a marked ligand such as, for example, a marked anti-AMH antibody so as to be able to be detected by means of *in vivo* magnetic resonance imaging techniques (figures 2 and 3).

In particular, the use of a ligand able to link the AMH, marked so as to be able to be detected by means of magnetic resonance imaging *in vivo* techniques, demonstrated to be effective in detecting not only the endometriosis lesions with appreciable sizes but even anatomic localizations of endometriosis with small sizes, lower than 0.5 centimetres of diameter. These data suggest that the ligand of the invention can be used advantageously not only for localizing the lesions but even for evaluating, by means of the sizes of the lesions themselves, the entity/gravity of endometriosis.

It follows that the ligand of the invention can be used even for learning the real extension of the endometriosis disease since the intra-organ lesions, which are not detectable by surgery, can be effectively localized too before performing the operation. From what said above, it appears clear that the use of the anti-AMH antibody of the invention can make the passages of diagnosing the endometriosis and/or surgical treatment of the pathology more selective and effective by defining the precise localization and extension of the endometriosic lesions.

Furthermore, as the here described approach is not invasive since it mainly consists in administrating the ligand able to link the AMH to the patient and displaying *in vivo* the sites wherein it accumulates as consequence of the link to the AMH deposits, the ligand of the invention can be advantageously used even to monitor in time the progress of the endometriosis pathology such as, for example but not only, in case the patient is subjected to schemes of pharmacological and/or surgical therapies.

[0014] Therefore the subject of the present application is:

- the isolated ligand of anti-Mullerian hormone suitable to be detected directly by means of magnetic resonance imaging for use in a *in vivo* method of diagnosing endometriosis comprising a passage of localization and/or evaluation of the entity of the endometriosis lesions in a patient;
- a formulation for use in a *in vivo* method of localizing and/or evaluating the entity of the endometriosis lesions in a patient comprising a ligand according to the invention and at least pharmaceutically acceptable carrier and/or excipient;
- a kit for use in a *in vivo* method for localizing and/or evaluating the entity of the endometriosis lesions in a patient comprising at least a ligand of the invention or a formulation of the invention and means useful to administer said ligand or said formulation to said patient.

[0015] In the present invention, the ligand of the anti-Mullerian hormone is an antibody (anti-AMH antibody).

[0016] Additional advantages, as well as the features and the use modes of the present invention will result evident from the following detailed description of some preferred embodiments, shown purely by way of example.

DETAILED DESCRIPTION OF THE FIGURES

[0017]

Figure 1A e 1B: Examples of the AMH hormone expression in the in vivo endometriotic structures, by means of immunohistochemistry method; the AMH expression is detected by the colouring of intense dark colour.

Figure 2A and 2B: total-body Magnetic Resonance Image of a small female mouse before (figure 2A) and after (figure 2B) the inoculation of gadolinium-conjugated antibody against AMH: the area corresponding to the sub-cutaneous ectopic transplant of connective solid endometriosic tissue and the tail area wherein the inoculation of the gadolinium-antibody compound for AMH took place are circled in white,

Figure 3A e 3B: Magnetic Resonance Image in cross-section of a female mouse before (figure 3A) and after (figure 3B) the inoculation of gadolinium-conjugated antibody against AMH: the area corre-

sponding to the ectopic transplant is circled in white, **Figure 4A-D:** histological and immunohistochemical analysis of the transplanted tissue. Figures A and B show the histological structure of the transplant with colouring by means of Hematoxylin-Van Gieson and Hematoxylin-Eosin; figures C and D show the expression (immunohistochemical colouring of intense black colour), respectively of CD10 and AMH in the transplanted tissue.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention, as already shown in the previous section, relates to an isolated ligand of the marked anti-Mullerian hormone (AMH) able to be detected by means of magnetic resonance imaging in the endometriosic lesions. Under ligand of the anti-Mullerian hormone of the present disclosure a natural or synthetic molecule is meant, able to link, preferably with high affinity, at least a specific epitope of AMH protein. In the context of the present invention and as it is defined in the claims, the isolated ligand consists of an antibody to the anti-Mullerian hormone (Ab anti AMH).

[0019] As it is known to the person skilled in the art the term epitope or antigenic determinant relates to a site on the antigen, in this case the AMH protein, which is specifically recognized and linked by an immunoglobulin. The epitopes can be formed by a sequence of contiguous aminoacids or by juxtaposed aminoacids in the three-dimensional shape of the protein. Preferably the ligand of the present invention is able to link an AMH epitope not present in other proteins, so as to avoid the aspecific link with proteins different from the anti-Mullerian hormone.

[0020] The anti-Mullerian hormone is a glycated protein of homodimeric 140 kDa belonging to the superfamily of the "Transforming Growth Factor-beta" (TGF-beta). The nucleotidic sequence and the coding aminoacidic sequence for the AMH of different origins (human, murine, bovine) is described in the known state of art. In particular, the aminoacid sequence of the monomeric AMH of human origin (sequence of 535 aa) is described in the bank UniProtKB/Swiss-Prot, version 133, last modification 16 May 2012; http://www.uniprot.org/) and identified with number P03971.

[0021] Preferably, the ligand of the invention is able to recognize and link an epitope of the human anti-Mullerian hormone.

[0022] The ligand of the invention can be an antibody able to link in specific way at least an epitope of the AMH hormone. (The Mullerian duct: recent insights into its development and regression Klattig J, Englert C. Sex Dev. 2007;1(5):271-8).

[0023] Under the term "antibody" in the present invention complete antibodies, antibodies with single chain, synthetic antibodies, chimeric antibodies, humanizing antibodies, not human antibodies, conjugates of antibodies and fragments or their derivatives are meant. In particular, under "complete antibodies" in the present invention proteins or glycoproteins are referred to, comprising at least two heavy chains and at least two light chains inter-connected by means of disulphide bridges. Each heavy chain is composed of a variable region (V_H) and a constant region (C_H). The constant region (C_H) comprises three domains C_H1, C_H2 and C_H3.

Each light chain is composed of a variable region (V_L) and a constant region (C_L). The variable regions of the heavy chain (V_H) and of the light chain (V_L) can be further divided into hyper-variable regions known as "Complementarity determining regions" (CDR). Such regions CDR are hypervariable with respect to the more preserved regions known as Framework region (FR). Each V_H and V_L is composed of 3 CDR and four FR, arranged from the terminal amino end to the terminal carboxy end in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the light and heavy chains contain the domain interacting with the antigen, by linking it. By way of pure example, such fragment can be: a Fab fragment consisting of the domains V_L,V_H,C_L and C_H1; a fragment consisting of the domains V_H and C_H1; a Fv fragment consisting of domains V_L and V_H; a fragment consisting of a single variable domain isolated by a CDR region; F(ab')2 fragment comprising two linked fragments Fab; Fv molecules with single chain wherein a domain V_L and V_H are linked by a connecting peptide promoting the association between the two domains so as to form a linkage site for the antigen. Examples of possible forms and structures of the antibodies are described in Holliger&Hudson (2006) Nature Biotechnology 23(9): 1126-1136; Carter (2006) Nature Reviews Immunology 6:343:357.

[0024] In the present invention for which the ligand is an antibody, alternative embodiments can be provided such as a human, humanized, murine, chimeric, rabbit, sheep antibody or however of any origin provided that it is able of recognizing and linking at least an epitope of the AMH hormone. Under the term "humanized" an antibody is meant comprising a human framework region (FR) and one or more regions determining the complementarity (CDR) of not human origin, for example murine. In a preferred embodiment of the invention, the antibody is of human origin. By purely way of example an antibody able to recognize the human AMH is the one commercialized by ABCAM, # cat. ab103233, MIS Antibody(C-20): sc-6886 of Santa Cruz; antibody against AMH # cat (MM0475-7H26) of Novus Biologicals; hormone against AMh # cat AM05878SU-N, of Acris Antibodies.

[0025] Furthermore, the antibody, able to link AMH, can be both a recombinant protein and a protein usually present in nature.

Under recombinant protein a molecule is meant which is produced in organisms and host cells which do not produce naturally the interest protein, for example an anti-AMH antibody.

The antibody can be both a monoclonal and a polyclonal antibody or, as it is known to the person skilled in the art,

an antibody with a single linking specificity or obtained from antibodies produced by different colonies of lymphocytes B.

[0026] The ligand of the AMH subject of the present invention is an isolated ligand marked so as to be directly detected by means of magnetic resonance imaging in the endometriosis lesions.

[0027] Under the term "isolated" in the present invention ligands in substantially free form are meant, for example in case of ligands present in the cells, free from any cellular material. In case of an anti-AMH antibody, instead, it will be free from antibodies having different antigenic specificity.

[0028] In particular, the detection of the marked ligand by means of magnetic resonance imaging can be performed by using techniques such as for example, and without being limited thereto: ecography, radiography, computed tomography, nuclear magnetic resonance, tomography with emission of positrons, scintigraphy or however any other imaging method useful to detect the antibody of the invention.

Such techniques are well known to the person skilled in the art and therefore do not request herein further examinations. A description of the magnetic resonance imaging techniques useful to the purpose of the present invention is however present in Sutton's Textbook of Radiology & Imaging 7th Edition, published by Churchill Livingstone.

[0029] To the detection purpose, the ligand can be marked by using any agent suitable to the detection by means of magnetic resonance imaging and, as it will be understood, the type of agent used to mark the ligand mainly will depend upon the type of techniques which will be chosen for displaying the endometriosis lesions. In the context of the invention and as defined in the claims, the ligand is marked with at least one of the agents chosen in the group comprising: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents, radioisotopes.

[0030] By purely way of example and not for limitative purposes, the paramagnetic contrast agents can be chosen among: gadolinium or manganese; the iodized contrast agents can be chosen among: iohexol, ioversol, iopromide, iopamidol, iodixanol; the intravenous contrast agents can be chosen among: sulphur hexafluoride; the radioisotopes can be chosen among: Technetium 99, Iodine 131, Thallium 201, Iodine 125, Fluorine 18, Carbon 14.

In particular, for the Nuclear Magnetic resonance, the ligand of the invention can be marked for example with: gadodiamide (Omniscan®), gadobenic acid (Multihance®), gadobutrol (Gadovist®), gadofosveset (Vasovist®), gadopentetic acid (Magnevist®), gadoteric acid (Dotaren®), gadoteridol (Prohance®) and gadoxetic acid (Primovist®). For the detection by means of the computerized tomography, iodized agents can be used such as: monomers such as ioexolo (Omnipaque®), ioversolo (Optiray®), iopromide (Ultravist®), iopamidol (for example Iopamiro®) or dimers such as iodixanol (Visipaque®). For the ecography, the ligand of the invention for example can be marked with intravenous contrast agents constituted by microbubbles of sulphur hexafluoride or other graphic contrast agents for ultrasounds.

In a preferred embodiment of the invention, the ligand is a polyclonal or monoclonal antibody able to recognize and link the AMH of human origin marked with gadolinium. The marking and conjugation of a protein with a detecting agent, such as those shown above, nowadays is performed by means of techniques well known to the person skilled in the art. By way of example, the methods which can be used for conjugating or marking the ligand of the invention are described in Kuriu Y et al. Monoclonal antibody conjugated to gadolinium as a contrast agent for magnetic resonance imaging of human rectal carcinoma. J Surg Oncol. 2006 Aug 1;94(2):144-148; and Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013 (available in: http://www.ncbi.nlm.nih.gov/books/NBK23053/).

[0031] As shown previously, the herein described ligand demonstrated to be useful in particular to the purpose of localizing and/or evaluating the entity of the endometriosis lesions directly *in vivo* in a patient suffering from, or supposed suffering from, endometriosis. Under endometriosis lesion, analogously to what reported in literature, the presence of an endometrial tissue, both glandular and stromal tissue, outside the cavity of uterus is meant. In the specific case, the anti-AMH antibody of the invention allows detecting *in vivo* both cystic and connective solid endometriosis lesions. The evaluation of the entity of the lesions, in this case, substantially relates to the analysis to the purposes of diagnosing or treating the size of the foci of the endometriosis disease. The size of the endometriosis lesions can be very variable and in case of neoformed lesions the sizes can be so reduced that they do not allow the localization thereof by the physician. Advantageously the ligand of the invention allows displaying even endometriosis lesions with diameter smaller or larger than 1 centimetre and 0.5 centimetre. Under evaluation of the entity of the endometriosis lesions herein the physician's determination of the sizes and/or the spreading level of the disease foci is meant in order to understand the endometriosis progress stage and, in case, to define the best therapeutic approach to be followed.

[0032] The subject of the present invention then is also a formulation for use in an *in vivo* method for localizing and/or evaluating the entity of the endometriosis lesions in a patient comprising at least a ligand of the invention, wherein said ligand is marked with at least one of the agents chosen in the group consisting of: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents, and radioisotopes, and at least a pharmaceutically acceptable carrier and/or excipient. In a preferred embodiment the formulation is administered to the patient wherein one wants to localize and/or evaluate the

entity of the endometriosis lesions, by injection or infusion or even in case by means of oral administration. A pharmaceutically acceptable carrier can be chosen, for example, among buffer aqueous solutions, sterile water, balanced saline physiological solutions, ions, additives. By pure way of example, the buffer aqueous solutions can be chosen among tris (hydroxyethyl) amino methane and the salts, phosphate, citrate and bicarbonates; the balanced ionic solutions, instead, can be selected among chlorides and bicarbonates of cations chosen among Ca, Na, K, Mg and other halides, carbonates, sulphates, phosphates and Na, K, Mg and Ca; the excipients can be chosen among glycerol, polyethylene glycol, and dextran. In any case, the carriers and the excipients which can be comprised in the formulation of the invention can be chosen among those commonly known and considered useful by the person skilled in the art for the present invention.

[0033] The subject of the present invention is also a kit for for use in an *in vivo* method localizing and/or evaluating the entity of the endometriosis lesions in a patient comprising at least a ligand of the invention or a formulation of the invention as defined above, wherein the ligand is marked with at least one of the agents chosen in the group consisting of: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents and radioisotopes, and means useful for the administration of said ligand or said formulation to the patient. By purely way of example, such means can comprise physiological solutions, needles, syringes, sterilizing solutions, etc.

Furthermore, herein also an *in vivo* method is described for localizing and/or evaluating the *in vivo* entity of the endometriosis lesions in a patient comprising a passage of administering the marked ligand or the marked formulation of the invention to the patient itself. As already designated previously, under localization the possibility of detecting precisely the site wherein there is the endometriosis lesion is meant, whereas under evaluation substantially the analysis of the sizes of the localized lesions is referred to. From this point of view, then, the *in vivo* method can even include an operating passage wherein the subject, thereto the ligand or the formulation of the invention was administered, is subsequently subjected to a technique of magnetic resonance imaging. By pure way of example and not for limitative purposes, such techniques can be: ecography, radiography, computed tomography, nuclear magnetic resonance, tomography with emission of positrons.

The just described method can be performed, if the person skilled in the art can consider to be useful, even on *in vitro* tissue samples and in this case then the *in vitro* method for localizing and/or evaluating the entity of the endometriosis lesions will include a passage of incubating a tissue sample, obtained from the patient under analysis, with a ligand or a formulation of the invention. In a way analogous to what described above, the sample can be subsequently subjected to a technique of magnetic resonance imaging with the purpose of allowing to display the site and the sizes of the endometriotic disease foci. In vitro methods are however not part of the claimed invention.

EXAMPLES

Example 1. *In vivo expression of AMH hormone in the* endometriosis *lesions by means of immunohistochemical methods.*

[0034] This experiment represents the first scientific demonstration of the fact that the AMH hormone is clearly and abundantly expressed in the endometriosis lesions, both in the glandular and in the stromal component. For this demonstration, collections of tissue were performed at the sub-peritoneal level from 10 patients affected by endometriosis; the tissues were fixed in 10%-buffered paraformaldehyde, included in paraffin and coloured with Hematoxylin and Eosin to highlight the glandular and stromal structures of endometriosis. On storied sections, immediately subsequent to the ones coloured with Hematoxylin and Eosin, immunohistochemical colourings were performed, by using with proper dilutions an antibody specific for AMH (anti-AMH antibody of ABCAM, # cat. ab103233) with the dilution of 1 to 100, the ABC system and the colouring with Diaminobenzidine to detect the antigen-antibody complexes. Such experiment allowed demonstrating that the AMH hormone is constantly and abundantly expressed in the glandular and stromal component of the endometriosis lesions. Figure 1 shows two examples of such expression.

Example 2. *Xenotransplant of human* endometriotic *tissue in nude mice*

[0035] Fragments of human connective solid endometriotic tissue (max diameter about 3 mm) collected from two different patients during surgical removal operation by laparoscopic way were transplanted subcutaneously in the left side of two female nude mice. After implanting endometriotic tissue, performed in total anaesthesia, the small female mice were stabled for two weeks with food and water ad libitum and, limited to the first week, with antibiotic therapy (5% enrofloxacin in the beverage water). The imaging evaluation was performed by means of using a 0.2-Tesla magnetic resonance for veterinary use. The small female mice were soothed with tiletamine + zolazepam + xylazine in order to be able to perform the imaging studies. After being positioned in the apparatus, total-body and local studies were performed for the abdominal area with sections of 2 mm. Subsequently the small female mice were removed from the apparatus for a second administration of sedative and in order to be able to perform the intravenous inoculation (tail vein) of the antibody (10 μl of a 0.2 mg/ml concentrated solution of anti AMH antibody conjugated with gadolinium). The small female mice were repositioned and total-body and loco-regional studies were performed. Both in the

total-body study (Figure 2) (wherein subcutaneous captation is found with residue of antibodies in the inoculation site in the caudal vein) and in some cross sections (Figure 3), antibody captation is highlighted in the site of transplanting the endometriosic tissue. In particular, in the cross section of an animal before the treatment, the subcutaneous mass not having captation signs is found. After the experiment, the animals were brought in animal house and sacrificed to explant the ectopic tissue. Such tissue was then analysed with histological and immunohistochemical examination. These examinations confirmed that the transplant histological aspect was that of a connective solid endometriotic tissue. At last, by means of immunohistochemical examination, performed by using the same method shown before, it was demonstrated that such transplanted tissue expressed CD10 (marker of endometriotic tissue) and the codifying protein for AMH (Figure 4).

Claims

1. An isolated ligand of anti-Mullerian hormone (AMH) suitable to be directly detected by means of imaging techniques for use in an *in vivo* method for the diagnosis of endometriosis comprising a passage of localizing and/or evaluating the entity of the endometriosis lesions in a patient, wherein said isolated ligand consists of an anti-AMH antibody marked with at least one of the agents chosen in the group consisting of: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents and radioisotopes.

2. The ligand for use according to claim 1, wherein said antibody is human, humanized, murine or chimeric.

3. The ligand for use according to anyone of claims 1 to 2, wherein said antibody is a polyclonal or monoclonal antibody.

4. The ligand for use according to anyone of claims 1 to 3, wherein said ligand is detected by means of: ecography, radiography, computed tomography, nuclear magnetic resonance, tomography with emission of positrons, scintigraphy.

5. The ligand for use according to any one of claims 1 to 4, wherein

   - said paramagnetic contrast agents are chosen among: gadolinium or manganese;
   - said iodized contrast agents are chosen among: iohexol. ioversol, iopromide, iopamidol, iodixanol;
   - said intravenous contrast agents are chosen among: sulphur hexafluoride;
   - said radioisotopes are chosen among: Technetium 99, Iodine 131, Thallium 201, Iodine 125, Fluorine 18, Carbon 14.

6. The ligand for use according to anyone of claims 1 to 5, wherein said lesions are endometriotic neoformations with diameter smaller or larger than 1 centimetre.

7. A formulation for use in an *in vivo* method for localizing and/or evaluating the entity of the endometriosis lesions in a patient comprising a ligand according to anyone of claims 1 to 6 and at least a pharmaceutically acceptable carrier and/or excipient, wherein said ligand is marked with at least one of the agents chosen in the group consisting of: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents, and radioisotopes.

8. A kit for use in an *in vivo* method for localizing and/or evaluating the entity of the endometriosis lesions in a patient comprising at least a ligand according to anyone of claims 1-6 or a formulation according to claim 7 and means useful to the administration of said ligand or said formulation to said patient, wherein said ligand is marked with at least one of the agents chosen in the group consisting of: paramagnetic contrast agents, iodized contrast agents, intravenous contrast agents, and radioisotopes.

Patentansprüche

1. Isolierter Ligand des Anti-Müller-Hormons (AMH), der geeignet ist mit Hilfe von bildgebenden Verfahren zur Verwendung in einem *In-vivo*-Verfahren zur Diagnose von Endometriose, das einen Durchlauf eines Lokalisierens und/oder Evaluierens der Entität der Endometriose-Läsionen in einem Patienten umfasst, direkt detektiert zu werden, wobei der isolierte Ligand aus einem Anti-AMH-Antikörper besteht, der mit mindestens einem Agens markiert ist, das aus der folgenden Gruppe ausgewählt wird: paramagnetische Kontrastmittel, jodierte Kontrastmittel, intravenöse Kontrastmittel und Radioisotope.

2. Ligand zur Verwendung gemäß Anspruch 1, wobei der Antikörper human, humanisiert, murin oder chimär ist.

3. Ligand zur Verwendung gemäß einem der Ansprüche 1 bis 2, wobei der Antikörper ein polyklonaler oder monoklonaler Antikörper ist.

4. Ligand zur Verwendung gemäß einem der Ansprüche 1 bis 3, wobei der Ligand mit Hilfe der folgenden Verfahren detektiert wird: Sonographie, Radiographie, Computertomographie, Kernspintomographie, Positronen-Emissions-Tomographie , Szintigra-

phie.

5. Ligand zur Verwendung gemäß einem der Ansprüche 1 bis 4, wobei

- die paramagnetischen Kontrastmittel aus der folgenden Gruppe ausgewählt werden: Gadolinium oder Mangan;
- die jodierten Kontrastmittel aus der folgenden Gruppe ausgewählt werden: Iohexol, Ioversol, Iopamidol, Iodixanol;
- die intravenösen Kontrastmittel aus der folgenden Gruppe ausgewählt werden: Schwefel, Hexafluorid;
- die Radioisotope aus der folgenden Gruppe ausgewählt werden: Technetium 99, Jod 131, Thallium 201, Jod 125, Fluor 18, Kohlenstoff 14.

6. Ligand zur Verwendung gemäß einem der Ansprüche 1 bis 5, wobei die Läsionen endometriotische Neubildungen mit einem Durchmesser von weniger oder mehr als einem Zentimeter sin,d.

7. Rezeptur zur Verwendung in einem *In-vivo*-Verfahren zum Lokalisieren und/oder Evaluieren der Entität der Endometriose-Läsionen in einem Patienten, die einen Liganden gemäß einem der Ansprüche 1 bis 6 und mindestens einen pharmazeutisch akzeptablen Träger und/oder Hilfsstoff umfasst, wobei der Ligand mit mindestens einem Agens markiert ist, das aus der folgenden Gruppe ausgewählt wird: paramagnetische Kontrastmittel, jodierte Kontrastmittel, intravenöse Kontrastmittel und Radioisotope.

8. Kit zur Verwendung in einem *In-vivo*-Verfahren zum Lokalisieren und/oder Evaluieren der Entität der Endometriose-Läsionen in einem Patienten, das mindestens einen Liganden gemäß einem der Ansprüche 1 - 6 oder eine Formulierung gemäß Anspruch 7 und Mittel, die der Verabreichung des Liganden oder der Formulierung an den Patienten zweckdienlich sind, umfasst, wobei der Ligand mit mindestens einem Agens markiert ist, das aus der folgenden Gruppe ausgewählt wird: paramagnetische Kontrastmittel, jodierte Kontrastmittel, intravenöse Kontaktmittel und Radioisotope.

Revendications

1. Ligand isolé d'hormone de régression müllérienne (HRM) adapté pour être directement détecté au moyen de techniques d'imagerie, pour une utilisation dans un procédé *in vivo* pour le diagnostic d'une endométriose, comprenant un passage de localisation et/ou d'évaluation de l'entité des lésions d'endométriose chez une patiente, lequel ligand isolé consiste en un anticorps anti-HRM marqué par au moins l'un des agents choisis dans le groupe constitué par : les agents de contraste paramagnétiques, les agents de contraste iodés, les agents de contraste intraveineux, et les isotopes radioactifs.

2. Ligand pour une utilisation selon la revendication 1, dans lequel ledit anticorps est humain, humanisé, murin ou chimère.

3. Ligand pour une utilisation selon l'une quelconque des revendications 1 et 2, dans lequel ledit anticorps est un anticorps polyclonal ou monoclonal.

4. Ligand pour une utilisation selon l'une quelconque des revendications 1 à 3, lequel ligand est détecté au moyen d'une échographie, radiographie, tomodensitométrie, résonance magnétique nucléaire, tomographie avec émission de positons, scintigraphie.

5. Ligand pour une utilisation selon l'une quelconque des revendications 1 à 4, dans lequel

- lesdits agents de contraste paramagnétiques sont choisis parmi : le gadolinium et le manganèse ;
- lesdits agents de contraste iodés sont choisis parmi : l'iohexol, l'ioversol, l'iopromide, l'iopamidol, l'iodixanol ;
- lesdits agents de contraste intraveineux sont choisis parmi l'hexafluorure de soufre ;
- lesdits isotopes radioactifs sont choisis parmi : le technétium 99, l'iode 131, le thallium 201, l'iode 125, le fluor 18, le carbone 14.

6. Ligand pour une utilisation selon l'une quelconque des revendications 1 à 5, dans lequel lesdites lésions sont des néoformations endométriotiques ayant un diamètre inférieur ou supérieur à 1 centimètre.

7. Formulation pour une utilisation dans un procédé *in vivo* pour localiser et/ou évaluer l'entité des lésions d'endométriose chez une patiente, comprenant un ligand de l'une quelconque des revendications 1 à 6 et au moins un véhicule et/ou excipient pharmaceutiquement acceptable, dans laquelle ledit ligand est marqué par au moins l'un des agents choisis dans le groupe constitué par : les agents de contraste paramagnétiques, les agents de contraste iodés, les agents de contraste intraveineux, et les isotopes radioactifs.

8. Trousse pour une utilisation dans un procédé *in vivo* pour localiser et/ou évaluer l'entité des lésions d'endométriose chez une patiente, comprenant un ligand de l'une quelconque des revendications 1 à 6 ou une formulation de la revendication 7 et des moyens utiles pour l'administration dudit ligand ou de ladite formulation à ladite patiente, dans laquelle ledit ligand

est marqué par au moins l'un des agents choisis dans le groupe constitué par : les agents de contraste paramagnétiques, les agents de contraste iodés, les agents de contraste intraveineux, et les isotopes radioactifs.

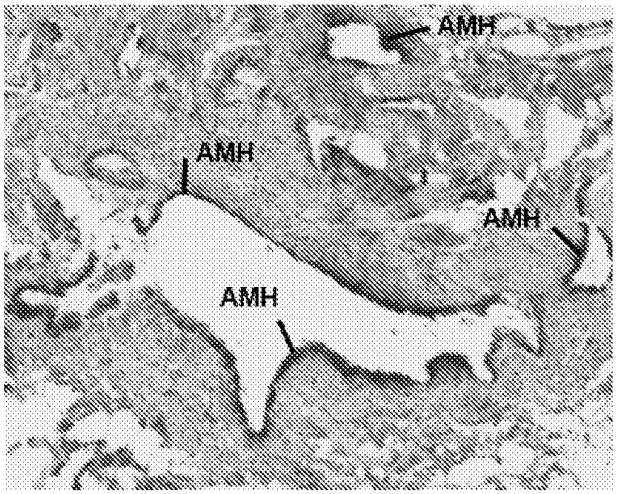

FIGURE 1A

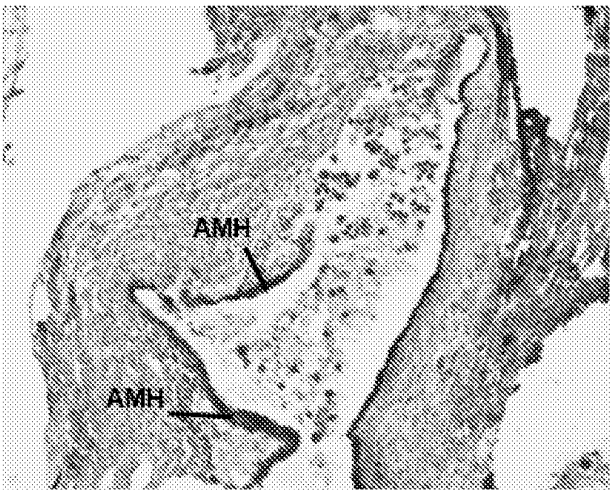

FIGURE 1B

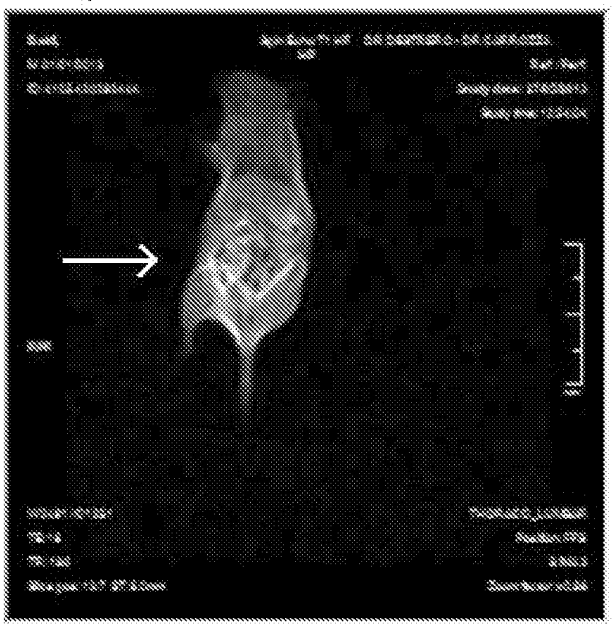

FIGURE 2A

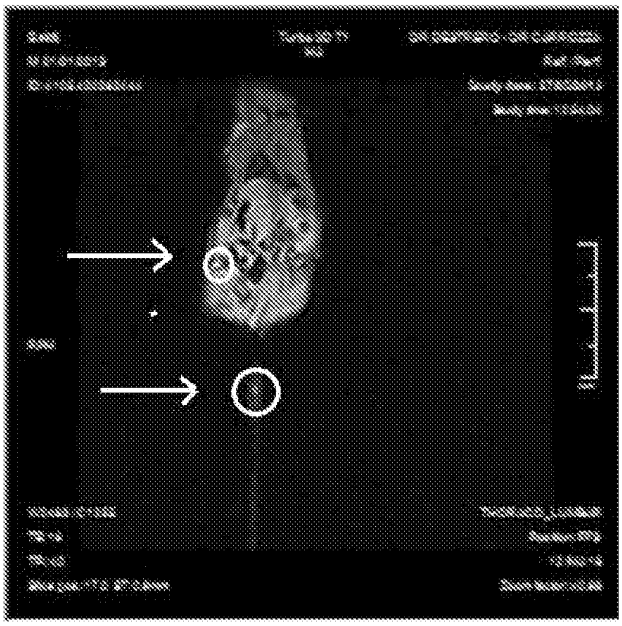

FIGURE 2B

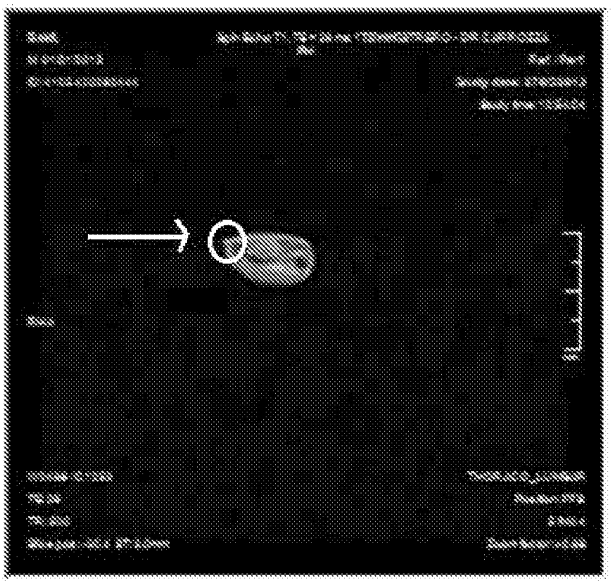

FIGURE 3A

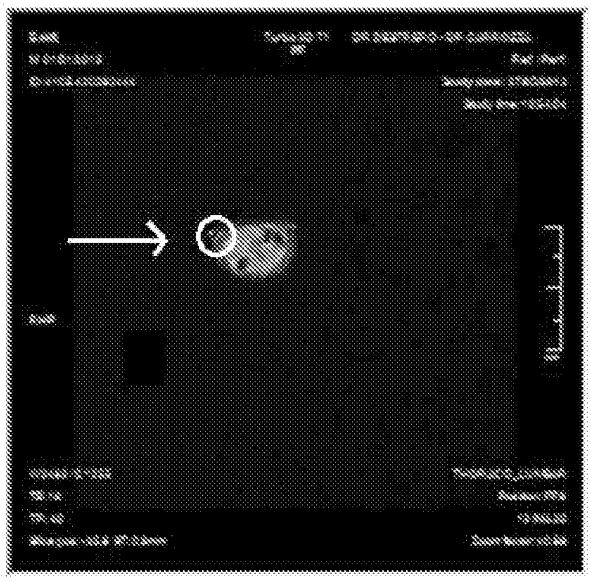

FIGURE 3B

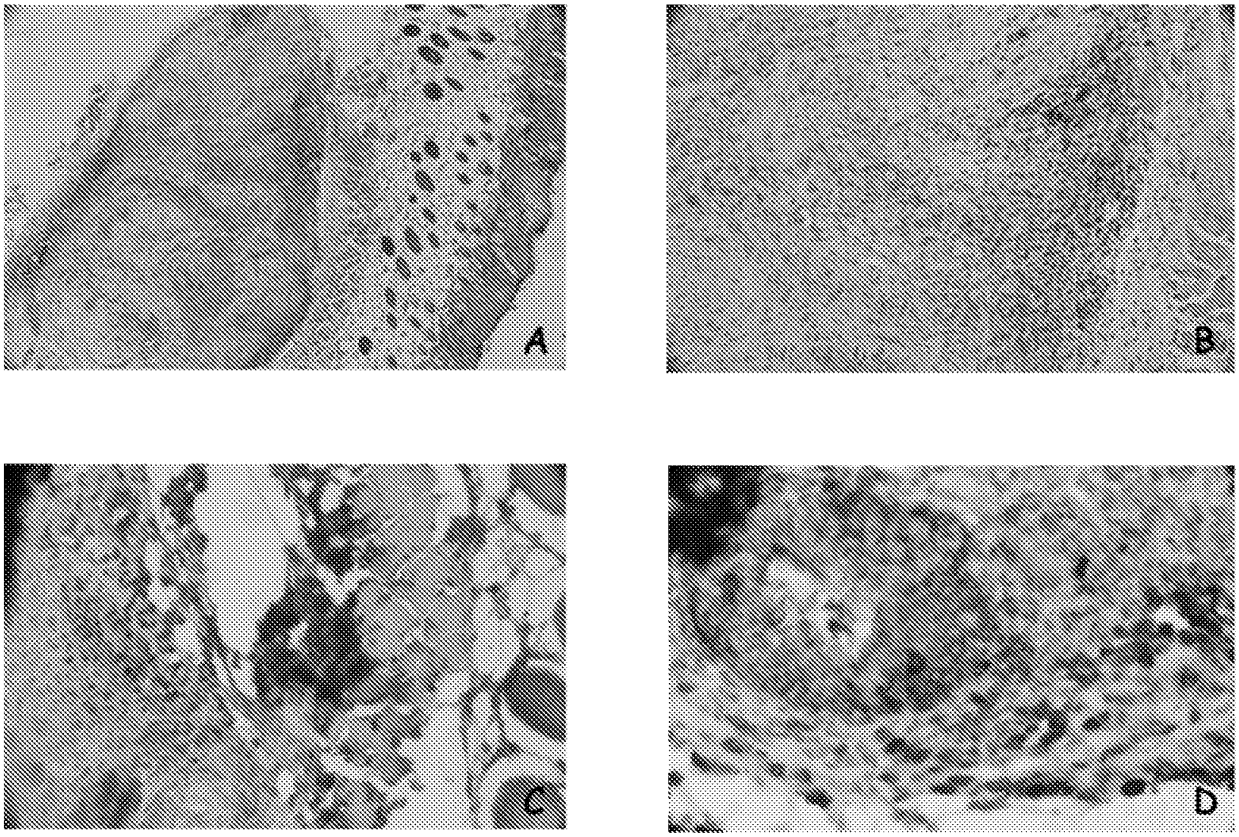

FIGURE 4

REFERENCES CITED IN THE DESCRIPTION

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