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(54) **Title**: MIRNAS IN JOINT DISEASE

(57) **Abstract**: The present invention concerns microRNAs (miRNA), in particular mir-199a, as an indicator of a tissue status or a disease such as osteoarthritis (OA) and as a target molecule for the discovery of a substance for the treatment of a joint disease. Further provided are methods and compounds for investigation, analysis and/or treatment of a joint disease, especially OA.

Description

miRNAs in Joint Disease

Field of the Invention

The present invention concerns microRNAs (miRNA), in particular mir-199a, as an indicator of a tissue status or a disease such as osteoarthritis (OA) and as a target molecule for the discovery of a substance for the treatment of a joint disease. Further provided are methods and compounds for investigation, analysis and/or treatment of a joint disease, especially OA.

Background of the Invention

Arthritis is the most common joint disease in humans which is marked by pain, inflammation, redness, and swelling of the joints. Several different forms of arthritis are known. Rheumatoid arthritis (RA), a chronic, systemic inflammatory disorder, primarily affects synovial joints, whereas ankylosing spondylitis (AS), also a chronic inflammatory arthritis, mainly affects the joints of spine and pelvis. Juvenile idiopathic arthritis (JIA) is characterised in an inflammation of the synovium of the joint and effects children of an age below 16 years. Gout is characterised by recurring attacks of acute inflammatory arthritis which are caused by elevated levels of uric acid in the blood. Septic arthritis is caused by an infection of the joint and psoriatic arthritis may develop in patients suffering from psoriasis. Osteoarthritis (OA) is characterised by mechanical abnormalities of the joint such as the degradation of the joints articular cartilage and subchondral bone.

OA is one of the most common diseases of the western world with more than 21 million diagnosed symptomatic cases in the USA, Japan, and Germany in 2000. Due to the aging of the population, an ever-increasing population of OA patients has to be faced, whose quality of life is severely affected. In addition, OA carries a tremendous socio-economic burden with direct and indirect costs. The current treatment modalities concentrate on the management of the pain associated with OA, since

pharmacological treatment modalities able to slow, stop or even reverse the course of OA and its effects, are long since sought for but still missing.

OA can be viewed as the clinical and pathological outcome of a range of disorders that result in structural and functional failure of synovial joints. It occurs when the dynamic equilibrium between the breakdown and the repair of joint tissues is overwhelmed. Structural failure of articular cartilage can result from abnormal mechanical strains injuring healthy cartilage, as well as from failure of pathologically impaired cartilage degenerating under the influence of physiological and mechanical strains. OA is characterised by a progressive degeneration of the articular cartilage, ultimately leading to a functional inability of the affected joint. During the chronical progression of OA the articular cartilage is destroyed setting free the underlying bone tissue, ultimately necessitating the surgical replacement of the affected joint.

Besides the destruction of the cartilage, pathological degenerations of the synovial membranes and the ligaments are taking place. Inflammation takes place in OA as a secondary effect.

Morphological changes observed in OA include cartilage erosion as well as a variable degree of synovial inflammation. These changes are attributed to a complex network of biochemical factors, including proteolytic enzymes that lead to a breakdown of the cartilage macromolecules, in particular collagen II, X and aggrecan. Cytokines such as IL-1 and TNF-α which are produced by activated synoviocytes, mononuclear cells or by articular cartilage itself, significantly up regulate matrix-metalloproteinases (MMPs) and cytokine gene expression, and blunt compensatory synthesis pathways.

The exact etiology of OA is still not resolved, but some contributing factors are associated with the onset and progression of the disease. The most important factor is age, but also periodic overload, obesity, joint laxity, and a genetic predisposition play an important role. The degradation of cartilage matrix components is generally agreed to be due to an increased synthesis and activation of extracellular proteinases, mainly MMPs, and cytokines amplifying degenerative processes.

Diagnosis of OA is commonly based on clinical examination of the joints in patients showing a gradual onset of symptoms and signs, with x-rays confirming the diagnosis. Typical changes observable via x-ray include a narrowing of the joint space, marginal osteophytes, subchondral sclerosis, subchondral cyst formation, bony remodelling, and

joint effusions. Laboratory tests for the diagnosis of OA are not available as the markers routinely used remain normal in OA. These tests are only useful in order to differentiate OA from others forms of arthritis, especially inflammatory types of arthritis. The diagnosis of OA is thus, only possible at a stage where the degradation of the joint already progressed far enough to be visible on an x-ray. Early onsets of the disease are not detectable as of yet, preventing a timely treatment that could avoid the destruction of the joint in the first place. Improved tools allowing for an identification of OA at an early stage are thus, still desired.

A causal pharmaceutical therapy leading to a decline of the degenerative symptoms is not possible, at present, because there exists no disease-modifying OA-drug (DMOAD). Accordingly, the contemporary disease management is reduced to the administration of analgesic pharmaceuticals, mainly non-steroidal anti-inflammatory drugs (NSAIDs), to relieve pain and thus, to improve quality of life.

As a consequence of the drawbacks of the state of the art there is a great need of improved diagnostic methods allowing for the identification of early stages of the disease and thus for the prevention and/or early treatment of OA.

Summary of the Invention

It is thus, the object of the present invention to provide improved methods for the identification of the disease as well as of substances and compositions suitable for an improved treatment. This is solved by the different aspects of present invention in providing methods and substances on the basis of miRNAs that are differentially expressed during the differentiation process of chondrocytes and cultured bone marrow stem cells (BMCSs).

The present invention is based on the surprising finding of the inventors that specific inhibition of mir-199a in chondrocytes increased chondrogenic markers whilst the overexpression of mir-199a in chondrocytes lead to an decrease of chondrogenic markers.

The invention provides in a first aspect a mir-199a for use as an indicator of a tissue status or a disease and in a second aspect, the use of mir-199a as an indicator of a tissue status or a disease.

In a third aspect, the invention provides a method of identifying an alteration of a tissue status or a risk for developing and/or identifying the presence and/or monitoring progression of a disease, preferably a joint disease, in an individual, comprising detecting the level of mir-199a in a sample.

In a fourth aspect, the present invention provides a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, preferably a joint disease, in an individual, comprising the steps of (a) determining the level of mir-199a in a sample of the individual, and (b) determining the dosage of a pharmaceutical depending on the level of mir-199a in the tested sample.

In a fifth aspect, the present invention provides a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a joint disease, comprising the steps of (a) determining the level of mir-199a in a sample, (b) determining the level of mir-199a in one or more reference samples, (c) examining the tested sample as to whether the level of mir-199a present in said sample is different from the level in the one or more reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-199a in the tested sample is different from the level in the one or more reference samples.

In a sixth aspect, the present invention provides a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a joint disease, comprising the steps of (a) determining the level of mir-199a in a tested sample, (b) determining the level of mir-199a in one or more reference samples, and (c) examining the tested sample as to whether the level of mir-199a present in said sample is different from the level in the one or more reference samples, wherein the tested sample was exposed differently to said substance than the one or more reference samples.

In a seventh aspect, the present invention provides the use of mir-199a in a method according to any of the aspects three to six.

In an eighth aspect, the present invention provides a kit for use in a method according to any of the aspects three to six., comprising one or more means of detecting mir-199a.

In a ninth aspect, the present invention provides for the use of the kit of the eighth aspect in a method according to any of the aspects three to six.

In a tenth aspect, the present invention provides one or more nucleic acids for detecting a mir-199a gene, gene product or functionally active variant thereof in a method according to any of the aspects three to six.

In an eleventh aspect, the present invention provides a peptide, polypeptide or protein for detecting a mir-199a gene, gene product or functionally active variant thereof in a method according to any of the aspects three to six.

In a twelfth aspect, the present invention provides for the use of a nucleic acid according to aspect ten, or of a peptide, polypeptide or protein according to aspect eleven in a method according to any of the aspects three to six.

In a thirteenth aspect, the present invention provides a method of screening for a mir-199a agonist, wherein the method comprises the steps of (a) providing mir-199a or the mir-199a gene, (b) providing a test compound, and (c) measuring or detecting the influence of the test compound on mir-199a or the mir-199a gene.

In a fourteenth aspect, the present invention provides a mir-199a agonist for the alteration of a tissue status or the prevention or treatment of a joint disease.

In a fifteenth aspect, the present invention provides a pharmaceutical comprising the mir-199a agonist according to the fourteenth aspect.

In a sixteenth aspect, the present invention provides a method of alteration of a tissue status or prevention or treatment of a joint disease, wherein a therapeutically effective amount of the pharmaceutical according to aspect fifteen is administered to an individual at risk of developing or suffering from a joint disease.

Brief Description of the Figures

Fig.1a: mir-199a expression in chondrocyte pellets during the cultivation period

Fig.1b: expression of mir-22, mir-140, mir-146a and mir-199a in chondrocyte pellets during the cultivation period

Fig.2: mir-199a expression in mesenchymal stem cell pellets during the cultivation period

Fig.3: Effects of mir-199a overexpression on the expression levels of two marker genes and two marker proteins in three different experiments: collagen II (A); aggrecan (B); ALP (C); MMP-1 (D);

Fig.4: Effects of mir-199a knock down on the expression levels of two marker genes and 1 marker protein in three different experiments: collagen II (A); aggrecan (B); ALP (C)

Fig.5: Relative changes in the expression of the two marker genes collagen II and aggrecan in response to mir-199a overexpression or knock-down

Detailed Description of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland). Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", is understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. "Nucleic acid" molecules are understood as a polymeric or oligomeric macromolecule made from nucleotide monomers. Nucleotide monomers are composed of a nucleobase, a five-carbon sugar (such as but not limited to ribose or 2'-deoxyribose),

and one to three phosphate groups. Typically, a polynucleotide is formed through phosphodiester bonds between the individual nucleotide monomers. In the context of the present invention referred to nucleic acid molecules include but are not limited to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and mixtures thereof such as e.g. RNA-DNA hybrids. The terms "polynucleotide" and "nucleic acid" are used interchangeably herein. The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584).

Nucleic acids may be degraded by endonucleases or exonucleases, in particular by DNases and RNases which can be found in the cell. It is, therefore, advantageous to modify the nucleic acids in order to stabilize them against degradation, thereby ensuring that a high concentration of the nucleic acid is maintained in the cell over a long period of time (Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Typically, such stabilization can be obtained by introducing one or more internucleotide phosphorus groups or by introducing one or more non-phosphorus internucleotides.

Suitable modified internucleotides are compiled in Uhlmann and Peyman (1990), supra (see also Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid which can be employed in one of the uses according to the invention contain, for example, methyl phosphonate, phosphorothioate, phosphoramidate, phosphorodithioate and/or phosphate esters, whereas non-phosphorus internucleotide analogues contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also the intention that this modification should improve the durability of a pharmaceutical composition which can be employed in one of the uses according to the invention.

Nucleic acids may be selected from the group consisting of a polynucleotide probe, a primer(s) (e.g. a primer pair), preferably a primer(s) for polymerase chain reaction (PCR), reverse transcription (RT) reaction, or DNA sequencing, a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a glycol nucleic acid (GNA), a threose nucleic acid (TNA), a microRNA (miRNA), and a small interfering RNA (siRNA).

The term "probe" as used herein refers to a single-strand oligonucleotide which is typically used for the detection of target RNA and/or DNA sequences that is complementary to the sequence of the probe. A probe hybridizes to single-stranded nucleic acid (DNA or RNA) whose nucleotide sequence allows for nucleotide pairing due to complementarity between the probe and the target sequence. The length of a probe depends on the intended use as well as the required specificity of the probe. Typically, a probe is 20-500 (i.e. 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500) nucleotides long, preferably 20-100 nucleotides, more preferably 20-50. For detection of microRNA probes are between 12 and 30 nucleotides. Probes are used in various experimental set ups such as but not limited to Southern and Northern Blots, for real-time PCR and In Situ Hybridization (ISH) as well as for microarray experiments. A probe may be unlabeled, directly labelled, or indirectly labelled, such as with biotin to which a streptavidin complex may later bind. Said label may be a molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, suitable labels include 32P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and other entities which are or can be made detectable. A label may be incorporated into nucleic acids at any position, e.g. at the 3' end, at the 5' end or internally. The term "probe" also encompasses nucleic acids differing in the composition of their backbone such as but not limited to peptide nucleic acids (PNAs), locked nucleic acids (LNAs), glycol nucleic acids (GNAs) and threose nucleic acids (TNAs).

The term "primer" as used herein refers to a single-strand oligonucleotide which typically serves as a starting point for DNA-replicating enzymes. A primer binds to or hybridises with a DNA template and typically comprises a sequence being complementary to the DNA sequence to which it is supposed to bind. A primer may also comprise additional sequences e.g. sequences serving as nuclease cleavage sites (e.g. Bam H1, Hind III, etc.). The length of a primer is chosen depending on the intended use. For instance, primers used for the amplification of DNA in Polymerase-Chain Reactions (PCR) typically have a length of at least 10 nucleotides, preferably between 10 to 50 (i.e. 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50) nucleotides, more preferably between 15 and

30 nucleotides. Shorter primers of at least 5 nucleotides are used for sequencing of DNA templates. Also encompassed in the term "primer" are "degenerate primers" which are a mixture of similar, but not identical primers. A primer may be tagged or labelled with a marker molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means.

As used herein, the term "microRNA" and variations such as "miRNA" and "miR" include human miRNAs, mature single stranded miRNAs, precursor miRNAs (pre-miR), and variants thereof, which may be naturally occurring. In some instances, the term "miRNA" also includes primary miRNA transcripts and duplex miRNAs. Unless otherwise noted, when used herein, the name of a specific miRNA refers to the mature miRNA. For example, mir-199a refers to a mature miRNA sequence derived from pre-mir-199a. Sequences for miRNAs, including human mature and precursor sequences (also called stem-loop sequences), are reported in the following database: miRBase - the microRNA Database, accessible under the following links: http://microrna.sanger.ac.uk or http://www.mirbase.org/

Publications comprising miRNA sequences are: Griffiths-Jones et al., Nucleic Acids Research, 2008, 36, Database Issue, D154-D158; Griffiths-Jones et al., Nucleic Acids Research, 2006, 34, Database Issue, D140-D144; Griffiths-Jones, Nucleic Acids Research, 2004, 32, Database Issue, D109-D111, Griffiths-Jones et al., Nucleic Acids Research, 2011, Vol. 39, Database Issue, D152–D157).

Lagos-Quintana et al. ["New microRNAs from mouse and human" Lagos-Quintana M, Rauhut R, Meyer J, Borkhardt A, Tuschl T RNA. 9:175-179(2003).] cloned miR-199 from human osteoblast sarcoma cells. They also reported identification of a miRNA from the opposite arm in mouse cells. This sequence was named miR-199-s and the sequence from the 3' arm of the homologous mouse precursor miR-199-as. Lim et al. ["Vertebrate microRNA genes" Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP Science. 299:1540(2003).] independently predicted this miRNA computationally using conservation with mouse and Fugu rubripes sequences ["Vertebrate microRNA genes" Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP Science. 299:1540(2003).]. Expression of the miR excised from the 5' arm was validated in zebrafish, and the ends mapped by cloning. The excised miR sequences are renamed miR-199a (to avoid confusion with the subsequently identified miR-199b (MI0000282)) and miR-199a*

(from the 3' arm). The two putative hairpin precursors in human map to chromosome 19 (mir-199a-1, MI0000242) and chromosome 1 (mir-199a-2, MI0000281). Landgraf et al. show that both mature products are expressed, prompting the renaming to miR-199a-5p and miR-199a-3p ["A mammalian microRNA expression atlas based on small RNA library sequencing" Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M Cell. 129:1401-1414(2007).]. (See also "Identification of human fetal liver miRNAs by a novel method" Fu H, Tie Y, Xu C, Zhang Z, Zhu J, Shi Y, Jiang H, Sun Z, Zheng X FEBS Lett. 579:3849-3854(2005).

Sequences of homo sapiens mir199a (the MI... identifier is the accession number in the mirbase database, see above)

1) stem-loop sequence of hsa-mir-199a-1 (MI0000242)

GCCAACCCAGUGUUCAGACUACCUGUUCAGGAGGCUCUCAAUGUGUACAGUAG UCUGCACAUUGGUUAGGC (SEQ ID NO: 1)

2) stem-loop sequence of hsa-mir-199a-2 (MI0000281)

AGGAAGCUUCUGGAGAUCCUGCUCCGUCGCCCCAGUGUUCAGACUACCUGUUC AGGACAAUGCCGUUGUACAGUAGUCUGCACAUUGGUUAGACUGGGCAAGGGA GAGCA (SEQ ID NO:4)

3) mature sequences:

hsa-miR-199a-5p (MIMAT0000231)

CCCAGUGUUCAGACUACCUGUUC (SEQ ID NO:2)

hsa-miR-199a-3p (MIMAT0000232)

ACAGUAGUCUGCACAUUGGUUA (SEQ ID NO:3)

The mature sequences of hsa (homo sapiens) mir199-a-1 and mir199-a-2 are identical.

This miRNA sequence is predicted based on homology to a verified miRNA from mouse ["Identification of novel genes coding for small expressed RNAs" Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T Science. 294:853-858(2001), "Reduced accumulation of specific microRNAs in colorectal neoplasia" Michael MZ, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ Mol Cancer Res. 1:882-891(2003)]. Its expression was later verified in human ["Kaposi's sarcoma-associated herpes virus expresses an array of viral microRNAs in latently infected cells" Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR Proc Natl Acad Sci U S A. 102:5570-5575(2005)., "A mammalian microRNA expression atlas based on small RNA library sequencing" Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M Cell. 129:1401-1414(2007).].

MicroRNAs (miRNAs) are endogenous, small RNA molecules that are implied in the regulation of gene expression at the translation level. Moreover, they are part of the cell's RNA interference (RNAi) mechanism. MiRNAs are encoded by genes transcribed from DNA but are not translated into protein (non-protein-coding RNA). They have been first discovered in *caenorhabditis elegans* and exist in both plants and animals. The sequences of many miRNAs are highly conserved among different species, suggesting that the miRNA pathway is part of a relatively old and important regulatory mechanism (Grosshans et al. J Cell Biol 156: 17-21 (2002)). MiRNAs have been found to regulate about 30% of mammalian genes (Czech, NEJM 354:1194-1195 (2006);

Mack, Nature Biotech. 25:631-638 (2007); Eulalio, et al., Cell 132:9-14 (2008)). Recently, it has been found that miRNAs repress protein production by blocking translation or causing transcript degradation, thereby regulating gene expression. A single miRNA may target 250-500 different mRNAs, proving this class of RNA to be an extremely important mediator of a wide range of cellular functions. Many of the genes regulated by miRNAs are disease-causing genes. Thus, any miRNA-modulating functionality has great therapeutic potential.

In animals, miRNAs are first expressed from the genome as RNA transcripts called primary miRNAs (pri-miRNAs). They are transcribed by RNA Polymerase II, and form hairpin structures. In the nucleus, the dsRNA-specific ribonuclease Drosha processes the pri-miRNAs into shorter, about 70- to 100-nucleotide long stem-loop structures known as pre-miRNAs, which are then exported out into the cytoplasm, likely by Exportin-5 (Exp5). (Yi, et al. Genes Dev. 17: 3011-3016 (2003)). In the cytoplasm, Dicer, a member of the RNase III ribonuclease family, cleaves the pre-miRNA into a double-stranded guide/passenger (miRNA/miRNA*) duplex with 3' overhangs at both ends. The two strands of the miRNA duplex often have mismatches from imperfect complementarity in their sequences and when they separate, a mature miRNA, in many cases, between about 19 and 23 nucleotides long, is bound by the RNA-induced silencing complex (RISC) or a similar protein complex. RISC is also the protein complex that effects target-specific mRNA degradation mediated by small or short interfering RNAs (siRNAs).

At present, it is believed that once it is selected by a catalytic component of RISC, argonaute, as the guide strand, the mature miRNA is integrated into the RISC complex, and binds to a messenger RNA (mRNA) molecule that has a significantly, though often not perfectly, complementary sequence. The passenger strand, miRNA*, may be degraded. Translation of the mRNA bound by the miRNA-RISC complex is then repressed, resulting in reduced expression of the corresponding gene. In some cases, the bound mRNA is cleaved or deadenylated and degraded. For certain miRNAs, a single precursor contains more than one mature miRNA sequence. In other instances, multiple precursor miRNAs contain the same mature sequence.

The terms "protein" and "polypeptide" are used interchangeably herein and refer to any peptide-linked chain of amino acids, regardless of length or post-translational

modification. Proteins usable in the present invention (including protein derivatives, protein variants, protein fragments, protein segments, protein epitopes and protein domains) can be further modified by chemical modification. This means such a chemically modified polypeptide comprises other chemical groups than the 20 naturally occurring amino acids. Examples of such other chemical groups include without limitation glycosylated amino acids and phosphorylated amino acids. Chemical modifications of a polypeptide may provide advantageous properties as compared to the parent polypeptide, e.g. one or more of enhanced stability, increased biological half-life, or increased water solubility. Chemical modifications applicable to the variants usable in the present invention include without limitation: PEGylation, glycosylation of non-glycosylated parent polypeptides, or the modification of the glycosylation pattern present in the parent polypeptide.

As used herein, the term "variant" is to be understood as a polynucleotide or protein which differs in comparison to the polynucleotide or protein from which it is derived by one or more changes in its length or sequence. The polypeptide or polynucleotide from which a protein or nucleic acid variant is derived is also known as the parent polypeptide or polynucleotide. The term "variant" comprises "fragments" or "derivatives" of the parent molecule. Typically, "fragments" are smaller in length or size than the parent molecule, whilst "derivatives" exhibit one or more differences in their sequence in comparison to the parent molecule. Also encompassed modified molecules such as but not limited to post-translationally modified proteins (e.g. glycosylated, biotinylated, phosphorylated, ubiquitinated, palmitoylated, or proteolytically cleaved proteins) and modified nucleic acids such as methylated DNA. Also mixtures of different molecules such as but not limited to RNA-DNA hybrids, are encompassed by the term "variant". Typically, a variant is constructed artificially, preferably by gene-technological means whilst the parent polypeptide or polynucleotide is a wild-type protein or polynucleotide. However, also naturally occurring variants are to be understood to be encompassed by the term "variant" as used herein. Further, the variants usable in the present invention may also be derived from homologs, orthologs, or paralogs of the parent molecule or from artificially constructed variant, provided that the variant exhibits at least one biological activity of the parent molecule, i.e. is functionally active.

Alternatively or additionally, a "variant" as used herein, can be characterised by a certain degree of sequence identity to the parent polypeptide or parent polynucleotide from which it is derived. More precisely, a protein variant in the context of the present invention exhibits at least 80% sequence identity to its parent polypeptide. A polynucleotide variant in the context of the present invention exhibits at least 80% sequence identity to its parent polynucleotide. The term "at least 80% sequence identity" is used throughout the specification with regard to polypeptide and polynucleotide sequence comparisons. This expression preferably refers to a sequence identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the respective reference polypeptide or to the respective reference polynucleotide.

The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5877), with hmmalign (HMMER package, http://hmmer.wustl.edu/) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673-80) available e.g. on http://www.ebi.ac.uk/Tools/clustalw/ or on http://www.ebi.ac.uk/Tools/clustalw2/index.html or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on http://www.ebi.ac.uk/Tools/clustalw/ or http://www.ebi.ac.uk/Tools/clustalw2/index.html. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12, to obtain polynucleotide sequences that are homologous to those nucleic acids which encode mir-199a. BLAST protein searches are performed with the BLASTP program, score = 50, word length = 3, to obtain amino acid sequences homologous to mir-199a. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in

Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1:I54-I62) or Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise.

"Hybridization" can also be used as a measure of sequence identity or homology between two nucleic acid sequences. A nucleic acid sequence encoding miRNAs or a portion thereof, can be used as a "hybridization probe" according to standard hybridization techniques. The hybridization of a mir-199a probe to DNA or RNA from a test source is an indication of the presence of the mir-199a DNA or RNA in the test source. Hybridization conditions are known to those skilled in the art and can be found, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y., 6.3.1-6.3.6, 1991. "Moderate hybridization conditions" are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1X SSC, 0.1% SDS at 50°C. "Highly stringent conditions" are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1 % SDS at 65°C.

The term "tissue" as used herein, refers to an ensemble of cells of the same origin which fulfil a specific function concertedly. Examples of a tissue include but are not limited to bone, cartilage, connective tissue, muscle tissue, nervous tissue, and epithelial tissue. Multiple tissues together form an "organ" to carry out a specific function. Examples of an organ include but are not limited to joint, skeleton, muscle, blood, brain, heart, liver, kidney, stomach, and skin. For example, a joint is formed of many different tissues such as but not limited to bone, cartilage, synovium, muscle, ligament, and tendon.

The term "joint" as used herein, refers to a location where two or more bones make contact. The term may refer to a movable joint or an unmovable joint. Further, a joint may be a fibrous joint wherein the bones are attached by dense connective tissue rich in collagen or a cartilaginous joint wherein the bones are connected by cartilage. A joint may also be a synovial joint wherein the bones are not directly connected but

have a synovial cavity and are attached by a capsule of articular cartilage which is associated with accessory ligaments and tendons. Synovial joints include but are not limited to ball-and-socket-joints, such as the hip or shoulder joints; ellipsoid joints and flat joints, such as the joints of the wrist; hinge joints, such as elbows, knees, ankles or the joints of fingers and toes; saddle joints such as the joint of the thumb; and pivot joint, such as the joint of atlas and axis.

The terms "tissue status", "status of a tissue", "tissue state" and "state of a tissue" are used interchangeably herein referring to the condition of a tissue. The state of a tissue may be characterised by a specific morphology of such tissue or may be characterised by the expression of one or more specific molecules such as but not limited to peptides, proteins, and nucleic acids, or combinations thereof. The status of a tissue may be regarded as "healthy" or "normal" in case it resembles the condition of such tissue when being free from illness or injury and efficiently fulfilling its specific function. The status of a tissue may be regarded as "degenerative", "diseased" or "abnormal" in case such tissue fails to fulfil its function due to an illness or injury. Additionally or alternatively, the status of a tissue may be regarded as "degenerative", "diseased" or "abnormal" in case the morphology of the tissue or its molecule expression pattern is "altered" or "changed" in comparison to normal tissue. Accordingly, the morphology of a tissue or the expression pattern of specific molecules in a tissue may be an indicator for the state of a tissue. Examples of a tissues status include but are not limited to tissue degradation such as cartilage degradation, bone degradation, and degradation of the synovium, tissue inflammation such as cartilage inflammation, or inflammation of the synovium, tissue remodelling such as bone remodelling or cartilage remodelling, sclerosis, liquid accumulation, or proliferative tissue such as proliferation in wound healing processes, cyst formations, or in cancer.

The term "disease" and "disorder" are used interchangeably herein, referring to an abnormal condition, especially an abnormal medical condition such as an illness or injury, wherein a tissue, an organ or an individual is not able to efficiently fulfil its function anymore. Typically, but not necessarily, a disease is associated with specific symptoms or signs indicating the presence of such disease. The presence of such symptoms or signs may thus, be indicative for a tissue, an organ or an individual suffering from a disease. An alteration of these symptoms or signs may be indicative

for the progression of such a disease. A progression of a disease is typically characterised by an increase or decrease of such symptoms or signs which may indicate a "worsening" or "bettering" of the disease. The "worsening" of a disease is characterised by a decreasing ability of a tissue, organ or organism to fulfil its function efficiently, whereas the "bettering" of a disease is typically characterised by an increase in the ability of a tissue, an organ or an individual to fulfil its function efficiently. A tissue, an organ or an individual being at "risk of developing" a disease is in a healthy state but shows potential of a disease emerging. Typically, the risk of developing a disease is associated with early or weak signs or symptoms of such disease. In such case, the onset of the disease may still be prevented by treatment. Examples of a disease include but are not limited to traumatic diseases, inflammatory diseases, infectious diseases, cutaneous conditions, endocrine diseases, intestinal diseases, neurological disorders, joint diseases, genetic disorders, autoimmune diseases, and various types of cancer.

The term "joint disease" as used herein, refers to the abnormal condition of the joints, in particular those due to injurious, traumatic, degenerative, inflammatory, infectious or autoimmune causes. Joint diseases include but are not limited to arthritis, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), juvenile idiopathic arthritis (JIA), gout, septic arthritis, psoriatic arthritis, and osteoarthritis (OA), cancer, such as chondrosarcoma, osteosarcoma, fibrosarcoma, and multiple myeloma, tendinitis, bursitis, fractures, and damage to cartilage or bone.

"Symptoms" of a disease are implication of the disease noticeable by the tissue, organ or organism having such disease and include but are not limited to pain, weakness, tenderness, strain, stiffness, and spasm of the tissue, an organ or an individual.

"Signs" or "signals" of a disease include but are not limited to the change or alteration such as the presence, absence, increase or elevation, decrease or decline, of specific indicators such as biomarkers or molecular markers, or the development, presence, or worsening of symptoms.

The term "indicator" as used herein, refers to a sign or signal for a condition or is used to monitor a condition. Such a "condition" refers to the biological status of a cell, tissue or organ or to the health and/or disease status of an individual. An indicator may be the presence or absence of a molecule, including but not limited to peptide, protein, and

nucleic acid, or may be a change in the expression level or pattern of such molecule in a cell, or tissue, organ or individual. An indicator may be a sign for the onset, development or presence of a disease in an individual or for the further progression of such disease. An indicator may also be a sign for the risk of developing a disease in an individual. For instance, indicators relating to arthritic diseases such as OA include but are not limited to transcription factors such as Sox-5, Sox-6, Sox-9, Nfat1, pitx1, FoxO, HIF2A, SAF-1, RUNX-2, cytokines such as IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, TNF-α, NF-κB, and INF-γ, phosphatases such as Alkaline Phosphatase (ALP), proteases such as metalloproteases (e.g. MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, MMP-14), aggrecanases (e.g. ADAM-8, ADAM-12, ADAM-TS4, ADAM-TS5) and cysteinproteasen (e.g. cathepsin B, cathepsin K, cathepsin S, calpain, caspases-3, caspase-9), tissue inhibitors of metalloproteinases (e.g. TIMP-1, TIMP-2, TIMP-3, TIMP-4), extracellular matrix components, such as collagens (e.g. collagen II, VI, IX, X, XI), proteoglycans (e.g. heparan sulfate, chondroitin sulfate, keratan sulfate), aggrecan, elastin, hyaluronic acid, fibronectin, laminin, CD-RAP, CDMPs, chondromodulin and pleiotrophin. Typically, in a tissue, organ, or individual suffering from arthritis, in particular OA, one or more of these indicators are expressed or activated to a higher or lower level than in a tissue, organ, or individual not suffering from arthritis. For instance, the levels of the extracellular matrix components collagen II and X, as well as aggrecan and CD-RAP are lowered in a tissue, organ, or individual suffering or at risk of developing OA in comparison a tissue, organ, or individual not suffering or at risk of developing OA. Further, the level of the transcription factor Sox9 as well as the tissue inhibitors of metalloproteinases TIMP-1 and TIMP-4 are decreased in a tissue, organ, or individual suffering or at risk of developing OA in comparison a tissue, organ, or individual not suffering or at risk of developing OA. In contrast, the levels of the cytokine IL-1, IL-6 and TNF-α as well as of the metalloproteases MMP-1, MMP-2, MMP-3 and MMP-9, and phosphatase ALP are enhanced in a tissue, organ, or individual suffering or at risk of developing OA in comparison a tissue, organ, or individual not suffering or at risk of developing OA.

In the context of the present invention, preferred indicators of a tissue status or a disease are miRNAs, including but not limited to mir-21, mir-22, mir-140, mir-146a, and

mir-199a. Mir-199a is particularly preferred as an indicator of diseases such as arthritis, in particular OA, in an individual.

As used herein, an "individual" means any mammal, reptile or bird that may benefit from the present invention. Preferably, an individual is selected from the group consisting of laboratory animals (e.g. mouse, rat or rabbit), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, duck, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including chimpanzees, bonobos, gorillas and human beings. It is particularly preferred that the "individual" is a human being.

The term "sample" or "sample of interest" are used interchangeably herein, referring to a part or piece of a tissue, organ or individual, typically being smaller than such tissue, organ or individual, intended to represent the whole of the tissue, organ or individual. Upon analysis a sample provides information about the tissue status or the health or diseased status of an organ or individual. Examples of samples include but are not limited to fluid samples such as blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, or solid samples such as tissue extracts, cartilage, bone, synovium, perichondrium, capsule, and connective tissue. Further examples of samples are cell cultures or tissue cultures such as but not limited to cultures of cartilage, bone, synovium, bone marrow cells, chondrocytes, chondroblasts, chondroclasts, synovial cells, osteocytes, osteoclasts, osteoblasts stem cells and/or mesenchymal stem cells. Analysis of a sample may be accomplished on a visual or chemical basis. Visual analysis includes but is not limited to microscopic imaging or radiographic scanning of a tissue, organ or individual allowing for morphological evaluation of a sample. Chemical analysis includes but is not limited to the detection of the presence or absence of specific indicators or alterations in their amount or level.

The term "reference sample" as used herein, refers to a sample which is analysed in a substantially identical manner as the sample of interest and whose information is compared to that of the sample of interest. A reference sample thereby provides a standard allowing for the evaluation of the information obtained from the sample of interest.

A reference sample may be derived from a healthy or normal tissue, organ or individual, thereby providing a standard of a healthy status of a tissue, organ or

individual. Differences between the status of the normal reference sample and the status of the sample of interest may be indicative of the risk of disease development or the presence or further progression of such disease or disorder.

A reference sample may be derived from an abnormal or diseased tissue, organ or individual thereby providing a standard of a diseased status of a tissue, organ or individual. Differences between the status of the abnormal reference sample and the status of the sample of interest may be indicative of a lowered risk of disease development or the absence or bettering of such disease or disorder.

A reference sample may also be derived from the same tissue, organ, or individual as the sample of interest but has been taken at an earlier time point. Differences between the status of the earlier taken reference sample and the status of the sample of interest may be indicative of the progression of the disease, i.e. a bettering or worsening of the disease over time. A reference sample was taken at an earlier or later time point in case a period of time has lapsed between taking of the reference sample and taking of the sample of interest. Such period of time may represent years (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 years), months (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months), weeks (e.g. 1, 2, 3, 4, 5, 6, 7, 8 weeks), days (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 days), hours (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours), minutes (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 minutes), or seconds (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 seconds).

The terms "lowered" or "decreased" level of an indicator, e.g. mir-199a, refer to the level of such indicator in the sample being reduced in comparison to the reference or reference sample. The terms "elevated" or "increased" level of an indicator, e.g. mir-199a, refer to the level of such indicator in the sample being higher in comparison to the reference or reference sample. E.g. a miRNA that is detectable in higher amounts in synovial fluid of one individual suffering from OA than in synovial fluid of individuals not suffering from OA, has an elevated level. For mir-199a, an elevated level in a sample (such as synovial fluid) indicates the presence of OA or increased susceptibility or increased probability to develop OA.

A reference sample may be "treated differently" or "exposed differently" than a sample of interest in case both samples are treated in a substantially identical way except from

a single factor. Such single factors include but are not limited to the time of exposure, the concentration of exposure, or the temperature of exposure to a certain substance. Accordingly, a sample of interest may be exposed to a different dosage of a certain substance than the reference sample or may be exposed for a different time interval than the reference sample or may be exposed at a different temperature than the reference sample. Different dosages to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold increased or decreased dosage of the dosage the reference sample is exposed to. Different exposure times to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold longer or shorter time period than the exposure of the reference. Different temperatures of exposure to which a sample of interest may be exposed to include but are not limited to the 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold and/or 1000-fold increased or decreased temperature than the exposure of the reference. In a non-limiting example a sample of interest may be exposed to a 10-fold increased concentration of a substance than the reference sample. The analysis of both samples is then conducted in a substantially identical manner allowing determining the effects, i.e. a beneficial or an adverse effect, of the increased concentration of such substance on the sample of interest. The skilled person will appreciate that this example applies mutatis mutandis to different ranges of concentrations, different exposure times, and/or different temperatures at exposure.

The term "agonist" as used herein refers to a substance that causes an action in a tissue, organ or individual such as receptor-signalling, gene expression, protein synthesis, and protein degradation. Typically, agonists act by binding to the active site or to allosteric sites of a receptor molecule thereby, triggering a specific reaction. Examples for agonists include but are not limited to nucleic acid molecules, such as mRNA or miRNA, or proteins, such as hormones, cytokines, growth factors, neurotransmitters, and transcription factors.

The term "antagonist" as used herein refers to a substance blocking the action of an agonist. Typically, antagonists act by binding to the active site or to allosteric sites of a receptor molecule, or interact with unique binding sites not normally involved in the regulation of the activity of the receptor. Typically, an antagonist competes with the

agonist at structurally-defined binding sites or alters the binding site of the agonist in a manner that the agonist is not able to cause the action it would normally cause due to its binding. The antagonist activity may be reversible or irreversible depending on the longevity of the interaction of the antagonist–receptor complex. Examples for antagonists include but are not limited to nucleic acid molecules, such as siRNAs or miRNAs, or proteins such as hormones, cytokines, growth factors or neurotransmitter, antibodies, or transcription factors.

The term "mimic" as used herein refers to a substance that imitates the action of a target molecule (e.g. a target molecule such as an agonist or an antagonist in a tissue, organ or individual) such as receptor-signalling, gene expression, protein synthesis, and protein degradation. Accordingly, a mimic may be structurally different from the target molecule (e.g. agonist or antagonist) it imitates but may still effect the same specific reaction, e.g. by binding to the active site or to allosteric sites of a receptor molecule (i.e. by exerting at least one of the activities of the agonist or antagonist). Typically, a mimic fulfils the same function as the respective agonist or antagonist, i.e. is functionally equivalent. For example, a mimic of mir-199a is a substance that exerts at least one of the activities of mir-199a, has the function of mir-199a and/or imitates the action of mir-199a.

The term "receptor" as used herein refers to a molecule such as a protein or polynucleotide, to which one or more specific signalling molecules bind. Signalling molecules may act as agonist or antagonist including without limitation nucleic acid molecules, such as siRNAs or miRNAs, or proteins such as hormones, cytokines, growth factors or neurotransmitter, antibodies or transcription factors. Receptors may be localised at the plasma membrane of a cell, within the cytoplasm and/or in intracellular compartments.

As used herein, "treat", "treating" or "treatment" of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in an individual that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in individuals that were previously symptomatic for the disorder(s).

As used herein, "prevent", "preventing", "prevention", or "prophylaxis" of a disease or disorder means preventing that such disease or disorder occurs in patient.

The terms "pharmaceutical", "medicament" and "drug" are used interchangeably herein referring to a substance and/or a combination of substances being used for the identification, prevention or treatment of a tissue status or disease.

"Pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "active ingredient" refers to the substance in a pharmaceutical composition or formulation that is biologically active, i.e. that provides pharmaceutical value. A pharmaceutical composition may comprise one or more active ingredients which may act in conjunction with or independently of each other.

The active ingredient can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as but not limited to those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The terms "preparation" and "composition" are intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it.

The term "carrier", as used herein, refers to a pharmacologically inactive substance such as but not limited to a diluent, excipient, or vehicle with which the therapeutically active ingredient is administered. Such pharmaceutical carrier can be liquid or solid. Liquid carrier include but are not limited to sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered intracavitary.

Solid form preparations are suitable for oral administration and include powders, tablets, pills, capsules, lozenges, cachets, suppositories, and dispersible granules. A solid excipient can be one or more substances, which may also act as diluents, flavouring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

In powders, the excipient is preferably a finely divided solid, which is in a mixture with the finely divided active ingredient. In tablets, the active ingredient of the present invention is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% to 80%, more preferably from 20% to 70% of the active compound.

Suitable pharmaceutical "excipients" include starch, glucose, lactose, sucrose, gelatine, pectin, dextrin, malt, rice, flour, chalk, silica gel, magnesium carbonate, sodium stearate, magnesium stearate, glycerol monostearate, talc, sodium chloride, tragacanth, methylcellulose, sodium carboxymethylcellulose, dried skim milk, glycerol, propylene, glycol, water, ethanol a low melting wax, cocoa butter, and the like.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Examples of suitable pharmaceutical carriers are also described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The term "adjuvant" refers to agents that augment, stimulate, activate, potentiate, or modulate the immune response to the active ingredient of the composition at either the cellular or humoral level, e.g. immunologic adjuvants stimulate the response of the immune system to the actual antigen, but have no immunological effect themselves. Examples of such adjuvants include but are not limited to inorganic adjuvants (e.g. inorganic metal salts such as aluminium phosphate or aluminium hydroxide), organic adjuvants (e.g. saponins or squalene), oil-based adjuvants (e.g. Freund's complete adjuvant and Freund's incomplete adjuvant), cytokines (e.g. IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, and INF-γ) particulate adjuvants (e.g. immuno-stimulatory complexes

(ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (e.g. monophosphoryl lipid A, or muramyl peptides), synthetic adjuvants (e.g. non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), or synthetic polynucleotides adjuvants (e.g polyarginine or polylysine).

As used herein, "administering" includes *in vivo* administration, as well as administration directly to tissue *ex vivo*, such as vein grafts. In the context of the present invention oral, topical, transdermal and/or intracavitary (e.g. intravenous) administration is preferred.

A "therapeutically effective amount" is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

Embodiments of the Invention

In a first aspect the present invention provides mir-199a for use as an indicator of a tissue status or a disease. The surprising finding of the inventors that the presence or absence of mir-199a effects the level of known disease indicators (such as transcription factors, extracellular matrix components, and proteases) evidences that the level of mir-199a correlates to the presence, absence or progression of a tissue status or a disease. Thus, mir-199a is indicative of a tissue status or a disease. The presence, absence or altered amounts of mir-199a are a sign for the presence, absence or progression of a tissue status or disease. In the context of the present invention, an altered tissue status is preferably characterised by the tissue exhibiting different mir-199a level than normal tissue or the same tissue at an earlier time point. A disease status is preferably characterised by an organ or individual exhibiting different mir-199a level in comparison to a normal organ or individual or in comparison to the same organ or individual at an earlier time point.

In a second aspect the present invention relates to the use of mir-199a as an indicator of a tissue status or a disease. Due to the different expression levels of mir-199a

during disease development and/or progression, mir-199a is used as an indicator of a tissue status or a disease.

In further embodiments of the first and/or second aspect of the present invention the level of mir-199a is indicative of (a) a tissue status or a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) the suffering of an individual from an altered tissue status or a disease and/or (d) the progression or a stage of a tissue status or a disease, in an individual. The level of mir-199a is the expression level of mir-199a or the amount of mir-199a present. Accordingly, the expression level of mir-199a and/or the amount of mir-199a present are indicative of (a) a tissue status or a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) the suffering of an individual from an altered tissue status or a disease and/or (d) the progression or a stage of a tissue status or a disease, in an individual. In more preferred embodiments the level of mir-199a is the level of mir-199a in an individual or in a sample of an individual.

In further embodiments an alteration in the mir-199a level indicates a change in tissue status or disease such as the worsening or bettering of a tissue status or disease. Preferably, a lowered level of mir-199a is indicative of a worsening of a tissue status or disease. An elevated level of mir-199a is indicative of a bettering of a tissue status or disease. An alteration in the mir-199a level is also indicative of the risk of developing an altered tissue status or a disease, more preferably a lowered level of mir-199a is indicative of the risk of developing a degenerative tissue status or disease. An altered mir-199a level, preferably a lowered mir-199a level, is also indicative of an individual suffering from an altered tissue status or a disease. Furthermore, an altered mir-199a level, e.g. an elevated or lowered level of mir-199a, indicates the progression or a stage of a tissue status or a disease, in an individual. Preferably a lowered mir-199a level is indicative of a worsening of a tissue status or disease.

It is particularly preferred that the level of mir-199a is indicative of a tissue status wherein the tissue is selected from the group consisting of bone, cartilage, connective tissue, muscle tissue, nervous tissue, and epithelial tissue. Preferably the tissue status is tissue degradation such as but not limited to cartilage degradation, bone degradation, and degradation of the synovium, tissue inflammation such as cartilage inflammation, or inflammation of the synovium, tissue remodelling such as bone remodelling or

cartilage remodelling, sclerosis, liquid accumulation, or proliferative tissue such as proliferation in wound healing processes, cyst formations, or in cancer. It is particularly preferred that the tissue status relates to tissue degradation or tissue inflammation, more preferably to cartilage degradation, degradation of the synovium, cartilage inflammation and/or inflammation of the synovium.

It is further preferred that the level of mir-199a is indicative of a disease wherein the disease is a joint disease such as but not limited to arthritis, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), juvenile idiopathic arthritis (JIA), gout, septic arthritis, psoriatic arthritis, and osteoarthritis (OA), cancer, such as chondrosarcoma, osteosarcoma, fibrosarcoma, and multiple myeloma, tendinitis, bursitis, fractures, and/or damage to cartilage or bone. In particularly preferred embodiments the disease is arthritis, more preferably osteoarthritis.

In a third aspect the present invention provides for a method of identifying (a) an alteration of a tissue status or the presence of a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-199a. Preferably, mir-199a is detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to mir-199a RNA or cDNA. Preferably, the level of mir-199a is the level of mir-199a in an individual or in a sample of an individual. Preferably, the level of mir-199a is the expression level of mir-199a or the amount of mir-199a present in an individual or in a sample of an individual.

It is further preferred that the individual is a mammal, reptile or bird. More preferably, the individual is selected from the group consisting of laboratory animals (e.g. mouse, rat or rabbit), domestic animals (including e.g. guinea pig, rabbit, horse, donkey, cow, sheep, goat, pig, chicken, duck, camel, cat, dog, turtle, tortoise, snake, or lizard), or primates including macacae, chimpanzees, bonobos, gorillas and human beings. It is particularly preferred that the individual is a human being.

The sample of an individual is one or more solid samples such as but not limited to tissue extracts, cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue, or one or more fluid samples such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid. In more preferred

embodiments the sample is selected from the group consisting of cartilage, synovium, capsule, blood, plasma, serum, urine, and synovial fluid. In particularly preferred embodiments the sample is cartilage, synovium, serum, and/or synovial fluid.

The level of mir-199a is detected via any known method using known means for detection of nucleic acids such as specific nucleic acids or polypeptides able to binding the miRNA in question. Suitable methods include but are not limited to Polaymerase-Chain-Reaction (PCR) techniques such as reverse transcriptase PCR or quantitative real time PCR, hybridisation techniques such as Northern Blot, in situ hybridization, or Chip hybridisation, immunohistological or immunohistochemical or immunocytochemical techniques, such as Western Blots, Immunofluorescence or ELISA, Mass Spectrometry (MS), such as LC/MS, High-Performance Liquid Chromatography (HPLC), in particular Ion Exchange HPLC after labelling with a complementary strand.

The means of detecting the level of mir-199a present in an individual or in a sample of an individual include but are not limited to those typically used in above mentioned methods. These include but are not limited to one or more sets of primers capable of specifically detecting mir-199a RNA or cDNA, (e.g. for use in a quantitative RT PCR), one or more nucleic acid probes able to specifically hybridise to mir-199a RNA or cDNA under standard conditions (e.g. for use in Northern Blot or Chip hybridisation techniques), one or more antibodies able to specifically detect mir-199a (e.g. for use in immunohistological or immunohistochemical or immunochemical techniques, e.g. detection of mir-199a in histological tissue sections or mir-199a immobilized on suitable carriers like membranes, chips, ELISA plates etc.).

In further preferred embodiments of the present invention the level of mir-199a in said individual or sample of an individual is compared to the level of mir-199a in one or more references and/or reference samples. Preferably, the reference is selected from the group consisting of a healthy individual, a diseased individual or the same individual as the tested individual at an earlier or later time point. Additionally or alternatively, the reference is a value representative for the level of mir-199a in absence of an altered tissue status or disease, in presence of an altered tissue status or disease or for an increased or decreased risk of developing an altered tissue status or disease.

The reference sample is preferably derived from a healthy individual, a diseased individual, or from the same individual as the sample of interest. Where the reference sample was taken from the same individual as the sample of interest, the reference sample was preferably taken at an earlier or later time point then the sample of interest. The time period which has lapsed between taking of the reference sample and taking of the sample of interest preferably represents years (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 years), months (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months), weeks (e.g. 1, 2, 3, 4, 5, 6, 7, 8 weeks), days (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 days), hours (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours), minutes (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 minutes), or seconds (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 seconds). Alternatively or additionally, the reference sample is a reference sample with a level of mir-199a representative for a healthy individual or representative for the presence or absence of an altered tissue status or disease or representative for an increased or decreased risk of developing an altered tissue status or disease.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, a lowered level of mir-199a in the sample of interest in comparison to said reference indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, a similar level of mir-199a in the sample of interest in comparison to said reference indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, an elevated level of mir-199a in the sample of interest in comparison to said reference indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, a similar level of mir-199a-a indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, a lowered level of mir-199a indicates (a) the presence of a deteriorated tissue status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, an elevated level of mir-199a indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a lowered level of mir-199a-a in the sample of interest indicates (a) the presence of a deteriorated tissue

status or a disease and/or (b) an increased risk to develop a deteriorated tissue status or a disease and/or (c) the progression of a deteriorated tissue status or a disease in the individual.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, an elevated level of mir-199a-a in the sample of interest indicates (a) an alteration of the tissue status or an improvement or absence of a disease and/or (b) a decreased risk to develop a deteriorated tissue status or a disease and/or (c) a declined progression of the deteriorated tissue status or the disease.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a similar level of mir-199a-a in the sample of interest indicates (a) a similar risk to develop a deteriorated tissue status or a disease and/or (b) a stagnation in the progression of a deteriorated tissue status or a disease, and/or (c) a persistence of the deteriorated tissue status or the disease in the individual.

In a fourth aspect the present invention provides a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of (a) determining the level of mir-199a in a sample of the individual, and optionally determining the level of mir-199a in a reference or reference sample for comparison with the level of mir-199a in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the level of mir-199a in the sample of interest, optionally depending on the comparison of the levels of mir-199a in the sample of interest and the reference or reference sample.

In the context of the present invention, levels of mir-199a are preferably detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to the mir-199a gene, gene product(s) and/or functionally active variant(s) thereof. Samples can be fluid or solid, preferably fluid sample are body fluid sample such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, and solid sample are a tissue sample, such as but not limited to cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue. The reference sample

is preferably derived from a healthy individual, a diseased individual, or from the same individual as the sample of interest. Where the reference sample was taken from the same individual as the sample of interest, the reference sample was preferably taken at an earlier or later time point then the sample of interest. Alternatively or additionally, the reference sample is a reference sample with a level of mir-199a representative for a healthy individual or representative for the presence or absence of an altered tissue status or disease or representative for an increased or decreased risk of developing an altered tissue status or disease.

In preferred embodiments the necessity for maintenance or alteration, i.e. an increase or decrease, of the dosage of the pharmaceutical is determined upon determination of the mir-199a levels in the sample, preferably in comparison to a reference or reference sample.

It is envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and a similar level of mir-199a in the

sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a lower level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the necessity of a maintained dosage or of an increased or of a decreased dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is determined.

In a fifth aspect the present invention provides a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of (a) determining the level of mir-199a in a sample, (b) determining the level of mir-199a in one or more references or reference samples, (c) examining the tested sample as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-199a in the sample of interest is different from the level in the one or more references or reference samples.

Levels of mir-199a are preferably detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to the mir-199a gene, gene product(s) and/or functionally active variant(s) thereof. Samples can be fluid or solid, preferably fluid sample are body fluid sample such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, and solid sample are a tissue sample, such as but not limited to cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue.

In preferred embodiments the dosage of the pharmaceutical is maintained or altered, i.e. increase or decrease, upon determination of the mir-199a levels in the sample in comparison to the one or more reference or reference sample.

It is envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or decreased.

In embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained, decreased or minimized.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or decreased.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or increased.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue status or the prevention or treatment of a disease in an individual, is maintained or decreased.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, the dosage of a pharmaceutical beneficial for the alteration of a tissue

status or the prevention or treatment of a disease in an individual, is maintained, increased or decreased.

In a sixth aspect the present invention provides a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of (a) determining the level of mir-199a in a sample of interest, (b) determining the level of mir-199a in one or more references or reference samples, and (c) examining the sample of interest as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples. Preferably, levels of mir-199a are detected on a RNA or DNA level, more preferably via known means such as but not limited to antibodies, primers or nucleic acid probes able to specifically bind and/or hybridise to the mir-199a gene, gene product(s) and/or functionally active variant(s) thereof. Samples can be fluid or solid, preferably fluid sample are body fluid sample such as but not limited to blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid, and solid sample are a tissue sample, such as but not limited to cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue.

In preferred embodiments the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease is determined upon determination of the mir-199a levels in the sample, preferably in comparison to a reference or reference sample.

It is envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, an adverse effect of said substance on a tissue status or the development of a disease is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing

an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, a beneficial effect of said substance on a tissue status or the development of a disease is determined.

It is also envisaged that in embodiments, wherein the reference or reference sample is derived from a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or with a level of mir-199a representative of the absence of an altered tissue status or disease, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, it is determined that said substance has no effect or only very limited effect on a tissue status or the development of a disease.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, it is determined that said substance has no effect or only very limited effect on a tissue status or the development of a disease.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, a beneficial effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease, and a lowered level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, an adverse effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, a lowered level of mir-

199a in the sample of interest in comparison to said reference or reference sample is determined, an adverse effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and an elevated level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, a beneficial effect of said substance on a tissue status or the development of a disease is determined.

In embodiments, wherein the reference or reference sample is derived from the same individual as the individual of interest at an earlier time point, and a similar level of mir-199a in the sample of interest in comparison to said reference or reference sample is determined, it is determined that said substance has no effect or only very limited effect on a tissue status or the development of a disease.

In a seventh aspect the present invention provides the use of mir-199a in a method according to any of the previous aspects of the present invention.

Accordingly, the use of mir-199a is provided in a method of identifying (a) an alteration of a tissue status or the presence of a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-199a, as described in detail above. The use of mir-199a is also provided in a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of (a) determining the level of mir-199a in a sample of the individual, and optionally determining the level of mir-199a in a reference or reference sample for comparison with the level of mir-199a in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the level of mir-199a in the sample of interest, optionally depending on the comparison of the levels of mir-199a in the sample of interest and the reference or reference sample, as described in detail above. Furthermore, the use of mir-199a is envisaged in a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of (a) determining the level of mir-199a in a sample, (b) determining the level

of mir-199a in one or more references or reference samples, (c) examining the tested sample as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-199a in the sample of interest is different from the level in the one or more references or reference samples, as described in detail above. Also the use of mir-199a in a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of (a) determining the level of mir-199a in a sample of interest, (b) determining the level of mir-199a in one or more references or reference samples, and (c) examining the sample of interest as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples, as described in detail above, is provided for. The use of mir-199a in either of the above indicated methods comprises the use of the mir-199a gene, mir-199a gene product(s) and/or functionally active variant(s) thereof.

In preferred embodiments of any of the aspects of the present invention, the sample of interest is a solid and/or fluid sample. The solid sample is preferably a tissue sample and the fluid sample a body fluid sample. It is further preferred that the tissue sample is selected from the group consisting of cartilage, bone, synovium, perichondrium, capsule, skin, and connective tissue, and the fluid sample is selected from the group consisting of blood, serum, plasma, synovial fluid, urine, saliva, and lymphatic fluid. In more preferred embodiments the sample is selected from the group consisting of cartilage, synovium, capsule, blood, plasma, serum, urine, and synovial fluid. In particularly preferred embodiments the sample cartilage, synovium, serum, and/or synovial fluid. Alternatively or additionally, the sample is a cell culture or tissue culture sample, preferably selected from the group consisting of cartilage, bone, synovium, bone marrow cells, chondrocytes, chondroblasts, chondroclasts, synovial cells, osteocytes, osteoclasts, osteoblasts, stem cells and/or mesenchymal stem cells.

In an eighth aspect the present invention provides a kit for use in any of the aspects of the present invention, comprising one or more means of detecting mir-199a. In

preferred embodiments of the kit, the one or more means for detecting mir-199a are means for determining the expression levels of mir-199a, more preferably on a gene and/or RNA level. It is particularly preferred that the one or more means for detecting mir-199a are selected from the group consisting of nucleic acid, preferably DNA or RNA, peptide, and protein, preferably monoclonal or polyclonal antibodies. Preferably, means for detecting mir-199a are either naturally occurring nucleic acid such as DNA or RNA or chemically modified nucleic acids with complementarity to the sequence of mir-199a and/or its precursor.

In further preferred embodiments the kit further comprises (a) a container, and/or (b) a data carrier. It is particularly preferred that the data carrier comprises information such as but not limited to (i) instructions concerning methods for identifying the risk for developing and/or identifying the presence and/or monitoring progression of a joint disease, (ii) instructions for use of the means for detecting mir-199a, preferably in a sample, more preferably in a sample from an individual and/or of the kit, (iii) quality information such as information about the lot/batch number of the means for detecting mir-199a and/or of the kit, the manufacturing or assembly site or the expiry or sell-by date, information concerning the correct storage or handling of the kit, (iv) information concerning the composition of the buffer(s), diluent(s), reagent(s) for detecting mir-199a and/or of the means for detecting mir-199a, (v) information concerning the interpretation of information obtained when performing the above-mentioned methods identifying and/or monitoring progression of a joint disease, (vi) a warning concerning possible misinterpretations or wrong results when applying unsuitable methods and/or unsuitable means, and/or (vii) a warning concerning possible misinterpretations or wrong results when using unsuitable reagent(s) and/or buffer(s).

In a ninth aspect the present invention provides the use of the kit of the eighth aspect in a method of any of the methods of the present invention is provided for. Accordingly, the use of said kit is provided in a method of identifying (a) an alteration of a tissue status or the presence of a disease and/or (b) the risk of developing an altered tissue status or a disease and/or (c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-199a, as described in detail above. The use of said kit is also provided in a method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the

prevention or treatment of a disease in an individual, comprising the steps of (a) determining the level of mir-199a in a sample of the individual, and optionally determining the level of mir-199a in a reference or reference sample for comparison with the level of mir-199a in the sample of interest, and (b) determining the dosage of a pharmaceutical depending on the level of mir-199a in the sample of interest, optionally depending on the comparison of the levels of mir-199a in the sample of interest and the reference or reference sample as described in detail above. Furthermore, the use of said kit is envisaged in a method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of (a) determining the level of mir-199a in a sample, (b) determining the level of mir-199a in one or more references or reference samples, (c) examining the tested sample as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, and (d) adapting the dosage of a pharmaceutical depending on whether the level of mir-199a in the sample of interest is different from the level in the one or more references or reference samples as described in detail above. Also the use of said kit in a method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of (a) determining the level of mir-199a in a sample of interest, (b) determining the level of mir-199a in one or more references or reference samples, and (c) examining the sample of interest as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples, as described in detail above, is provided for.

In a tenth aspect the present invention provides for one or more nucleic acids for detecting a mir-199a gene, gene product(s) or functionally active variant(s) thereof in a method of any of the aspects of the present invention as described in detail above. In preferred embodiments the nucleic acid is a nucleic acid either occurring naturally such as DNA or RNA, or chemically modified. Preferably, the nucleic acid is selected from the group consisting of a nucleic acid probe, a polyamide or peptide nucleic acid (PNA), microRNA (miRNA), small interfering RNA (siRNA), locked nucleic acid (LNA),

primers for polymerase chain reaction (PCR), primers for reverse transcription (RT) reaction, and primers for DNA sequencing. It is particularly preferred that such nucleic acid exhibits a strong binding to mir-199a. Thus, exemplary LNAs of about 8 nucleotides are suitable for detecting mir-199a, as long as the sequence is unique within the entirety of microRNA sequences.

In an eleventh aspect the present invention provides a peptide, polypeptide or protein for detecting a mir-199a gene, gene product(s), functionally active variant(s) thereof and/or a label on a microRNA in a method of any of the aspects of the present invention as described in detail above. In preferred embodiments the peptide, polypeptide or protein is a protein ligand, preferably an antibody, a fragment or derivate thereof, a darpin or an anticalin, or the polypeptide or peptide is a probe, preferably a mass spectrometry probe.

In preferred embodiments the protein ligand is an antibody that has been prepared recombinantly and, where appropriate, modified, such as chimaeric antibodies, humanized antibodies, multifunctional antibodies, bispecific or oligospecific antibodies, single-stranded antibodies and F(ab) or $F(ab)_2$ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213 or WO 98/24884). Additionally or alternatively to the classical antibodies protein scaffolds against mir-199a are used as protein ligands, e.g. anticalins which are based on lipocalin (Beste et al. (1999) Proc. Natl. Acad. Sci. USA, 96, 1898-1903). The natural ligand-binding sites of the lipocalins, for example the retinol-binding protein or the bilin-binding protein, can be altered, for example by means of a “combinatorial protein design” approach, in such a way that they bind to selected haptens, here to mir-199a (Skerra, 2000, Biochim. Biophys. Acta, 1982, 337-50). Other known protein scaffolds are known as being alternatives to antibodies for molecular recognition (Skerra (2000) J. Mol. Recognit., 13, 167-187).

In a twelfth aspect the present invention provides for the use of the nucleic acid of the tenth aspect of the present invention and/or of a peptide, polypeptide or protein of the eleventh aspect of the present invention in a method of any of the aspects of the present invention. In preferred embodiments the nucleic acids is used in naked form, in the form of gene transfer vectors or complexed with liposomes or gold particles. Examples of gene transfer vectors are viral vectors, for example adenoviral vectors or

retroviral vectors (Lindemann et al. (1997), Mol. Med., 3, 466-76; Springer et al. (1988) Mol. Cell., 2, 549-58). Complexes with liposomes usually achieve a very high efficiency of transfection, in particular of skin cells (Alexander and Akhurst, 1995, Hum. Mol. Genet. 4:146-a79-85). In lipofection, small, unilamellar vesicles composed of cationic lipids are prepared by ultrasonicating the liposome suspension. The DNA is bound ionically on the surface of the liposomes in a ratio which is such that a positive net charge remains and all the plasmid DNA is complexed by the liposomes. In addition to the DOTMA (1,2-dioleyloxypropyl-3-trimethylammonium bromide) and DOPE (dioleoylphosphatidylethanolamine) lipid mixtures employed by Felgner, P. L. et al. (1987), Proc. Natl. Acad. Sci USA, 84, 7413 - 7414, a large number of lipid formulations have by now been synthesized and tested for their efficiency in transfecting a variety of cell lines (Behr et al. (1989) Proc. Natl. Acad. Sci. USA, 86, 6982-6986; Zhao and Huang (1991), Biochim. Biophys. Acta, 1189, 195-203; Felgner et al. (1994) J. Biol. Chem., 269, 2550-2561). Examples of the lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulphate or DOGS (dioctadecylamidoglycylspermine). Auxiliary substances which increase the transfer of nucleic acids into the cell can, for example, be proteins or peptides which are bound to the DNA or synthetic peptide-DNA molecules which enable the nucleic acid to be transported into the nucleus of the cell (Schwartz et al. (1999) Gene Therapy 6:282; Brandén et al. (1999) Nature Biotech., 17, 784). Auxiliary substances also include molecules which enable nucleic acids to be released into the cytoplasm of the cell (Planck et al. (1994) J. Biol. Chem., 269, 12918; Kichler et al. (1997) Bioconj. Chem, 8, 213) or, for example liposomes (Uhlmann and Peyman (1990), supra). Another, particularly suitable form can be obtained by applying the above-described nucleic acids to gold particles and firing these particles into tissue or cells using what is termed a “gene gun” (Wang et al. (1999) J. Invest. Dermatol. 112:775-81, Tuting et al. (1998) J. Invest. Dermatol. 111:183-8).

Mir-199a or the mir-199a gene or a functionally active variant thereof may be used as a target molecule for the discovery of a mir-199a agonist or mimic thereof. In particular mir-199a or the mir-199a gene or a functionally active variant thereof are brought into direct or indirect contact with a test compound and the influence of the test compound on mir-199a or the mir-199a gene is measured or detected. Preferably the test

compound is a chemical molecule such as but not limited to non-polymeric organic compounds, lipids, carbohydrates, peptides, preferably peptides with about 10 to about 80 amino acids, in particular with 10 to 25 amino acids and oligonucleotides, preferably with about 10 to about 90 nucleotides, in particular with 15 to 25 nucleotides. Especially preferred are small chemical molecules, in particular non-polymeric organic compounds, either synthesized in a laboratory or found in nature, with a preferred molecular weight of about 200 g/mole to about 1500 g/mole, in particular 400 g/mole to 1000 g/mole.

Preferably, the test compound alters the level of mir-199a such as increases or decreases the expression level or the amount of mir-199a present. In the present context test compounds increasing the level of mir-199a are particularly preferred.

A method of screening for a mir-199a agonist or mimic thereof comprises the steps of (a) providing mir-199a or the mir-199a gene, (b) providing a test compound, and (c) measuring or detecting the influence of the test compound on mir-199a or the mir-199a gene.

A mir-199a agonist or mimic thereof may be used for the alteration of a tissue status or the prevention or treatment of a joint disease .

An agonist for mir-199a or mimic thereof can be any molecule or composition that elevates or increases the function or elevates the expression level or amount of mir-199a present. Examples comprise, but are not limited to: 1. A nucleic acid, such as an oligonucleotide, oligoribonucleotides, especially dsRNA, ssRNA, siRNA, miRNA, shRNA etc, as known in the art, 2. An agonistic peptide or protein, such as a protein stabilising or promoting the function of the miRNA in question, an agonistic antibody or fragment thereof or a transcriptional agonist of the miRNA in question, and/or 3. a small molecule compound with agonistic action. Alternatively or additionally, the agonist of the present invention can be in the form of a natural product extract, either in crude or in purified form. The extract can be produced according to standard procedures, such as water and/or alcohol and/or organic solvent extraction and/or column chromatography and/or precipitation from an animal, plant or microbial source, like snake poison, leaves or microbial fermentation broths.

Such agonist or mimic thereof may be used for the treatment of a joint disease, in particular a degenerative joint disease such as osteoarthritis. The mir-199a agonist or

mimic thereof may be used to treat joint pain, in particular by reducing the joint pain in degenerative joint diseases. In addition, the mir-199a agonist or mimic thereof may be used for the production of a medicament for the treatment of a joint disease and/or for the treatment of the joint pain as specified above.

A pharmaceutical may comprising a mir-199a agonist or mimic thereof for use in the prevention or treatment of a joint disease,. The pharmaceutical may further comprises a pharmaceutically acceptable carrier and/or excipient and optionally one or more additional active substances. The additional active substances may be effective against the same or against a different disease or disorder or condition. Accordingly, additional active substances comprise those having a beneficial effect in the treatment of a joint disease and/or for the treatment of the joint pain as specified above. Furthermore, additional active substances directed at the treatment of different diseases or disorder or conditions suitable to be administered in combination with the active ingredient of the present invention are comprised. Exemplified, additional active substances affecting further miRNAs and/or their gene product(s), such as agonist or antagonists to individual miRNAs, are encompassed. Additional active substances also comprise food supplements such as but not limited to vitamins, minerals, fiber, fatty acids, or amino acids, considered beneficial in a persons diet and/or in the treatment of a joint disease and/or for the treatment of the joint pain as specified above and/or in any other disease, disorder or condition.

For the production of the pharmaceutical the mir-199a agonists or mimic thereof are usually formulated with one or more pharmaceutically acceptable additives or auxiliary substances, such as physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease antagonists, preferably aprotinin, ε-aminocaproic acid or pepstatin A or sequestering agents such as EDTA, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc. depending on the kind of administration. Suitable further additives are, for example, detergents, such as, for example, Triton X-100 or sodium desoxycholate, but also polyols, such as, for example, polyethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic compounds, such as, for example, amino acids such as glycine or in particular taurine or betaine and/or a protein, such as, for

example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

The physiological buffer solution preferably has a pH of approx. 6.0-8.0, expecially a pH of approx. 6.8-7.8, in particular a pH of approx. 7.4, and/or an osmolarity of approx. 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pH of the medicament is in general adjusted using a suitable organic or inorganic buffer, such as, for example, preferably using a phosphate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4-(2-hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3-morpholino-1-propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

For the production of the pharmaceutical the mir-199a agonists or mimic thereof may also be formulated with a nanoparticle, a liposome or other lipid complexation, or may be conjugated to a protein, peptide, nucleic acid (e.g. an aptamer) or gel, e.g. based on hyaluronic acid, or similar slow release formulations.

The pharmaceutical is preferably in unit dosage form. In such form the preparation may be subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packaged tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, an injection vial, a tablet, a cachet, or a lozenge itself, or it can be the appropriate number of any of these in packaged form.

A method of alteration of a tissue status or prevention or treatment of a joint disease, comprises the administration of a therapeutically effective amount of the pharmaceutical comprising a mir-199a agonist or mimic thereof to an individual at risk of developing or suffering from a joint disease. In particular, the pharmaceutical is administered orally, topically, transdermal or intracavitary. Intracavitary administration includes but is not limited to intravenous and intra-articular administration.

The pharmaceutical can be administered in a conventional manner, e.g. by means of oral dosage forms, such as, for example, tablets or capsules, by means of the mucous membranes, for example the nose or the oral cavity, in the form of dispositories

implanted under the skin, by means of injections, infusions or gels which contain the medicaments according to the invention. It is further possible to administer the medicament topically and locally in order to treat the particular joint disease as described above, if appropriate, in the form of liposome complexes. Furthermore, the treatment can be carried out by means of a transdermal therapeutic system (TTS), which makes possible a temporally controlled release of the medicaments. TTS are known for example, from EP 0 944 398 A1, EP 0 916 336 A1, EP 0 889 723 A1 or EP 0 852 493 A1. Injection solutions are in general used if only relatively small amounts of a solution or suspension, for example about 1 to about 20 ml, are to be administered to the body. Intracavitary administration includes but is not limited to infusions and injections. Infusion solutions are in general used if a larger amount of a solution or suspension, for example one or more litres, are to be administered. Since, in contrast to the infusion solution, only a few millilitres are administered in the case of injection solutions, small differences from the pH and from the osmotic pressure of the blood or the tissue fluid in the injection do not make themselves noticeable or only make themselves noticeable to an insignificant extent with respect to pain sensation. Dilution of the formulation according to the invention before use is therefore in general not necessary. In the case of the administration of relatively large amounts, however, the formulation according to the invention should be diluted briefly before administration to such an extent that an at least approximately isotonic solution is obtained. An example of an isotonic solution is a 0.9% strength sodium chloride solution. In the case of infusion, the dilution can be carried out, for example, using sterile water while the administration can be carried out, for example, via a so-called bypass

In preferred embodiments of any aspect of the present invention is the tissue status is tissue damage, preferably selected from the group consisting of damage to bone, cartilage, synovium, perichondrium, capsule, and connective tissue and/or is characterised by altered level of regulatory molecules, signalling molecules, and/or degenerative molecules present in the tissue.

In preferred embodiments of any aspect of the present invention is the disease is a joint disease, preferably selected from the group consisting of osteoarthritis, rheumatoid arthritis, psoriatic arthritis, septic arthritis, Ankylosing spondylitis, Bursitis

(inflammation), Dermatomyositis, Fibromyalgia, Gouty arthritis, Juvenile chronic arthritis (Still's disease), Mixed Connective Tissue Disease, Polymyalgia rheumatica, Polymyositis, Reactive arthritis (Reiter's Syndrome), Scleroderma, Shoulder tendinitis, Sjorgen's Sydrome, Systemic Lupus Erythemetosis and/or is characterised by a physical or metabolic injury of the joint indicated by an inflammation of the joint or a degradation of the joint, more preferably wherein the disease is osteoarthritis.

Further, non-limiting aspects and embodiments of the present invention are described below.

The diagnostic methods involve measuring the levels of one or more miRNAs in patient samples and using the test results to diagnose and/or predict an optimal treatment regimen for the patient. Compositions described in the invention include antagonists of mir-199a., that function as miRNA antagonists that can be introduced to a patient to treat and reduce one or more of the conditions associated with joint diseases, especially with osteoarthritis.

The diagnostic methods involve measuring the levels of one or more miRNAs in patient samples and using the test results to diagnose and/or predict an optimal treatment regimen for the patient. Compositions described in the invention include agonists of mir-199a / antagonists of mir-199a, that function as miRNAs / miRNA agonist/antagonists that can be introduced to a patient to treat and reduce one or more of the conditions associated with inflammatory diseases, especially with osteoarthritis.

In the context of one or more of the present aspects of the invention (e.g. such as using the miRNA as biomarker), the detection of the miRNA can be performed by any known method using known means for detection of nucleic acids such as specific nucleic acids able to binding the miRNA in question. One aspect of present invention concerns the use of a means for detecting the miRNA in question amount in a biological sample for the manufacture of a diagnostic means for diagnosing a malfunction of the carbohydrate or lipid metabolism.

The means to detect the amount (i.e. the steady state level) of the miRNA in question present in a biological sample can be any means to detect the miRNA in question. This can be for example a set of primers capable of specifically detecting the miRNA in question or the cDNA, e.g. for use in a quantitative RT PCR. Another means can e.g. be a nucleic acid probe able to specifically hybridise to miRNA in question mRNA or cDNA under standard conditions, e.g. for use in Northern Blot or Chip hybridisation techniques. Another means can e.g. be an antibody able to specifically detect the miRNA in question e.g. for use in immunohistological or immunohistochemical or immunochemical techniques (e.g. detection of miRNA in question in histological tissue sections or miRNA in question immobilized on suitable carriers like membranes, chips, ELISA plates etc.).

According to one embodiment, the invention concerns a method for identifying a joint disease such as OA or an increased risk for developing a joint disease, such as OA in an individual, which comprises examining a sample taken from the individual, as to whether the level of mir-199a and/or the activity of mir-199a present in said sample is different from that of one or more reference samples. The presence of an elevated level indicating an increased risk of said individual to suffer from or to develop a joint disease such as OA.

The terms "lowered level" or "elevated level" refer to the amount of the miRNA in a sample of tissue or tissue fluid known to have, under certain circumstances, detectable levels lf the miRNA in question (such as cellular or tissue extract, synovial fluid, serum or plasma), especially from an individual to be tested, compared to the amount of the miRNA in comparable sample from a cohort or cohorts that do not have the joint disease, especially OA. E.g. a miRNA that is detectable in higher amounts in synovial fluid of one individual suffering from OA than in synovial fluid of individuals not suffering from OA, has an elevated level. For mir-199a, an elevated level in a patient sample (such as synovial fluid) indicates the presence of OA or increased susceptibility or increased probability to develop OA.

In some cases, the level of the miRNA marker will be compared to a control to determine whether the level is reduced or elevated. The control may be an external control, such as the miRNA in a sample from a patient known to be free of the joint

disease especially OA. In other circumstances, the external control may be the miRNA in a sample from a tissue/tissue fluid known not to have detectable amounts (or always the same amount, irrespective of the disease-state of the individual) of the miRNA in question or a known amount of a synthetic RNA. An internal control may be a miRNA from the tissue/tissue fluid sample being tested. The identity of a miRNA control may be the same as or different from the patient serum or plasma miRNA being measured.

In the context of present invention, an antagonist for a miRNA can be any molecule or composition that lowers or inhibits the function or amount of the miRNA in question. Examples comprise, but are not limited to:

1. A nucleic acid, such as an oligonucleotide, oligoribonucleotides, especially dsRNA, ssRNA, siRNA, miRNA, shRNA, ssDNA, ssDNA/RNA hybrids or chemically modified nucleic acids etc., as known in the art.

2. An antagonistic peptide or protein, such as a protein destabilising or inhibiting the function of the miRNA in question, an antagonistic antibody or fragment thereof or a transcriptional antagonist of the miRNA in question,

3. A small molecule compound with antagonistic action,

4. A composition comprising one or more of 1-4 and possibly an excipient.

One subject matter of the present invention is, therefore, the use of a antagonist for the treatment of a joint disease, in particular a degenerative joint disease such as osteoarthritis. The miRNA antagonist can also be used to treat the joint pain, in particular by reducing the joint pain in degenerative joint diseases. In addition, the miRNA antagonist can be used for the production of a medicament for the treatment of a joint disease and/or for the treatment of the joint pain as specified above.

According to the present invention the term “chemical molecule” encompasses non-polymeric organic compounds, lipids, carbohydrates, peptides, preferably peptides with about 10 to about 80 amino acids, in particular with 10 to 25 amino acids and oligonucleotides, preferably with about 10 to about 90 nucleotides, in particular with 15 to 25 nucleotides. Especially preferred are small chemical molecules, in particular non-polymeric organic compounds, either synthesized in a laboratory or found in nature,

with a preferred molecular weight of about 200 g/mole to about 1500 g/mole, in particular 400 g/mole to 1000 g/mole.

Alternatively the antagonist of the present invention can be in the form of a natural product extract, either in crude or in purified form. The extract can be produced according to standard procedures, such as water and/or alcohol and/or organic solvent extraction and/or column chromatography and/or precipitation from an animal, plant or microbial source, like snake poison, leaves or microbial fermentation broths.

The term “binding protein” or “binding peptide” refers to a class of proteins or peptides which bind and inhibit mir-199a including, without limitation, polyclonal or monoclonal antibodies, antibody fragments and protein scaffolds directed against mir-199a, e.g. anticalins which are directed against mir-199a.

The procedure for preparing an antibody or antibody fragment is effected in accordance with methods which are well known to the skilled person, e.g. by immunizing a mammal, for example a rabbit, with mir-199a, where appropriate in the presence of, for example, Freund’s adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al. (1981) The New England Journal of Medicine: 1344-1349). The polyclonal antibodies which are formed in the animal as a result of an immunological reaction can subsequently be isolated from the blood using well known methods and, for example, purified by means of column chromatography. Monoclonal antibodies can, for example, be prepared in accordance with the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293-299).

According to the present invention the term antibody or antibody fragment is also understood as meaning antibodies or antigen-binding parts thereof, which have been prepared recombinantly and, where appropriate, modified, such as chimaeric antibodies, humanized antibodies, multifunctional antibodies, bispecific or oligospecific antibodies, single-stranded antibodies and F(ab) or $F(ab)_2$ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213 or WO 98/24884).

As an alternative to the classical antibodies it is also possible, for example, to use protein scaffolds against mir-199a, e.g. anticalins which are based on lipocalin (Beste et al. (1999) Proc. Natl. Acad. Sci. USA, 96, 1898-1903). The natural ligand-binding

sites of the lipocalins, for example the retinol-binding protein or the bilin-binding protein, can be altered, for example by means of a "combinatorial protein design" approach, in such a way that they bind to selected haptens, here to mir-199a (Skerra, 2000, Biochim. Biophys. Acta, 1982, 337-50). Other known protein scaffolds are known as being alternatives to antibodies for molecular recognition (Skerra (2000) J. Mol. Recognit., 13, 167-187).

The term "nucleic acids against the mir-199a gene or mir-199a itself" refers to double-stranded or single stranded DNA or RNA which, for example, inhibit or activate the expression of the mir-199a gene or the activity of mir-199a and includes, without limitation, antisense nucleic acids, aptamers, siRNAs (small interfering RNAs), miRNAs, shRNAs (short hairpin RNAs) and ribozymes.

The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584). Aptamers are nucleic acids which bind with high affinity to a polypeptide, here mir-199a. Aptamers can be isolated by selection methods such as SELEX (see e.g. Jayasena (1999) Clin. Chem., 45, 1628-50; Klug and Famulok (1994) M. Mol. Biol. Rep., 20, 97-107; US 5,582,981) from a large pool of different single-stranded RNA molecules. Aptamers can also be synthesized and selected in their mirror-image form, for example as the L-ribonucleotide (Nolte et al. (1996) Nat. Biotechnol., 14, 1116-9; Klussmann et al. (1996) Nat. Biotechnol., 14, 1112-5). Forms which have been isolated in this way enjoy the advantage that they are not degraded by naturally occurring ribonucleases and, therefore, possess greater stability.

Nucleic acids may be degraded by endonucleases or exonucleases, in particular by DNases and RNases which can be found in the cell. It is, therefore, advantageous to modify the nucleic acids in order to stabilize them against degradation, thereby ensuring that a high concentration of the nucleic acid is maintained in the cell over a long period of time (Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Typically, such a stabilization can be obtained by introducing one or more internucleotide phosphorus groups or by introducing one or more non-phosphorus internucleotides.

Suitable modified internucleotides are compiled in Uhlmann and Peyman (1990), supra (see also Beigelman et al. (1995) Nucleic Acids Res. 23:3989-94; WO 95/11910; WO 98/37240; WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid which can be employed in one of the uses according to the invention contain, for example, methyl phosphonate, phosphorothioate, phosphoramidate, phosphorodithioate and/or phosphate esters, whereas non-phosphorus internucleotide analogues contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also the intention that this modification should improve the durability of a pharmaceutical composition which can be employed in one of the uses according to the invention.

The use of suitable antisense nucleic acids is further described e.g. in Zheng and Kemeny (1995) Clin. Exp. Immunol., 100, 380-2; Nellen and Lichtenstein (1993) Trends Biochem. Sci., 18, 419-23, Stein (1992) Leukemia, 6, 697-74 or Yacyshyn, B. R. et al. (1998) Gastroenterology, 114, 1142).

The production and use of siRNAs as tools for RNA interference in the process to down regulate or to switch off gene expression, here mir-199a gene expression, is e.g. described in Elbashir, S. M. et al. (2001) Genes Dev., 15, 188 or Elbashir, S. M. et al. (2001) Nature, 411, 494.

Ribozymes are also suitable tools to inhibit the translation of nucleic acids, here the RAK gene, because they are able to specifically bind and cut the mRNAs. They are e.g. described in Amarzguioui et al. (1998) Cell. Mol. Life Sci., 54, 1175-202; Vaish et al. (1998) Nucleic Acids Res., 26, 5237-42; Persidis (1467) Nat. Biotechnol., 15, 921-2 or Couture and Stinchcomb (1996) Trends Genet., 12, 510-5.

The nucleic acids described are preferably meant to be used or act as antagonists of mir-199a.

For the production of the medicament the mir-199a antagonists of the present invention are usually formulated with one or more pharmaceutically acceptable additives or auxiliary substances, such as physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease antagonists, preferably aprotinin, ε-aminocaproic acid or pepstatin A or sequestering

agents such as EDTA, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc. depending on the kind of administration.

Suitable further additives are, for example, detergents, such as, for example, Triton X-100 or sodium desoxycholate, but also polyols, such as, for example, polyethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic compounds, such as, for example, amino acids such as glycine or in particular taurine or betaine and/or a protein, such as, for example, bovine or human serum albumin. Detergents, polyols and/or zwitterionic compounds are preferred.

The physiological buffer solution preferably has a pH of approx. 6.0-8.0, expecially a pH of approx. 6.8-7.8, in particular a pH of approx. 7.4, and/or an osmolarity of approx. 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pH of the medicament is in general adjusted using a suitable organic or inorganic buffer, such as, for example, preferably using a phosphate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4-(2-hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3-morpholino-1-propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer is suitable, for example, for injection and infusion solutions.

The medicament can be administered in a conventional manner, e.g. by means of oral dosage forms, such as, for example, tablets or capsules, by means of the mucous membranes, for example the nose or the oral cavity, in the form of dispositories implanted under the skin, by means of injections, infusions or gels which contain the medicaments according to the invention. It is further possible to administer the medicament topically and locally in order to treat the particular joint disease as described above, if appropriate, in the form of liposome complexes. Furthermore, the treatment can be carried out by means of a transdermal therapeutic system (TTS), which makes possible a temporally controlled release of the medicaments. TTS are known for example, from EP 0 944 398 A1, EP 0 916 336 A1, EP 0 889 723 A1 or EP 0 852 493 A1.

Injection solutions are in general used if only relatively small amounts of a solution or suspension, for example about 1 to about 20 ml, are to be administered to the body.

Infusion solutions are in general used if a larger amount of a solution or suspension, for example one or more litres, are to be administered. Since, in contrast to the infusion solution, only a few millilitres are administered in the case of injection solutions, small differences from the pH and from the osmotic pressure of the blood or the tissue fluid in the injection do not make themselves noticeable or only make themselves noticeable to an insignificant extent with respect to pain sensation. Dilution of the formulation according to the invention before use is therefore in general not necessary. In the case of the administration of relatively large amounts, however, the formulation according to the invention should be diluted briefly before administration to such an extent that an at least approximately isotonic solution is obtained. An example of an isotonic solution is a 0.9% strength sodium chloride solution. In the case of infusion, the dilution can be carried out, for example, using sterile water while the administration can be carried out, for example, via a so-called bypass.

The above-described nucleic acids can be used in naked form, in the form of gene transfer vectors or complexed with lipids (especially cationic lipids) or gold particles, they may be formulated as liposomes (wherein the nucleic acid is e.g. carried as cargo inside a membraneous lipidlayer such as a lipid bilayer), or may be based on nanoparticles e.g. on dextrane, peptides, or other polymers.
Examples of gene transfer vectors are viral vectors, for example adenoviral vectors or retroviral vectors (Lindemann et al. (1997), Mol. Med., 3, 466-76; Springer et al. (1988) Mol. Cell., 2, 549-58). Complexes with liposomes usually achieve a very high efficiency of transfection, in particular of skin cells (Alexander and Akhurst, 1465, Hum. Mol. Genet. 4:146-a79-85). In lipofection, small, unilamellar vesicles composed of cationic lipids are prepared by ultrasonicating the liposome suspension. The DNA is bound ionically on the surface of the liposomes in a ratio which is such that a positive net charge remains and all the plasmid DNA is complexed by the liposomes. In addition to the DOTMA (1,2-dioleyloxypropyl-3-trimethylammonium bromide) and DOPE (dioleoylphosphatidylethanolamine) lipid mixtures employed by Felgner, P. L. et al. (1987), Proc. Natl. Acad. Sci USA, 84, 7413 - 7414, a large number of lipid formulations have by now been synthesized and tested for their efficiency in transfecting a variety of cell lines (Behr et al. (1989) Proc. Natl. Acad. Sci. USA, 86, 6982-6986; Zhao and Huang (1991), Biochim. Biophys. Acta, 1189, 195-203; Felgner et al. (1994) J. Biol.

Chem., 269, 2550-2561). Examples of the lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulphate or DOGS (dioctadecylamidoglycylspermine).

Auxiliary substances which increase the transfer of nucleic acids into the cell can, for example, be proteins or peptides which are bound to the DNA or synthetic peptide-DNA molecules which enable the nucleic acid to be transported into the nucleus of the cell (Schwartz et al. (1999) Gene Therapy 6:282; Brandén et al. (1999) Nature Biotech., 17, 784). Auxiliary substances also include molecules which enable nucleic acids to be released into the cytoplasm of the cell (Planck et al. (1994) J. Biol. Chem., 269, 12918; Kichler et al. (1997) Bioconj. Chem, 8, 213) or, for example liposomes (Uhlmann and Peyman (1990), supra).

Another, particularly suitable form can be obtained by applying the above-described nucleic acids to gold particles and firing these particles into tissue or cells using what is termed a “gene gun” (Wang et al. (1999) J. Invest. Dermatol. 112:775-81, Tuting et al. (1998) J. Invest. Dermatol. 111:183-8).

Another subject matter of the present invention is the use of mir-199a or the mir-199a gene as a target for the discovery of a mir-199a antagonist for the treatment of a joint disease, in particular a degenerative joint disease such as osteoarthritis or and joint disease such as rheumatoid arthritis, and/or for the treatment of joint pain, in particular by reducing the joint pain in degenerative joint diseases. Preferably the mir-199a antagonist can be used in form of a medicament as described above.

Accordingly, the present invention refers also to a method of screening a mir-199a antagonist, wherein the method comprises the steps of:

(a) providing mir-199a or the mir-199a gene,

(b) providing a test compound, and

(c) measuring or detecting the influence of the test compound on mir-199a or the mir-199a gene.

In general, mir-199a or the mir-199a gene is provided e.g. in an assay system and brought directly or indirectly into contact with a test compound, in particular a biochemical or chemical test compound, e.g. in the form of a chemical compound library. Then, the influence of the test compound on mir-199a or the mir-199a gene is measured or detected. Thereafter, suitable antagonists can be analyzed and/or

isolated. For the screening of chemical compound libraries, the use of high-throughput assays are preferred which are known to the skilled person or which are commercially available.

According to the present invention the term “chemical compound library” refers to a plurality of chemical compounds that have been assembled from any of multiple sources, including chemically synthesized molecules and natural products, or that have been generated by combinatorial chemistry techniques.

In general, the influence of the test compound on mir-199a or the mir-199a gene is measured or detected in a heterogeneous or homogeneous assay. As used herein, a heterogeneous assay is an assay which includes one or more washing steps, whereas in a homogeneous assay such washing steps are not necessary. The reagents and compounds are only mixed and measured.

Suitable functional assays may be based on the gene expression of mir-199a.

In particular, the present invention relates to the following aspects:

1. Mir-199a for use as an indicator of a tissue status or a disease.

2. Use of Mir-199a as an indicator of a tissue status or a disease.

3. Mir-199a according to aspect 1 or use of mir-199a according to aspect 2 , wherein the level of mir-199a is indicative of

(a) a tissue status or a disease and/or

(b) the risk of developing an altered tissue status or a disease and/or

(c) the suffering of an individual from an altered tissue status or a disease and/or

(d) the progression or a stage of a tissue status or a disease, in an individual.

4. A method of identifying

(a) an alteration of a tissue status or the presence of a disease and/or

(b) the risk of developing an altered tissue status or a disease and/or

(c) monitoring the progression or the stage of a tissue status or a disease

in an individual, comprising detecting the level of mir-199a.

5. Mir-199a or use of mir-199a according to aspect 3 or method of aspect 4, wherein the level of mir-199a is the level of mir-199a in an individual or in a sample of an individual.

6. Mir-199a or use of mir-199a or method according to aspect 5 further comprising comparing the level of mir-199a in said individual or sample to the level of mir-199a in one or more references or reference samples.

7. Mir-199a or use of mir-199a or method according to aspect 6, wherein the reference is selected from the group consisting of a healthy individual, a diseased individual or the same individual as the tested individual at an earlier or later time point or a value representative for the level of mir-199a in absence of the altered tissue status or disease, in presence of an altered tissue status or disease or for an increased or decreased risk of developing an altered tissue status or disease.

8. Mir-199a or use of mir-199a or method according to aspect 7, wherein the reference sample is selected from the group consisting of a reference sample derived from a healthy individual, a reference sample derived from a diseased individual, a reference sample derived from the same individual as the sample of interest taken at an earlier or later time point, and a reference sample with a level of mir-199a representative for a healthy individual or representative for the presence or absence of an altered tissue status or disease or representative for an increased or decreased risk of developing an altered tissue status or disease.

9. Mir-199a or use of mir-199a or method according to aspect 7, wherein the reference is a healthy individual or an individual with a decreased risk of developing an altered tissue status or a disease or a level of mir-199a representative of the absence of an altered tissue status or disease, or mir-199a or use of mir-199a or method according to aspect 8, wherein the reference sample is derived from a healthy individual or from an individual with a decreased risk of developing an altered tissue status or disease or comprises a level of mir-199a representative of a healthy

individual or of a status of disease-absence or for a decreased risk of developing an altered tissue status or disease, wherein a lowered level of mir-199a indicates

(a) the presence of a deteriorated tissue status or a disease and/or

(b) an increased risk to develop a deteriorated tissue status or a disease and/or

(c) the progression of a deteriorated tissue status or a disease

in the individual.

10. Mir-199a or use of mir-199a or method according to aspect 7, wherein the reference is a diseased individual or an individual with an increased risk of developing an altered tissue status or a disease or a value representative of the presence of an altered tissue status or disease or mir-199a or use of mir-199a or method according to aspect 8, wherein the reference sample is derived from a diseased individual or from an individual with an increased risk of developing an altered tissue status or disease or comprises a level or amount of mir-199a representative for a diseased individual or for a status of disease-presence or for an increased risk of developing an altered tissue status or disease, wherein a similar level of mir-199a indicates

(a) the presence of a deteriorated tissue status or a disease and/or

(b) an increased risk to develop a deteriorated tissue status or a disease and/or

(c) the progression of a deteriorated tissue status or a disease

in the individual.

11. Mir-199a or use of mir-199a or method according to aspect 7, wherein the reference is the same individual at an earlier time point, or mir-199a or use of mir-199a or method according to aspect 8 wherein the reference sample is derived from the same individual as sample of interest and was taken at an earlier time point, wherein

(i) a lowered level of mir-199a in the sample of interest indicates

(a) the presence of a deteriorated tissue status or a disease and/or

(b) an increased risk to develop a deteriorated tissue status or a disease and/or

(c) the progression of a deteriorated tissue status or a disease

in the individual.

,

(ii) an elevated level of mir-199a in the sample of interest indicates

(a) an alteration of the tissue status or an improvement or absence of a disease and/or
(b) a decreased risk to develop a deteriorated tissue status or a disease and/or
(c) a declined progression of the deteriorated tissue status or the disease,

(iii) a similar level of mir-199a in the sample of interest indicates
(a) a similar risk to develop a deteriorated tissue status or a disease and/or
(b) a stagnation in the progression of a deteriorated tissue status or a disease, and/or
(c) a persistence of the deteriorated tissue status or the disease
in the individual.

12. A method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of
(a) determining the level of mir-199a in a sample of the individual, and optionally determining the level of mir-199a in a reference or reference sample for comparison with the level of mir-199a in the sample of interest, and
(b) determining the dosage of a pharmaceutical depending on the level of mir-199a in the sample of interest, optionally depending on the comparison of the levels of mir-199a in the sample of interest and the reference or reference sample.

13. A method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of
(a) determining the level of mir-199a in a sample,
(b) determining the level of mir-199a in one or more references or reference samples,
(c) examining the tested sample as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, and
(d) adapting the dosage of a pharmaceutical depending on whether the level of mir-199a in the sample of interest is different from the level in the one or more references or reference samples.

14. A method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of

(a) determining the level of mir-199a in a sample of interest,

(b) determining the level of mir-199a in one or more references or reference samples, and

(c) examining the sample of interest as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples,

wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples.

15. Mir-199a or use of mir-199a according to any of aspects 5 to 11 or method according to any of aspects 5 to 14, wherein the sample of interest is tissue and/or fluid.

16. Mir-199a or use of mir-199a or method of aspect 15, wherein the tissue sample is selected from the group consisting of tissue extract, synovial tissue and cartilage, and the body fluid sample is selected from the group consisting of synovial fluid, serum plasma and urine.

17. Use of mir-199a in a method according to any of aspects 4 to 16.

18. A kit for use in a method according to any of aspects 4 to 16, comprising one or more means of detecting mir-199a.

19. The kit according to aspect 18 wherein the one or more means for detecting mir-199a are means for determining the expression levels of mir-199a, preferably on a gene and/or RNA level.

20. The kit according to aspect 18 or 19, wherein the one or more means for detecting mir-199a are selected from the group consisting of nucleic acid, preferably

DNA or RNA, a mixture of both or chemically modified nucleic acids, peptide, and protein, preferably monoclonal or polyclonal antibodies.

21. The kit according to any of aspects 18 to 20, wherein the kit further comprises
(a) a container, and/or
(b) a data carrier, wherein the data carrier comprises information such as
(i) instructions concerning methods for identifying the risk for developing and/or identifying the presence and/or monitoring progression of a joint disease
(ii) instructions for use of the means for detecting mir-199a, preferably in a sample, more preferably in a sample from an individual and/or of the kit,
(iii)quality information such as information about the lot/batch number of the means for detecting mir-199a and/or of the kit, the manufacturing or assembly site or the expiry or sell-by date, information concerning the correct storage or handling of the kit,
(iv)information concerning the composition of the buffer(s), diluent(s), reagent(s) for detecting mir-199a and/or of the means for detecting mir-199a,
(v)information concerning the interpretation of information obtained when performing the above-mentioned methods identifying and/or monitoring progression of a joint disease,
(vi)a warning concerning possible misinterpretations or wrong results when applying unsuitable methods and/or unsuitable means, and/or
(vii) a warning concerning possible misinterpretations or wrong results when using unsuitable reagent(s) and/or buffer(s).

22. Use of the kit of any of aspects 18 to 21 in a method according to any of aspects 4 to 16.

23. One or more nucleic acids for detecting a mir-199a gene, gene product or functionally active variant thereof in a method according to any of aspects 4 to 16.

24. The nucleic acid according to aspect 23, wherein the nucleic acid is selected from the group consisting of a locked nucleic acid (LNA), nucleic acid probe, a polyamide or peptide nucleic acid (PNA), microRNA (miRNA), small interfering RNA

(siRNA), primers for polymerase chain reaction (PCR), primers for reverse transcription (RT) reaction, and primers for DNA sequencing.

25. The nucleic acid according to aspect 24 comprising the nucleotide sequence of at least 8 nucleotides.

26. A peptide, polypeptide or protein for detecting a mir-199a gene, gene product or functionally active variant thereof in a method according to any of aspects 4 to 16.

27. The peptide, polypeptide or protein according to aspect 25, wherein the protein or polypeptide is a protein ligand, preferably an antibody, a fragment or derivate thereof, a darpin or an anticalin, or wherein the polypeptide or peptide is a probe, preferably a mass spectrometry probe.

28. Mir-199a or use of mir-199a according to any of aspects 5 to 11 or method according to any of aspects 5 to 14, and 35 to 36, use of the kit of aspect 22, the nucleic acid of any of aspects 23 or 25, the peptide polypeptide or protein of aspect 26 or 27, or the use of the nucleic acid, peptide, polypeptide or protein of aspect 28, wherein the tissue status is tissue damage, preferably selected from the group consisting of damage to bone, cartilage, synovium, perichondrium, capsule, and connective tissue and/or is characterised by altered level of regulatory molecules, signalling molecules, and/or degenerative molecules present in the tissue.

29. Mir-199a or use of mir-199a according to any of aspects 5 to 11 or method according to any of aspects 5 to 14, and 35 to 36, use of the kit of aspect 22, the nucleic acid of any of aspects 23 to 25, the peptide polypeptide or protein of aspect 26 or 27, or the use of the nucleic acid, peptide, polypeptide or protein of aspect 28, wherein the disease is a joint disease, preferably selected from the group consisting of osteoarthritis, rheumatoid arthritis, psoriatic arthritis, septic arthritis, Ankylosing spondylitis, Bursitis (inflammation), Dermatomyositis, Fibromyalgia, Gouty arthritis, Juvenile chronic arthritis (Still's disease), Mixed Connective Tissue Disease, Polymyalgia rheumatica, Polymyositis, Reactive arthritis (Reiter's Syndrome),

Scleroderma, Shoulder tendinitis, Sjorgen's Sydrome, Systemic Lupus Erythemetosis and/or is characterised by a physical or metabolic injury of the joint indicated by an inflammation of the joint or a degradation of the joint, more preferably wherein the disease is osteoarthritis.

Further aspects of the present invention are the following:

1. [Prognostic Method] A method of identifying an increase in risk for joint disease in a subject comprising:

a) measuring the level of mir-199a in a biological sample from the subject; and

b) comparing the level of mir-199a in the sample to the level of mir-199a in a suitable control

wherein a decrease in the level of mir-199a in the sample is determined as compared to the control, thereby identifying the subject as having an increased risk for joint disease.

2. [Diagnostic Method] A method for diagnosing a joint disease in a subject, the method comprising:

a) measuring the level of mir-199a in a biological sample from the subject; and

b)comparing the level of mir-199a in the sample to the level of mir-199a in a suitable control

wherein a decrease in the level of mir-199a in the sample is determined as compared to the control, thereby identifying the subject as having the joint disease.

3. [Patient Stratification] A method for treating a subject having a joint disease, the method comprising:

a) measuring the level of mir-199a in a biological sample from the subject;

b)comparing the level of mir-199a in the sample to the level of mir-199a in a suitable control; and

c) administering to the patient a pharmaceutical composition for treatment of joint disease when a decrease in the level of mir-199a in the sample is determined as compared to the control, thereby treating the subject.

4. [Dosage Optimization] A method for determining the dosage of a pharmaceutical for treating a subject having a joint disease, the method comprising:

a) measuring the level of mir-199a in a biological sample from the subject;

b)comparing the level of mir-199a in the sample to the level of mir-199a in a suitable control; and

c) decreasing the dosage of a pharmaceutical composition when an increase in the level of mir-199a in the sample is determined as compared to the control or increasing the dosage of the pharmaceutical composition when a decrease in the level of mir-199a in the sample is determined as compared to the control,

thereby determining the dosage of the pharmaceutical in the subject.

5. [Screening Assays] A method for determining the effect of a substance or test compound on a joint disease, the method comprising:

a) measuring the level of mir-199a in a sample treated with the substance or test compound;

b) comparing the level of mir-199a in the treat sample to the level of mir-199a in a suitable control; and

c) identifying the substance as having an adverse effect if a decrease in the level of mir-199a in the sample is determined as compared to the control or identifying the substance as a having a beneficial effect if an increase in the level of mir-199a in the sample is determined as compared to the control,

thereby determining the effect of the substance or test compound on joint disease.

6. The method of any one of aspects 1-4, wherein the level of mir-199a is detected using one or more nucleic acid probes.

7. The method of any one of aspects 1-6, wherein the suitable control is a biological sample from a healthy subject.

8. The method of aspect 6, wherein the suitable control is a sample that is not treated with substance.

9. The method of any one of the previous aspects, wherein the joint disease is osteoarthritis.

10. [Method of treatment with mir-199a antagonist] A method of treating a joint disease in a subject comprising administering a mir-199a agonist to the subject, thereby treating joint disease in the subject.

11. The method of aspect 10, wherein the mir-199a agonist is selected from the group consisting of an siRNA, a shRNA, an antisense RNA, and a ribozyme.

12. The method of aspect 10, wherein the mir-199a agonist, is substantially complementary to at least a portion of the precursor form of mir-199a (SEQ ID NO:1).

13. The method of aspect 10, wherein the mir-199a agonist is substantially complementary to at least a portion of the mature form of mir-199a (SEQ ID NO:2) or mir-199a* (SEQ ID NO:3).

14. The method of aspect 10, wherein the miRNA-146a agonist is a nucleic acid molecule of about 6 to about 20 nucleotides in length.

15. The method of aspect 10, wherein miRNA-146a agonist is a LNA-modified or PNA-modified nucleic acid molecule.

16. The method of aspect 10, wherein the joint disease is osteoarthritis.

17. The method of aspect 10, wherein administration of the miR-146 agonist results in the increase in one or more chondrogenic markers in chondrocytes of the subject.

18. The method of aspect 17, wherein the one or more chondrogenic markers are selected from the group consisting of collagen II, aggrecan, Sox9, collagen X, ALP, CD-Rap, MMP-1 and MMP-3.

19. The method of aspect 11, wherein the mammal is a human.

20. A kit comprising one or more means of detecting mir-199a and a suitable control.

The invention is described in more detail in the examples and figures that are not to be understood as limiting the scope of present invention.

Examples

Example 1: mir-199a expression profile in chondrocyte and stem cells during their cultivation

The expression of mir-199a was determined in primary chondrocytes and bone marrow derived mesenchymal stem cells (BMSC) during their cultivation in order to assess the normal fluctuation in the expression level of mir-199a in these cells over the indicated time periods.

The chondrocytes were cultivated in pellet culture for 0, 1, 4, 7 or 14 days in DMEM medium supplemented with 2mM L-glutamine (Sigma-Aldrich), 1× non essential amino acids (Sigma-Aldrich), 10nM dexamethasone (Sigma-Aldrich), 10µg/ml insulin, 5.5µg/ml transferrin, 5ng/ml sodium selenite (Sigma-Aldrich), 44µg/ml ascorbic acid (Sigma-Aldrich) and 10ng/ml TGF-β1 (R&D Systems). BMSC were cultivated in pellet culture for 0, 1, 4, 7, 14 or 21 days in the same medium. Chondrocytes and BMSC were harvested and each pellet was processed individually for RNA isolation. For the analysis of secreted proteins supernatants were collected and pooled over the entire culture period. For extraction of the RNA, Pellets were harvested after seven days of culture and separately homogenized with the Mixermill (Retsch). A proteinase K digest was performed onto the homogenized cells. Total RNA including miRNA was eluted from the Qiagen miRNeasy Plus Micro kit. The RNA was reverse transcribed into cDNA using the Reverse Transcriptase Kit and RNase Inhibitor (Life Technologies)

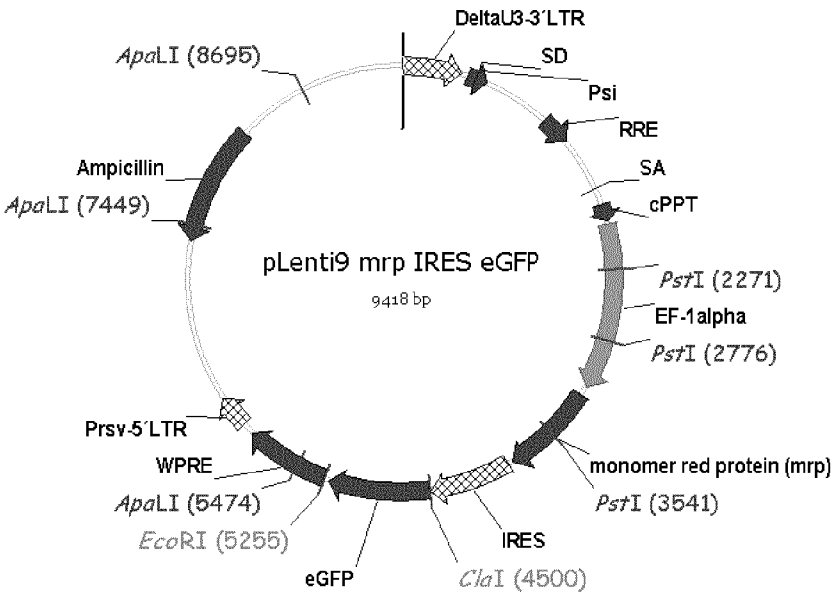
according to the manufacturer's instructions. The mir-199a expression profile in chondrocyte and BMSC was determined via miRNA specific quantitative real time PCR using Taqman probes according to protocols well-known to the skilled person. The ABI Taqman® microRNA Low density arrays (TLDA, Applied Biosystems, Foster City, CA) were used to profile 668 miRNAs and additional reference genes. The assay includes three steps: a multiplex RT, a preamplification and a singleplex TaqMan PCR. All steps were performed according to the manufacturer's instructions. The Real-time PCR was done on an AB 7900HT Sequence Detection System. For expression analysis the comparative Ct method was applied.

Fig. 1a illustrates the mir-199a expression during the cultivation period in chondrocyte pellets and Fig. 2 illustrates the mir-199a expression during the cultivation period in BMSC. An Expression of mir-199a could be detected in chondrocytes at a cycle threshold (ct) of about 16, which increased during to cultivation period to 22 cycles (ct = 16-22) whilst the ct for BMSCs increased from 18 to 20 during to indicated cultivation period. Thus, the expression level of mir-199a increased in both cell lines with increased term of cultivation. The cultivation conditions were chosen such as to reflect conditions of healthy differentiated cartilage. The increase in mir-199a expression over time is thus, indicative of an increased mir-199a expression in differentiated, healthy cartilage. Accordingly, mir-199a is an indicator of the physiological, differentiated cartilage. This increase in expression level over cultivation time stands in contrast to the relatively stable expression levels of mir-22 and mir-146a in parallel experiments conducted under the same conditions (Fig. 1b), both micro RNAs identified by the inventors as markers of osteoarthritic cartilage. Moreover, a similar increase in expression under similar conditions over time could be observed for mir-140, a micro RNA identified by present inventors to be associated with the physiological, differentiated cartilage state. Thus, an increased expression of mir-199a in comparison to certain controls, e.g. markers of osteoarthritic cartilage such as mir-22 and/or mir-146a is indicative for a physiological cartilage state and the absence of OA. Accordingly, mir-199a is a indicator of differentiated cartilage.

Example 2: Overexpression and Knock down of mir-199a in BMSC

The expression of the proteins collagen II, aggrecan, ALP, MMP-1 and MMP-3 is know to be altered in arthritic joints. Their respective expression levels are thus, indicative of the presence or absence of OA. To investigate the role of mir-199a in OA, the effects of its overexpression or knockdown on these molecules, measured either on mRNA (collagen II, aggrecan) or on protein level (MMP-1, and MMP-3) or as activity (ALP) are analysed in BMSC after chondrogenic differentiation.

In three independent experiments (V15, V18, V20) BMSC were infected with pseudoviral particles comprising either the DNA encoding for mir-199a (herein further referred to as: mir-199a) or MRP ((herein further referred to as: empty vector) Mir-199a had been cloned into pLenti9, a lentiviral vector containing partial sequences of HIV1 LTRs (see map below). Pseudoviral particles were produced as a custom service by Vectalys (Toulouse, France). Infection of BMSCs was carried out with a multiplicity of infection (MOI) of five. For enhancement of infection 8µg/ml Polybrene was added and the plates were centrifuged for 90 min at 500×g at 32°C. On the following day high-density pellets consisting of 2.5×10^5 cells were formed by centrifugation (300×g, 5 min) in 96 well conical bottom plates (Eppendorf).



Cells were harvested after 7 days of pellet culture and their RNA isolated following the same protocol as given in Example 1. cDNA synthesis was performed using the Reverse Transcriptase Kit and RNase Inhibitor (Life Technologies) according to the manufacturer's instructions. The expression levels of the four chondrogenic markers collagen II, aggrecan, Sox9, and collagen X were determined via quantitative real time PCR. TaqMan reactions were performed using TaqMan® Universal PCR Master Mix and the FAMTM-labelled gene specific assays for the target genes ACAN, COL2A1, COL10A1, and SOX9 (see table). All gene specific assays consist of FAM™ dye-labeled TaqMan® MGB probes. PCR was performed using the ABI Prism 7900 (Life Technologies). Ribosomal protein L37a (RPL37a) was used as reference gene. For RPL37a the following sequences were selected: reference forward primer: GGCACTGTGGTTCCTGCAT, reference reverse primer: ACAGCGGAAGTGGTATTGTACGT. The MGB reference probe was labeled with VIC and had the sequence: CCGCCAGCCACTGTCT. For expression analysis the comparative Ct method was applied.

Target gene	Gene symbol	Accession No.	Gene specific assay ID
Aggrecan	ACAN	NM_013227	Hs00153936_m1
Collagen, type II, alpha 1	COL2A1	NM_033150	Hs00264051_m1

The effects of mir-199a overexpression on the expression levels of two different marker proteins (i.e. collagen II and aggrecan) in three different, independent experiments (i.e. V15, V18, V20) are illustrated in Fig. 3 (see Fig. 3 A-B). Each experiment comprised four samples (n = 4).

The expression levels of the marker protein ALP, and MMP-1 were tested in experiments V15 and V18 (see Fig. 3 C-D) using ELISA according to protocols well-known to the skilled person. Protein levels were quantified in pellet culture supernatants using ELISA kits according to the manufacturer's instructions: MMP-1 (R&D Systems). For each single pellet culture supernatants obtained at every medium change within the whole culture period of three weeks were collected and pooled for

determination of protein levels by ELISA for each single pellet. Alkaline phosphatase (ALP) activity levels were quantified spectrophotometrically in pellet culture supernatants using ALP buffer and p-nitrophenyl phosphate (p-NPP) substrate concentrate (Millipore). Culture supernatants were collected at day 20. The amount of culture medium applied to the pellets during culture was identical for each pellet. Cells over-expressing mir-199a also expressed increased levels of collagen II, aggrecan and ALP, whilst the levels of MMP-1 were decreased. These results confirm that an increased mir-199a expression can be associated with differentiated cartilage.

In three different experiments (V21, V22, V23) mir-199a was knocked-down in BMSC (herein further referred to as: mir-199a inh) using miRIDIAN Hairpin Inhibitor hsa-mir-199a (Dharmacon) and the effects on the expression of the three marker proteins collagen II, aggrecan and ALP were observed in comparison to control cells. BMSCs were treated with miRIDIAN Hairpin Inhibitor hsa-mir-199a at a concentration of 30 nM in monolayer culture. miRIDIAN microRNA Hairpin Inhibitor Negative Control #1 (Dharmacon) served as negative control. On the following day high-density pellets consisting of 2.5×10^5 cells were formed by centrifugation (300×g, 5 min) in 96 well conical bottom plates (Eppendorf). Pellets were cultured for a maximum of 3 week. All following steps of analysis were performed as already described for over-expression of mir-199a.

The effects of the mir-199a knock-down on the expression levels of the three marker proteins (i.e. collagen II, aggrecan, and ALP) in three different, independent experiments (i.e. V21, V22, V23) are illustrated in Fig. 4 A-C.

Per experiment four samples were examined (n = 4). The expression levels of all three marker proteins tested were decreased upon knock-down of mir-199a. Thus, lowering of mir-199a levels led to decreased levels of marker proteins of physiological cartilage. Accordingly, lowered levels of mir-199a are indicative of the presence of OA. The expression levels of mir-199a are thus indicative of the physiological or pathological state of cartilage, especially with respect to the presence or absence of osteoarthritis. It can further be concluded that agonists of mir-199a (expression or function) can be used for the treatment or prevention of OA by increasing the expression of proteins that are indicative and beneficial for the physiological state of cartilage.

Fig. 5 further illustrates the percentage changes of the expression levels of the marker proteins collagen II and aggrecan due to overexpression or knock-down of mir-199a in comparison to the non-treated controls.

5

Sequence Listing – Free Text Information

SEQ ID NO: 1 Homo sapiens stem-loop sequence of hsa-mir-199a-1 (accession number: MI0000242)

SEQ ID NO: 2 hsa-miR-199a-5p (accession number: MIMAT0000231)

SEQ ID NO: 3 hsa-miR-199a-3p (accession number: MIMAT0000232)

SEQ ID NO: 4 Homo sapiens stem-loop sequence of hsa-mir-199a-2 (accession number: MI0000281)

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Claims

1. Mir-199a for use as an indicator of a tissue status or a disease.

2. Use of mir-199a as an indicator of a tissue status or a disease.

3. A method of identifying
(a) an alteration of a tissue status or the presence of a disease and/or
(b) the risk of developing an altered tissue status or a disease and/or
(c) monitoring the progression or the stage of a tissue status or a disease in an individual, comprising detecting the level of mir-199a.

4. A method for determining the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease in an individual, comprising the steps of
(a) determining the level of mir-199a in a sample of the individual, and optionally determining the level of mir-199a in a reference or reference sample for comparison with the level of mir-199a in the sample of interest, and
(b) determining the dosage of a pharmaceutical depending on the level of mir-199a in the sample of interest, optionally depending on the comparison of the levels of mir-199a in the sample of interest and the reference or reference sample.

5. A method for adapting the dosage of a pharmaceutical for the alteration of a tissue status or the prevention or treatment of a disease, comprising the steps of
(a) determining the level of mir-199a in a sample,
(b) determining the level of mir-199a in one or more references or reference samples,

(c) examining the tested sample as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples, and

(d) adapting the dosage of a pharmaceutical depending on whether the level of mir-199a in the sample of interest is different from the level in the one or more references or reference samples.

6. A method of determining the beneficial and/or adverse effects of a substance on a tissue status or the development of a disease, comprising the steps of

(a) determining the level of mir-199a in a sample of interest,

(b) determining the level of mir-199a in one or more references or reference samples, and

(c) examining the sample of interest as to whether the level of mir-199a present in said sample of interest is different from the level in the one or more references or reference samples,

wherein the sample of interest was exposed differently to said substance than the one or more references or reference samples.

7. Use of mir-199a in a method according to any of claims 3 to 6.

8. A kit for use in a method according to any of claims 3 to 6, comprising one or more means of detecting mir-199a.

9. Use of the kit of claim 8 in a method according to any of claims 3 to 6.

10. One or more nucleic acids for detecting a mir-199a gene, gene product or functionally active variant thereof in a method according to any of claims 3 to 6.

11. A peptide, polypeptide or protein for detecting a mir-199a gene, gene product or functionally active variant thereof in a method according to any of claims 3 to 6.

12. Use of a nucleic acid according to claim 10, or of a peptide, polypeptide or protein according to claim 11 in a method according to any of claims 3 to 6.

5

Fig. 1a

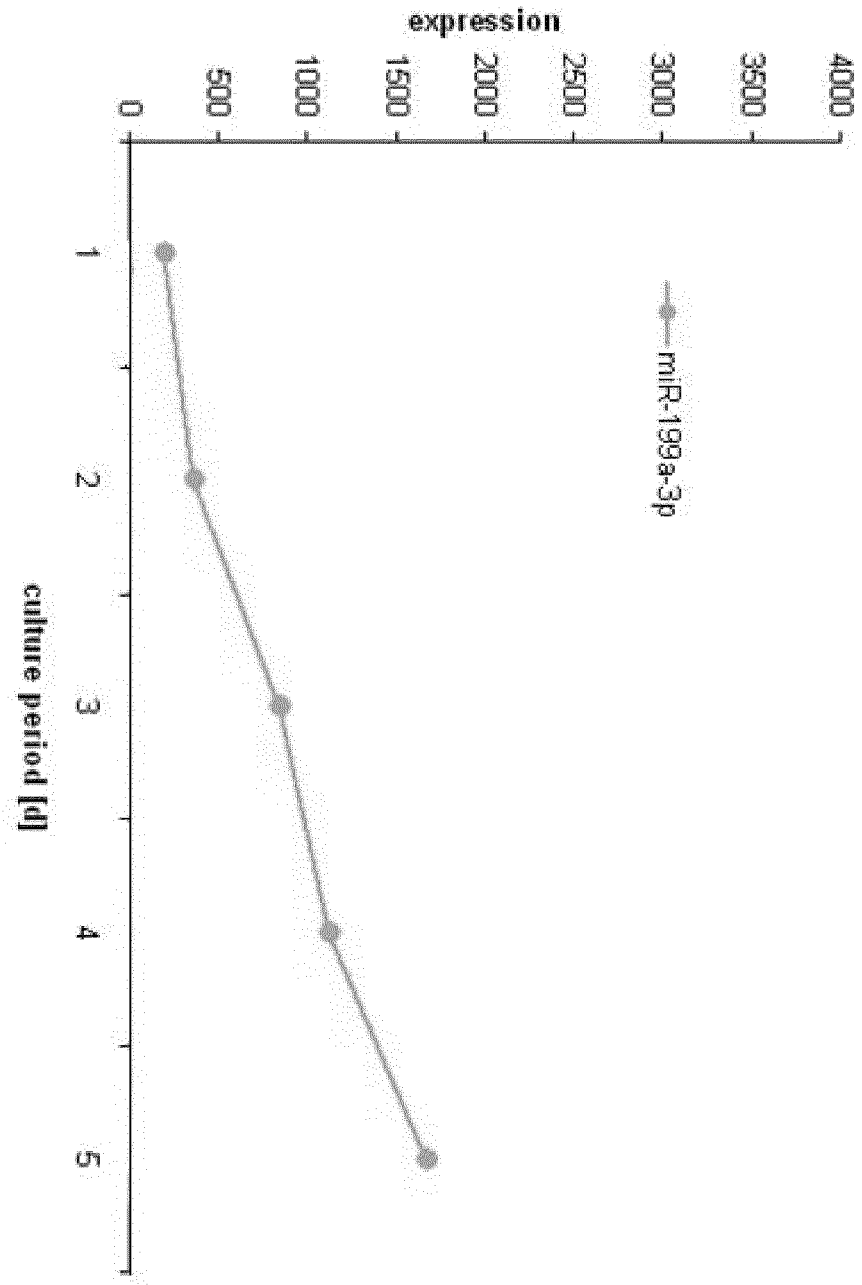

expression
0
500
1000
1500
2000
2500
3000
3500
4000
culture period [d]
1
2
3
4
5
miR-199a-3p

Fig. 1b

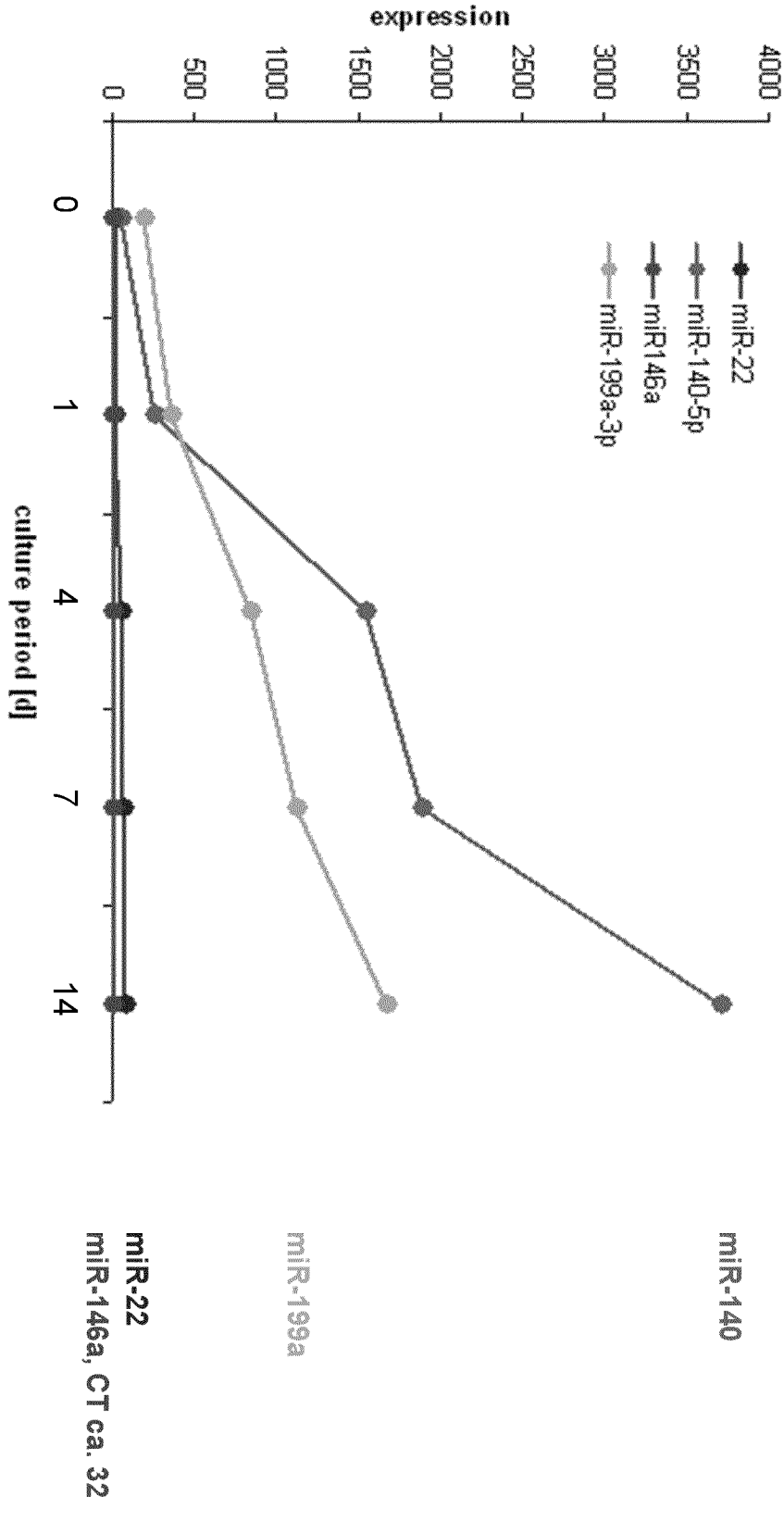

expression
4000
3500
3000
2500
2000
1500
1000
500
0
miR-22
miR-140-5p
miR146a
miR-199a-3p
0
1
4
7
14
culture period [d]
miR-140
miR-199a
miR-22
miR-146a, CT ca. 32

Fig. 2

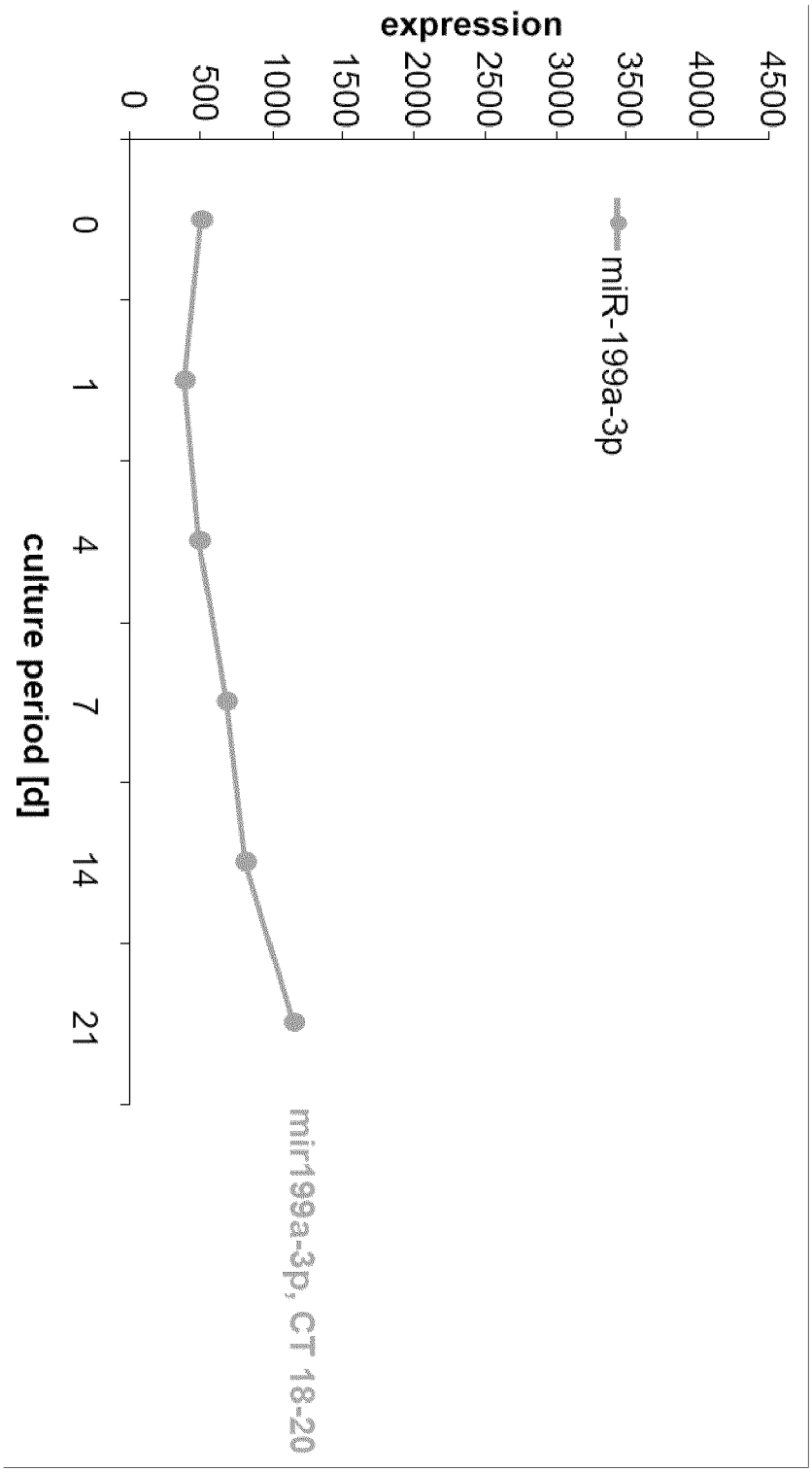

expression
4500
4000
3500
3000
2500
2000
1500
1000
500
0
miR-199a-3p
0
1
4
7
14
21
culture period [d]
mir199a-3p, CT 18-20

Fig. 3

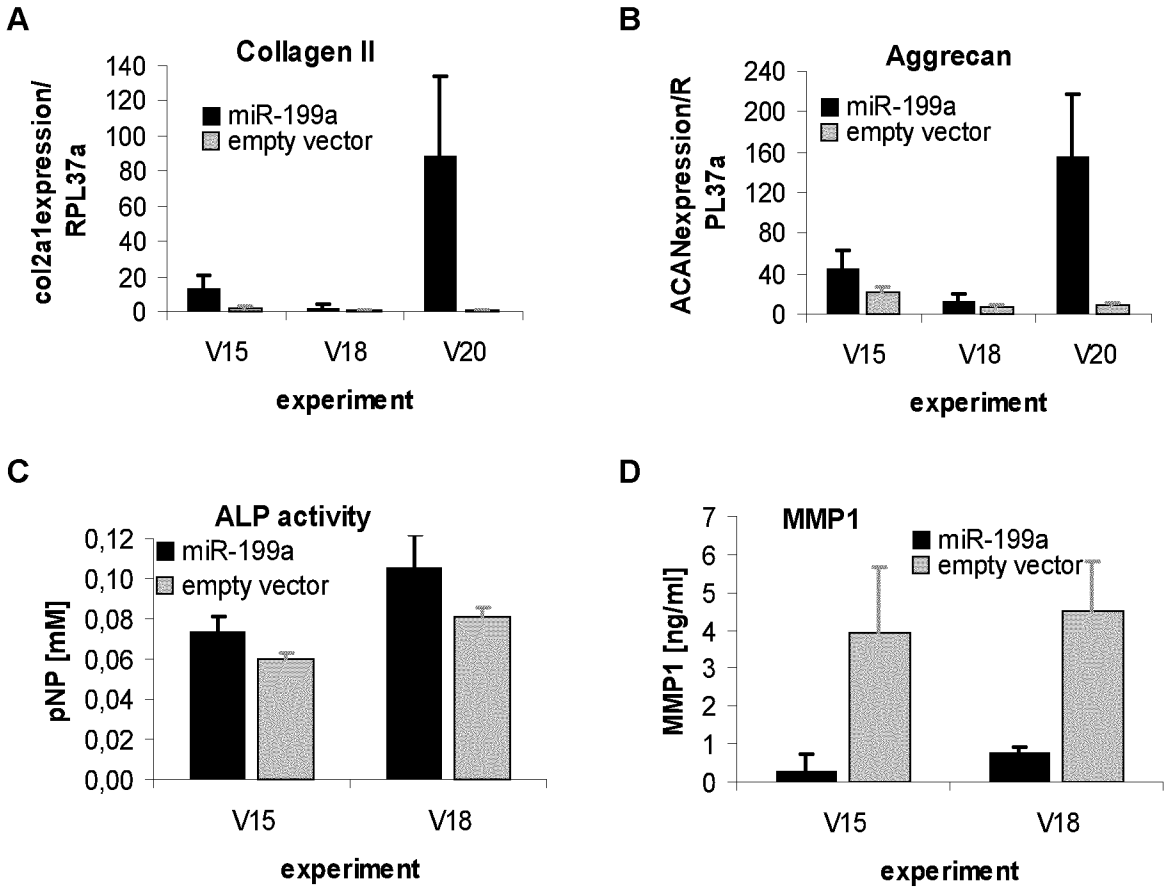

A
Collagen II
col2a1expression/ RPL37a
140
120
100
80
60
40
20
0
miR-199a
empty vector
V15
V18
V20
experiment
B
Aggrecan
ACANexpression/R PL37a
240
200
160
120
80
40
0
miR-199a
empty vector
V15
V18
V20
experiment
C
ALP activity
pNP [mM]
0,12
0,10
0,08
0,06
0,04
0,02
0,00
miR-199a
empty vector
V15
V18
experiment
D
MMP1
MMP1 [ng/ml]
7
6
5
4
3
2
1
0
miR-199a
empty vector
V15
V18
experiment

Fig. 4

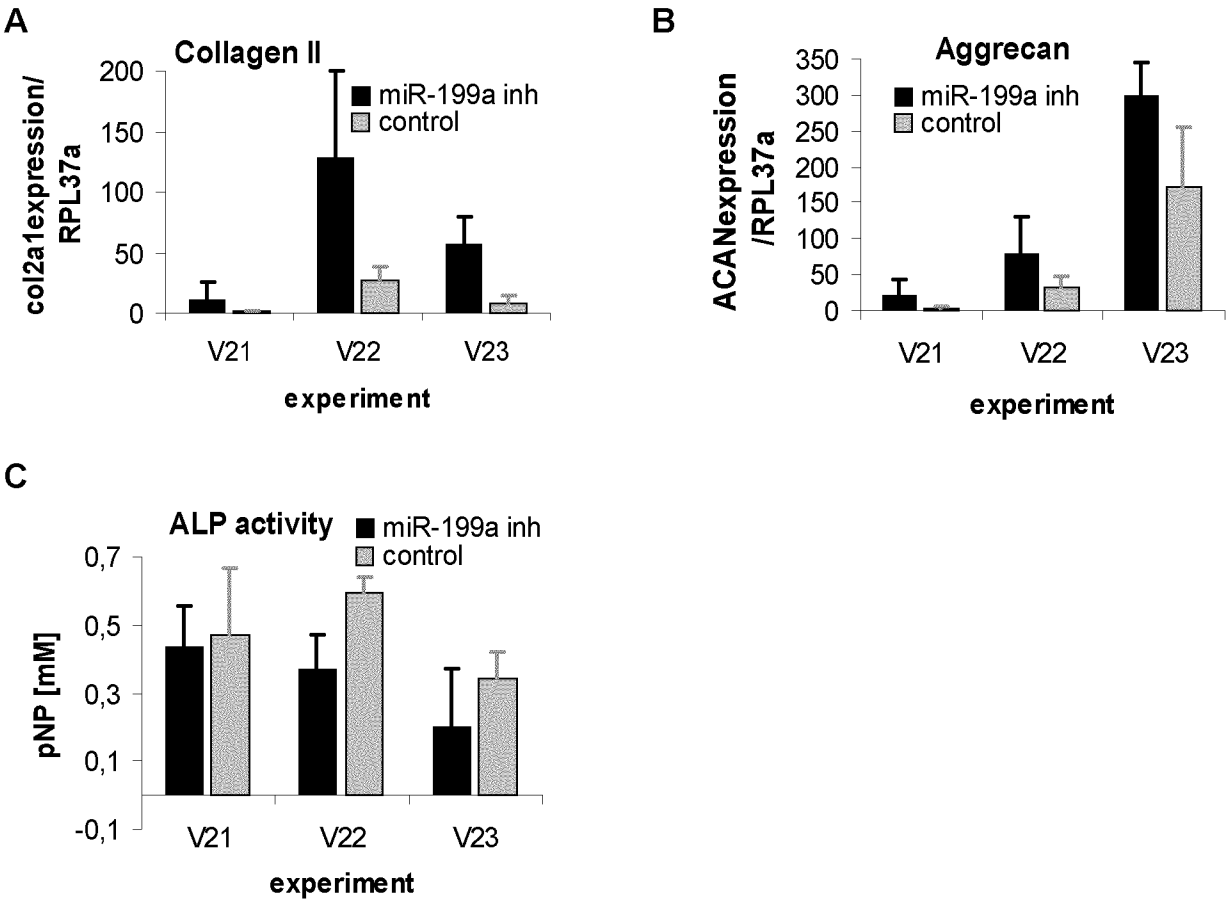

A
Collagen II
col2a1expression/ RPL37a
200
150
100
50
0
miR-199a inh
control
V21
V22
V23
experiment
B
Aggrecan
ACANexpression /RPL37a
350
300
250
200
150
100
50
0
miR-199a inh
control
V21
V22
V23
experiment
C
ALP activity
pNP [mM]
0,7
0,5
0,3
0,1
-0,1
miR-199a inh
control
V21
V22
V23
experiment

Fig. 5

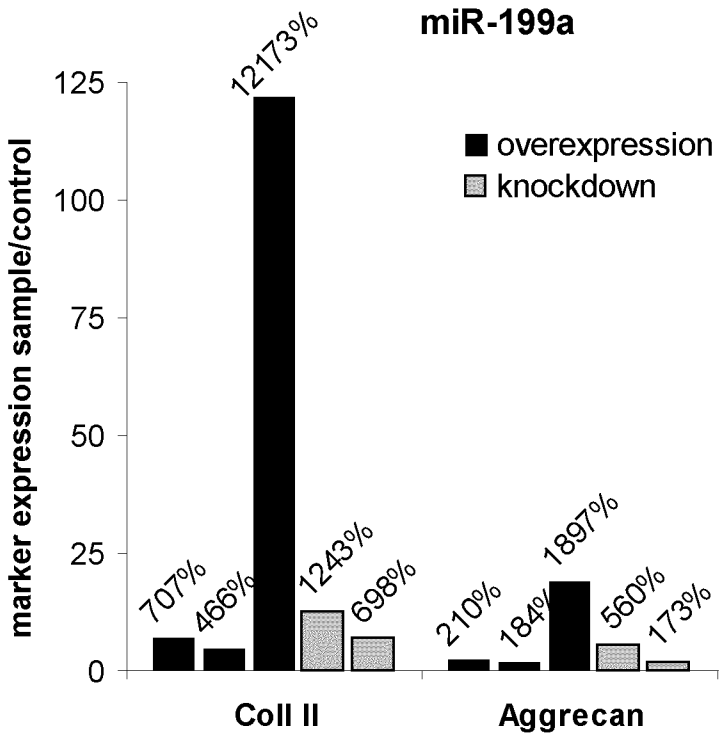
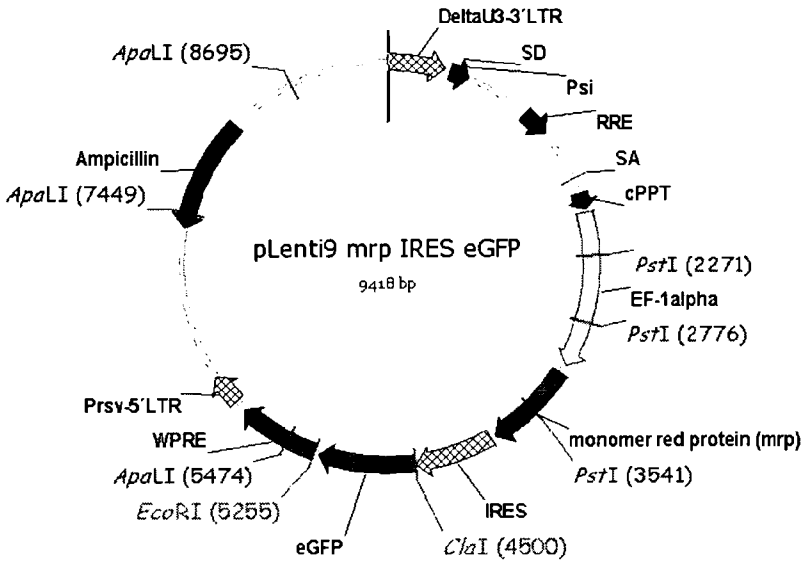

miR-199a
marker expression sample/control
125
100
75
50
25
0
overexpression
knockdown
707%
466%
12173%
1243%
698%
210%
184%
1897%
560%
173%
Coll II
Aggrecan

Fig. 6


DeltaU3-3'LTR
SD
Psi
RRE
SA
cPPT
PstI (2271)
EF-1alpha
PstI (2776)
monomer red protein (mrp)
PstI (3541)
IRES
ClaI (4500)
eGFP
EcoRI (5255)
ApaLI (5474)
WPRE
Prsv-5'LTR
ApaLI (7449)
Ampicillin
ApaLI (8695)
pLenti9 mrp IRES eGFP
9418 bp

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/073036

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/713
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, Sequence Search, EMBL, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/097136 A1 (UNIV LELAND STANFORD JUNIOR [US]; CLARKE MICHAEL [US]; SHIMONO YOHEI []) 6 August 2009 (2009-08-06) claims 1,7,8	1-12
X	WO 2009/151600 A2 (UNIV TUFTS [US]; TUFTS MEDICAL CT INC [US]; HATA AKIKO [US]; DAVIS BRA) 17 December 2009 (2009-12-17) claims 1,55,62,145,150,152,156; example 8; table 1	1-12

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2012	26/03/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. A. LIN ET AL: "miR-199a*, a Bone Morphogenic Protein 2-responsive MicroRNA, Regulates Chondrogenesis via Direct Targeting to Smad1", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 284, no. 17, 1 January 2008 (2008-01-01), pages 11326-11335, XP55022015, ISSN: 0021-9258, DOI: 10.1074/jbc.M807709200 figures 1-8	1-10,12
A	TOKUMITSU WATANABE ET AL: "Dnm3os , a non-coding RNA, is required for normal growth and skeletal development in mice", DEVELOPMENTAL DYNAMICS, vol. 237, no. 12, 1 December 2008 (2008-12-01), pages 3738-3748, XP55022186, ISSN: 1058-8388, DOI: 10.1002/dvdy.21787 page 3744, right-hand column - page 3745, left-hand column	1-12
A	ANNALISA PALMIERI ET AL: "Peptide-15 Changes miRNA Expression in Osteoblast-Like Cells", IMPLANT DENTISTRY, vol. 17, no. 1, 1 January 2008 (2008-01-01), pages 100-108, XP55022180, ISSN: 1056-6163, DOI: 10.1097/ID.0b013e318166d182 right-hand column	1-12
A	A. Z. OSKOWITZ ET AL: "Human multipotent stromal cells from bone marrow and microRNA: Regulation of differentiation and leukemia inhibitory factor expression", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 105, no. 47, 1 November 2008 (2008-11-01), pages 18372-18377, XP55022187, ISSN: 0027-8424, DOI: 10.1073/pnas.0809807105 figure 3	1-12

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NORIHISA FURUKAWA ET AL: "Optimization of a microRNA expression vector for function analysis of microRNA", JOURNAL OF CONTROLLED RELEASE, ELSEVIER, AMSTERDAM, NL, vol. 150, no. 1, 2 December 2010 (2010-12-02), pages 94-101, XP028148659, ISSN: 0168-3659, DOI: 10.1016/J.JCONREL.2010.12.001 [retrieved on 2010-12-10] figure 1; table 1 -----	1-12
A	FURER V ET AL: "The role of microRNA in rheumatoid arthritis and other autoimmune diseases", CLINICAL IMMUNOLOGY, ACADEMIC PRESS, US, vol. 136, no. 1, 1 July 2010 (2010-07-01), pages 1-15, XP027074866, ISSN: 1521-6616, DOI: 10.1016/J.CLIM.2010.02.005 [retrieved on 2010-06-03] the whole document -----	1-12

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/073036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 2713469 A1	06-08-2009
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		EP 2247750 A1	10-11-2010
		JP 2011511634 A	14-04-2011
		US 2011021607 A1	27-01-2011
		WO 2009097136 A1	06-08-2009
WO 2009151600 A2	17-12-2009	US 2011152352 A1	23-06-2011
		WO 2009151600 A2	17-12-2009