

Use of Cathepsin H

Present invention concerns the use of Cathepsin H. Other aspects of the invention concern methods for screening pharmaceuticals, for diagnosing pain susceptibility and for the treatment of pain.

In the western world, chronic pain is a major unsolved health problem undermining the health and welfare of millions of citizens. Chronic pain severely afflicts the well-being of the individual experiencing it and it is frequently accompanied or followed by vegetative signs, which often result in depression. Chronic pain results in individual suffering and social economic costs of tremendous extent. Existing pharmacological pain therapies are widely unsatisfying both in terms of efficacy and of safety.

In light of the severe drawbacks connected with state of the art pain treatments, there is a great need for novel options for treatment of ongoing pain, and for diagnosis and prognosis concerning the potential development of chronic pain. Especially in light of the vast gap between the fast advancing understanding of the neurobiology of pain and the unmet clinical need to provide effective treatments without the drawbacks of state of the art treatments, efforts need to be directed to the discovery of new targets for novel classes of analgesics.

Thus, it is the object of the present invention to provide a new means for the development and provision of new classes of pain modulating drugs.

This object is solved by the use of Cathepsin H or functional fragments or derivatives thereof for identifying compounds that modulate pain and especially neuropathic pain.

The invention is based on the surprising finding of the inventors, demonstrating for the first time that Cathepsin H expression closely correlates with pain susceptibility in mouse models of neuropathic pain.

Pain is, per definition of the international association for the study of pain, an unpleasant sensory and emotional experience associated with actual or potential

tissue damage, or described in terms of such damage. Pain is normally the consequence of an activation of the nociceptive nervous system, that is specialized to detect and encode damage or potential damage of tissue. Pain is thus part of a warning system of the body to initiate reactions for minimizing actual or potential damage to the body. Pain can be the primary symptom of a medical condition or can be secondary effect of a diseased state, often without any biological meaning.

Pain may be acute or chronic. Acute pain is a physiological signal indicating a potential or actual injury. It occurs accompanying tissue damage, infection, inflammation or other acute causes, alerting the individual after bodily damage or malfunction. If acute pain is not treated properly, it may lead to chronic pain.

Chronic pain is a diseased state with varying origin, duration, intensity and specific symptoms.

Chronic pain may be of nociceptive origin, inflammatory or neuropathic. Nociceptive pain is judged to be commensurate with ongoing activation of somatic or visceral pain-sensitive nerve fibers. Neuropathic pain is pain resulting from any kind of damage to peripheral or central neuronal tissue; it is believed to be sustained by aberrant somatosensory processes in the peripheral nervous system, the CNS, or both. (For an overview of pain mechanisms, see for example Scholz and Woolf, 2002; Julius and Basbaum, 2001, Woolf and Mannion, 1999; Wood, J.D., 2000; Woolf and Salter, 2000.)

Chronic neuropathic pain is variable from patient to patient. Recent data indicate that individual pain susceptibility plays an important role for the amount of individual suffering, i.e. there is an important heritable predisposition to pain, particularly to the development of neuropathic pain. Present invention is based on extensive studies of the inventors which aimed to identify pain susceptibility genes (i.e. genes that determine the amount of pain felt in the presence of a given, fixed degree of tissue injury) in rodent models of chronic pain. The rodent models and experimental settings used by the inventors allowed for experimental conditions where among the different

individuals a) nature and uniformity of the neural lesion can be precisely controlled and b) genetic and environmental variability can be minimized.

Cathepsin H (CTSH; alternative titles according to the Atlas of Genetics and

5 Cytogenetics in Oncology and Haematology (<http://atlasgeneticsoncology.org>): ACC-4, ACC-5, CPSB, DKFZp686B24257, EC 3.4.22.16, MGC1519, aleurain, minichain) is a lysosomal protease.

The gene locus of human Cathepsin H is on chromosome 15q24-125 (see e.g. Atlas of Genetics and Cytogenetics in Oncology and Haematology). The gene contains 12
10 exons spanning over 23 kb genomic sequences. The nucleotide sequence of the human preprocathepsin H was e.g. published by Fuchs and Gassen, 1989, Nucleic Acids Res. 17: 9471-9471. The gene structure of rat cathepsin H was published e.g. by Ishidoh et al, FEBS Letters, 1989, Vol. 253, number 1,2, 103-107. The genomic sequence of homo sapiens CTSH on chromosome 15 can e.g. be retrieved at the
15 NCBI homepage under accession number NG_009614.1 (SEQ ID NO.1).

The gene of Cathepsin H has a TATA- and CAAT-less promoter and upstream of exon 1 one GC box. Two different forms of cathepsin H cDNA have been detected in prostate tissues and cancer cell lines: a full-length form (CTSH) and a truncated form with deletion of 12 amino acids at the signal peptide region (CTSHdelta 10-21) (see
20 Atlas of Genetics and Cytogenetics in Oncology and Haematology). The length of the preproenzyme transcript is 1005 bp. The coding polynucleotide sequence of Cathepsin H is publicly available at the NCBI nucleotide database under several accession numbers, such as: BC006878 (CTSH, complete coding sequence, mus musculus, SEQ ID NO.4, NM_004390.3 (Homo sapiens CTSH transcript variant1
25 mRNA (SEQ ID NO.2), NM_148979.2 (Homo sapiens CTSH, transcript variant 2 mRNA (SEQ ID NO.3)),. The skilled person knows how to retrieve further coding or genomic sequences of Cathepsin H from the NCBI database (Cathepsin H of other species; mutants or different isoforms of Cathepsin H, if existing). If in the following, it is referred to the Cathepsin H coding sequence, this can mean any of the above
30 mRNA or coding sequences; with sequences according to SEQ ID. NOs. 2, 3 and 4 being preferred examples.

- The protein sequence of Cathepsin H is publicly available at the NCBI protein database, e.g. under the following accession numbers: homo sapiens (hs) Cathepsin H isoform a preproprotein: NP_004381 (SEQ ID No.8); hs isoform b precursor: NP:683880 (SEQ ID NO.9); murine (mus musculus) Cathepsin H: preproprotein: NP_031827; mus musculus isoform CRA_a: EDL20900, mus musculus isoform CRA_b: EDL20901, rat (Rattus norvegicus) Cathepsin H: NP_037071; cathepsin H isoform CRA-a (Rattus norvegicus): EDL77562, isoform CRA-b (Rattus norvegicus) of Cathepsin H: EDL77563, cathepsin H, Moreover the protein sequence is publicly available at the UniProtKB database (www.beta.uniprot.org), under accession numbers: P09668 (HUMAN_CATH, human Cathepsin H, SEQ ID NO.5) If in the following, it is referred to the Cathepsin H protein or amino acid sequence, this can mean any of the above protein sequences or translated protein sequences from the above listed coding sequences; such as the following sequences: SEQ ID NO.s 5, 6, 7, 8 or 9.
- NCBI is the national centre for biotechnology information (postal address: National Centre for Biotechnology Information, National Library of Medicine, Building 38A, Bethesda, MD 20894, USA; web-adress: www.ncbi.nlm.nih.gov). More sequences (e.g. sequences carrying SwissProt or EMBL accession numbers) can be retrieved in the UniProtKB database under www.beta.uniprot.org.
- Cathepsin H is a lysosomal protease with aminopeptidase and weaker endopeptidase function. It belongs to the family of C1 (papain-like) cystein proteases. Cathepsin H protein is synthesized as a preproenzyme of 335 amino acids and proteolytically processed into an active single chain inside of endosomes or lysosomes. In addition to the heavy and light chain (together denominated long chain), Cathepsin H contains a so-called mini chain "EPQNCSAT" that is derived from the propeptide. (For the structure of Cathepsin H, see also Turk et al., Biological Chemistry, 1997). The mini chain appears to be involved in the aminipeptidase activity of Cathepsin H and play a key role in substrate recognition. A recombinant form of human Cathepsin H lacking the mini-chain was shown to be an endopeptidase (Valiljeva et al, 2003). Endopeptidase substrates of Cathepsin H are e.g.: Bz-Arg+NHNap, Bz-Arg+NH-Mec, Bz-Phe-Val-Arg+NHMec,. It has dipeptidyl-peptidase activity for the substrates: Pro-Gly+Phe and Pro-Arg+NHNap. It has

aminopeptidase activity for the substrates: H-Arg-NH-Mec, Bz-Arg-NH-Mec, Bz-Arg-NH-Mec and H-Cit-NH-Mec (see e.g. Rothe and Dodt, 1992; Bz=benzoyl, NH-Mec = e-methylcoumaryl-7-amide; Cit= citrulline). Naturally occurring inhibitors of Cathepsin H are the cystatins, alpha2-macroglobulin as well as antigens from mouse cytotoxic lymphocytes CTLA-2beta.

Cathepsin H is ubiquitously expressed with highest level in the kidney. Its expression may be increased in some cancerous tissues. Cathepsin is predominantly located in the endosomal-lysosomal compartment, but also secreted to some extent and circulating in the blood.

- 10 The functions of Cathepsin H comprise in general: protease activity, e.g hydrolase activity, peptidase activity, e.g. aminopeptidase, dipeptidylpeptidase, exopeptidase or endopeptidase activity, transacylase activity (see Koga et al., 1991); cleavage of the above-listed substrates; cleavage of native C5 and generation of chemotaxin C5a, processing of hydrophobic surfactant-associated protein C, cleavage and/or
- 15 degradation of fibronectin and fibrinogen. More specifically, it is involved as a lysosomal cystein protease in the intracellular protein degradation. It is involved in the generation of chemotaxin C5a by cleavage of native C5. Cathepsin H is involved in the processing of hydrophobic surfactant-associated protein C. It furthermore appears to be involved in the development of unstable atherosclerotic plaques, and
- 20 possibly also in early atherogenesis.

- The use according to present invention allows for the identification of novel substances for the prevention and/or treatment of pain and especially of neuropathic pain. The use according to present invention comprises the identification of compounds with desired characteristics (i.e. lowering the pain sensation) as well as
- 25 the identification of compounds with undesired characteristics (i.e. increasing the pain sensation). Moreover, present invention allows for the further characterisation of compounds already identified of being useful for the prevention and/or treatment of any disease or diseased state. In this case, present invention can e.g. be used for excluding identified active compounds having unwanted side-effects (i.e. the increase
- 30 of pain sensation): Candidate compounds for a given disease can e.g. be profiled for

their influence on Cathepsin H (protein and/or nucleic acid, expression and/or function, etc.).

5 A compound / test compound / active compound as to be employed for the different aspects of present invention can be any biological or chemical substance or natural product extract, either purified, partially purified, synthesized or manufactured by means of biochemical or molecular biological methods.

10 A compound considered as being active in modulating pain in the sense of the different aspects of present invention can be any substance having an influence on one of the functions of Cathepsin H or on the Cathepsin H amount (protein or nucleic acid) in a cell, on Cathepsin H expression, posttranslational modification (e.g. N-glycosilation or processing (e.g. cleavage), protein folding or activation.

15 To this end, the substance can modulate any of the functions of Cathepsin H (e.g. those as listed above or below). Cathepsin H protein activity can be modulated by the substance e.g by direct interaction and interference of Cathepsin H polypeptide/protein or fragments thereof. The substance can also modulate Cathepsin H expression, e.g. on the level of transcription (initiation, elongation,
20 processing, etc), transcript stability, translation. Moreover it can modulate the posttranslational modification, the processing from the inactive to the active form (cleavage of the prepro-form to the the active protein), as well as protein folding etc. of Cathepsin H. The substance can exert the above effects directly or indirectly (indirectly meaning i.e. by interfering (positively or negatively) with natural signalling
25 cascades having influence on Cathepsin H function / protein activity / expression etc.)

Functions of Cathepsin H comprise those as listed above, e.g. protease activity; the ability of removing dipeptides from the amino terminus of one or more protein
30 substrates; the ability to interact specifically with one or more protein substrates (protein-protein interaction), such as those listed above; the ability to cleave and/or activate one or more protein substrates, such as those listed above; the ability to process protein or peptide substrates, such those listed above, the ability to be inhibited by one of the inhibitors as listed above.

Functions of Cathepsin H comprise also generally the ability of Cathepsin H protein or nucleic acid or fragments thereof to interact with other molecules (comprising, but not limited to: proteins, peptides, nucleic acids, synthetic molecules) and preferably concern its capability of interacting and cleaving protein substrates.

Substrate of an enzyme is understood, within the terms of present application, to be any molecule that can be modified by the enzyme. Naturally occurring substrates in the scope of present invention are molecules that correspond to the form in which they occur in the natural physiological or pathological context (such as those listed above), and which are also capable of being modified by the respective enzyme.

The modulation of pain and especially neuropathic pain can be either a decrease or an increase.

According to one aspect of present group of inter-related inventions, a fragment or derivative of Cathepsin H can be used. A fragment can be a fragment of a protein, polypeptide or polynucleic acid.

A fragment of a protein or polypeptide is a protein or polypeptide that carries one or more end-terminal (n- and/or c-terminal) and/or internal deletions of one, two or more amino acids, when compared to the full-length Examples of Cathepsin H; fragments comprise, e.g. the domains and/or fragments as listed in the description of Figure 7 (see below).

A functional fragment of Cathepsin H protein is any fragment of this protein having at least one or more of the functional characteristics of the full-length protein, especially as listed above.

A fragment of a polynucleotide acid is a polynucleotide acid or an oligonucleotide carrying one or more end-terminal (5'- and/or 3'-) and/or internal deletions of one, two or more nucleotides, when compared to the full-length genomic or coding sequence. A functional fragment of Cathepsin H nucleic acid is any fragment having at least one

or more of the functional characteristics of the full-length polynucleic acid (mRNA, genomic or coding sequence).

The term derivative of Cathepsin H comprises any type of modification of Cathepsin H in comparison to the naturally-occurring form, and especially in comparison to Cathepsin H according to SEQ IDs NO: 5, 6, 7, 8 or 9, that is not a deletion. A functional derivative of Cathepsin H is any derivative of this protein having at least one and preferably two or more of the functional characteristics of the unmodified protein. Derivatives comprise, e.g. modifications of the amino acid or nucleotide sequence or any other kind of modification such as a chemical or biological modification leading e.g. to the stabilization of the polypeptide or polynucleotide (such as phosphorothioate modifications of the nucleic acid backbone or of exchanges of the bonds between amino acids, etc), or enabling a specific targeting of the polypeptide or polynucleotide to certain cells or facilitating its entry into or its uptake by cells (such as cell-permeant phosphopeptides, ortho coupling to cell-permeant peptide vectors, e.g. based on the antennapedia/penetrating, TAT and signal-peptide based sequences; or coupling to parts of ligands for specific transporters or importers).

Another aspect of present invention concerns the use of a non-human transgenic animal heterologously expressing Cathepsin H or a functional fragment thereof for identifying or analyzing compounds that modulate pain and especially neuropathic pain.

The non human animal can be any non human animal. Preferred are rodents, such as rats or mice.

A transgenic animal is an animal that carries in its genome foreign DNA, which has deliberately been transferred thereto. The introduction of the foreign DNA into the animal genome can be performed according to standard procedures (see e.g. Transgenic Animal Technology A Laboratory Handbook. C.A. Pinkert, editor; Academic Press Inc., San Diego, California, 1994 (ISBN: 0125571658)).

The term heterologous expression refers to an expression differing from the normal gene expression in the the host organism (concerning steady state level, amount, timing or tissue distribution of the expressed gene or concerning the type of expressed gene (i.e. the gene is normally not expressed in the host at all)). The
5 heterologous expression can be constitutive or inducible. Suitable inducible expression systems are well known in the art (e.g. the Tetracycline inducible system or the like). The organism can be a cell or a non-human animal.

According to another aspect, present invention concerns the use of a non-human
10 transgenic animal heterologously expressing Cathepsin H or a functional fragment thereof as a model system for enhanced pain sensitivity, especially neuropathic pain sensitivity.

Yet another aspect of present invention concerns the use of a non-human Cathepsin
15 H knock-out animal for identifying or analyzing compounds that modulate pain and especially neuropathic pain.

A knock-out organism (such as an animal or a cell) refers to an organism in which the expression or function of a gene is partially or completely deleted and comprises
20 genomic as well as functional knock outs, inducible as well as constitutive knock outs. The generation of knock out organisms is well known in the art, as well as cells or animals which can be used for generating knock out organisms. The generation of Cathepsin H – knock out mice is described in Pham and Ley, 1999.

A further aspect of present invention concerns use of a non-human Cathepsin H
25 knock-out animal as a model system for lowered pain and especially lowered neuropathic pain sensitivity.

The use of a cell heterologously expressing Cathepsin H or a functional fragment
30 thereof for identifying compounds that modulate pain, especially neuropathic pain, is another aspect of present invention.

The cell can be any prokaryotic or eucaryotic cell, such as cells capable of being transfected with a nucleic acid vector and of expressing a reporter gene. These

comprise principally primary cells and cells from a cell culture, such as a eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *s. pombe* or *s. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E.coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue punction or surgical techniques.

According to one embodiment, a modified cell, having a lower Cathepsin H activity as compared to its unmodified state, is used. This way, it can e.g. be tested, if the compounds to be tested are able to enhance or restore the lowered or totally abolished Cathepsin H activity. Or it can be tested whether the substances are able to perform their function (e.g. pain modulation or even a function in the context of another diseased state or disease) in the context of lowered pain sensitivity.

The modification can be any type of modification (stable or transient, preferably stable), that leads to a decrease of Cathepsin H activity, Cathepsin H transcript steady state level (i.e. by activation of Cathepsin H transcription or transcript stabilisation) or Cathepsin H protein steady state level (i.e. by activation of Cathepsin H translation or its posttranslational processing; by modulation of Cathepsin H posttranslational modification or by activation of its stabilisation or by inhibition of its degradation). This can for example be achieved by using dominant negative mutants of Cathepsin H, antisense oligonucleotides, RNAi constructs of Cathepsin H, by generating functional or genomic Cathepsin H knock outs (which can e.g. be inducible) or other suitable techniques known within the state of the art. For an overview of the above techniques, see for example: Current protocols in Molecular biology (2000) J.G. Seidman, Chapter 23, Supplement 52, John Wiley and Sons, Inc.; Gene Targeting: a practical approach (1995), Editor: A.L. Joyner, IRL Press; Genetic Manipulation of Receptor Expression and Function, 2000; Antisense Therapeutics, 1996; Scherr et al, 2003.

According to one embodiment, a Cathepsin H knock-out cell is used. Suitable cell lines for the generation of knock-outs are well known in the state of the art, see e.g., Current protocols in Molecular Biology (2000) J.G. Seidman, Chapter 23, Supplement 52, John Wiley and Sons, Inc; or Gene Targeting a practical approach.

(1995) Ed. A.L. Joyner, IRL Press. The generation of Cathepsin H (DPPI) knock-out cells is also published in Pham and Ley, 1999 (the generation of DPPI murine embryonic stem cell knock out clones, see page 8628, left column, upper half).

- 5 Another aspect of the invention concerns thus the use of a Cathepsin knock-out cell for identifying or analyzing compounds that modulate pain, especially neuropathic pain.

10 Furthermore, the use of a Cathepsin knock-out cell as a model system for lowered pain, especially lowered neuropathic pain sensitivity, is another aspect of present group of inter-related inventions.

15 According to another embodiment of present invention, the cell can have a higher amount of Cathepsin H as compared to a reference cell (e.g. the same cell in its unmodified state). This cellular system can serve to mimick a state of enhanced pain sensitivity, as the amount of Cathepsin H expression is related to pain sensitivity.

20 Present invention relates thus also to the use of a cell heterologously expressing Cathepsin H or a functional fragment thereof as a model system for enhanced pain sensitivity, especially neuropathic pain sensitivity.

25 The use of a cell heterologously expressing a reporter gene expressibly linked to the Cathepsin H promoter and/or enhancer for identifying or analyzing compounds that modulate pain, especially neuropathic pain, is another embodiment of present set of related inventions.

30 The above aspect of present invention is based on a typical reporter gene assay commonly known in the art. To this end, the promoter of choice is inserted into an expression vector suitable for the type of host cell chosen, upstream of the reporter gene of choice in such a way as to allow for an expression of the reporter gene if the promoter is active. The construct is subsequently introduced into the host cell of choice. Suitable methods for transformation or transfection are well known in the art as well as conditions for cell cultivation and detection of reporter gene expression (see e.g. standard literature listed below). Suitable conditions are well known in the

art as well as vectors, reporter genes and necessary reagents, which are also commercially available.

A vector is a circular or linear polynucleotide molecule, e.g. a DNA plasmid, bacteriophage or cosmid, by aid of which polynucleotide fragments (e.g. cut out from other vectors or amplified by PCR and inserted in the cloning vector) can specifically be amplified in suitable cells or organisms. Expression vectors enable the heterologous expression of a gene of interest (e.g. a reporter gene), in the host cell or organism. The type of cell or organism largely depends on the aim and the choice lies within the knowledge of the skilled artisan. Suitable organisms for the amplification of a nucleic acid are e.g. mostly single cell organisms with high proliferation rates, like e.g. bacteria or yeast. Suitable organisms can also be cells isolated and cultivated from multicellular tissues, like e.g. cell lines generated from diverse organisms (e.g. SF9 cells from *Spodoptera Frugiperda*, etc.). Suitable cloning vectors are known in the art and commercially available at diverse biotech suppliers like, e.g. Roche Diagnostics, New England Biolabs, Promega, Stratagene and many more. Suitable cell lines are e.g. commercially available at the American Type Culture Collection (ATCC).

For the heterologous expression of a protein or polypeptide, the cell can be any prokaryotic or eucaryotic cell capable of being transfected with a nucleic acid vector and of expressing the gene of interest, e.g. a reporter gene. These comprise principally primary cells and cells from a cell culture, preferably an eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HEK293, RIN-5F, HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *S. pombe* or *S. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E. coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue puncture or surgical techniques.

Within the context of present application, the term "transfection" refers to the introduction of a nucleic acid vector into a host cell (either prokaryotic or eucaryotic) and comprises thus the term "transformation".

The transfection can be a stable or transient transfection.

The Cathepsin H promoter is a part of the Cathepsin H gene able to drive expression of a gene product of interest if introduced into a suitable expression vector upstream of the coding sequence of the gene product. The cathepsin H promoter is part of the genomic upstream of 5'-sequence of the Cathepsin H gene that steers transcriptional activation of the downstream, transcribed region and can e.g. be derived from Ishidoh et al., FEBS letters, 1989 or Ishidoh et al, Biomed. Biochim. Acta, 1991, 50(4): 541-7.

A reporter gene can be any gene that allows for an easy quantification of its gene product. A vast variety of reporter genes for eukaryotic or prokaryotic hosts as well as detection methods and necessary reagents are known in the art and commercially available. These comprise e.g. the genes of beta Lactamase (lacZ), Luciferase, Green or Blue fluorescent protein (GFP or BFP), DsRed, HIS3, URA3, TRP1 or LEU2 or beta Galactosidase. These genes encode proteins which can be easily detected by means of a visible (colour or luminescent) reaction (e.g. lacZ, Luciferase). These comprise gene-products which can be easily detected by means of a visible (colour or luminescent) reaction or gene-products conferring resistance towards antibiotics like Ampicillin or Kanamycin when expressed. Other reporter gene-products enable the expressing cells to grow under certain conditions like e.g. auxotrophic genes.

A functional fragment of a reporter gene is any fragment of a given reporter gene that allows for an easy quantification of its gene product.

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The transfection can be a stable or transient transfection.

The cell can be any prokaryotic or eucaryotic cell capable of being transfected with a nucleic acid vector and of expressing a reporter gene. These comprise principally primary cells and cells from a cell culture, preferably a eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as

5 HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *S. pombe* or *S. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E. coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue puncture or surgical techniques.

10 Within the context of the above aspect of present invention the control vector can be any suitable vector which comprises a reporter gene or functional fragment thereof, but wherein reporter gene expression is not driven by a (functional) Cathepsin H promoter. This can e.g. mean that the reporter gene or functional fragment thereof is not operationally coupled to a functional Cathepsin H promoter (i.e. either totally

15 devoid of a Cathepsin H promoter, comprises a non functional Cathepsin H promoter or promoter fragment or wherein the coupling of promoter and reporter gene is not functional). Another possibility is that the reporter gene or functional fragment thereof is operationally coupled to another promoter than the Cathepsin H promoter (e.g. SV40 or another standard promoter). The functional vector and the control vector can

20 also be transfected to the same cell, but in which case the reporter genes need to be different.

The identification of compounds according to the above uses can e.g. be performed according to assays as described below or as known in the art.

25 An assay is any type of analytical method or system to monitor a biological process. Suitably, molecular cascades and mechanisms representing parts of physiological metabolic pathways but also of pathological conditions are reproduced in cellular or biochemical (in vitro) systems. The pharmacological activity of a potential

30 pharmaceutical compound can thus be determined according to its capability of interfering with or modulating these cascades or mechanisms.

For the use in drug screening, especially the high throughput screening for novel pharmaceutical compounds, the assay needs to be reproducible and is preferably

also scalable and robust. In the scope of present invention, high throughput screen means, that a method according to present invention is performed in a very small scale, e.g. on 96, 386 or 1536 well plates in samples of very small volume in the range of few millilitres down to few nanoliters or even less. Thus, a very large amount of samples can be analysed in a short time. High throughput screening mostly comprises the screening of approximately 500.000 different compounds for a certain ability by means of one single assay. The assay is preferably suitable for high throughput screening of chemical substances for their ability of modulating the activity of the target molecule under investigation. The type of assay depends e.g. on the type of target molecule used (e.g. polypeptide or polynucleotide) and the “read out”, i.e. the parameter, according to which the activity of the target molecule is determined (see below).

Different types of such assays are commonly known in the state of the art and commercially available from commercial suppliers.

Suitable assays for different purposes encompass radioisotopic or fluorescent assays, for example fluorescence polarization assays (such as those offered commercially by Panvera) or Packard BioScience (HTRF; ALPHAScreenTM) for measuring the interaction of a labeled member with a non-labeled member (e.g. the interaction of labeled proteins with their unlabeled protein-ligands).

More examples include cell based assays, wherein a cell line stably (inducibly or not; chromosomal or episomal) or transiently expresses a recombinant protein of interest. These assays comprise e.g. reporter gene assays, wherein the regulation of a certain promotor or a signal transduction pathway of a member of a signal transduction cascade is measured according to the activity of a reporter enzyme, the expression of which is under the control of said certain promotor. For this type of assay, a recombinant cell line has to be constructed containing the reporter gene under the control of a defined promotor that is to be investigated itself or that is regulated by the signaling cascade under investigation. Suitable reporter enzymes are commonly known within the state of the art and comprise firefly luciferase, renilla luciferase (e.g. commercially available by Packard reagents), β -Galactosidase. Suitable cell lines

depend on the aim of the assay but comprise mostly cell lines that are easy to transfect and easy to cultivate, such as, e.g. HeLA, COS, CHO, NIH-3T3, etc.

For determination of protease activity, typical protease assay formats are known: e.g.

- 5 using a substrate carrying a reporter tag (e.g. a luminescent/fluorescent or other signal emitting protein/peptide or entity) at one position of the substrate and a quencher (an entity (e.g. another peptide inhibiting the signal emission of the reporter tag as long as the substrate is intact/uncleaved) at another position; the substrate is incubated with Cathepsin H under suitable conditions to allow for the
- 10 cleavage of the substrate leading to the emission of a detectable signal (e.g. light-emission), because of the separation of quencher and reporter tag.

Other types of assays and other types of “read out” are well known in the state of the art. One assay for the detection of cathepsin H activity in cells can e.g. be gained

15 from Rüttger et. Al., BioTechniques, 2006.

Assays according to present invention concern e.g.:

20 A method of identifying or analyzing compounds modulating and/or preventing pain, preferably neuropathic pain, comprising the steps

- a. Providing at least two samples;
- b. Contacting one sample containing Cathepsin H or a functional fragment or derivative thereof with a compound,
- c. determining the activity of Cathepsin H in the presence of compound,
- 25 d. determining the activity of Cathepsin H in the absence of compound, and
- e. comparing the activity of Cathepsin H according to c) with that according to d).

30 A method for identifying or analyzing compounds that modulate and/or prevent pain, preferably neuropathic pain, comprising:

- a. Contacting a Cathepsin H protein or functional fragment or derivative thereof with a test compound; and

- b. Determining whether the test compound modulates the activity of the Cathepsin H protein or functional fragment or derivative thereof.

A method for identifying or analyzing compounds that modulate and/or prevent pain, preferably neuropathic pain, comprising:

- a. Contacting a cell, which has a detectable amount or activity of Cathepsin H or of a functional fragment or derivative thereof, with a test compound;
- b. Determining whether the test compound is able to modulate the amount or activity of Cathepsin H or the functional fragment or derivative thereof present in the cell.

A method for identifying or analyzing compounds that modulate and/or prevent pain, preferably neuropathic pain comprising:

- a. Contacting a nucleic acid coding for a Cathepsin H protein or a functional fragment or derivative thereof with a test compound in a transcriptionally active system, and
- b. Determining the amount of mRNA coding for the Cathepsin H protein or the functional fragment or derivative thereof present in said system in presence of said compound, and
- c. Determining whether the compound is capable of modulating the amount of mRNA coding for the Cathepsin H protein or functional fragment or derivative present in said system.

A transcriptionally active system is any biochemical or cellular system which at least has the ability to perform a transcription reaction of a transcription unit. Such systems are well known in the art and comprise cells as well as in vitro transcription systems or kits (e.g. on basis of cell extracts) commercially available.

A method for identifying compounds or analyzing compounds that modulate and/or prevent pain, preferably neuropathic pain, comprising:

- a. Providing a cell transfected with a nucleic acid vector comprising the promoter of a Cathepsin H gene or a functional fragment thereof

operationally coupled to a reporter gene or a functional fragment thereof:

b. Providing a cell transfected with a control vector which comprises a reporter gene or a functional fragment thereof not being operationally coupled to a functional Cathepsin H promoter;

c. Determining the reporter gene activity of the cell according to a) and b) in the presence of a test compound;

d. Determining the reporter gene activity of the cell according to a) and b) in absence of the test compound.

A method for identifying or analyzing a compound that modulates pain, preferably neuropathic pain comprising

a. Selecting a compound that modulates the activity of Cathepsin H as a test compound, and

b. Administering said test compound to a subject in sensation of pain to determine whether the pain is modulated.

A method of identifying or analyzing a compound that modulates and/or prevents pain, preferably neuropathic pain in a subject comprising:

c. Assaying a biological activity of Cathepsin H or a functional fragment or derivative thereof in the presence of one or more test compounds to identify one or more modulating compounds that modulate the biological activity of Cathepsin H, and

d. Testing one or more of the modulating compounds for their ability to reduce pain (especially neuropathic pain) and/or pain sensation (especially neuropathic pain sensation) and/ or pain sensitivity (especially neuropathic pain sensitivity) in a subject.

Further aspects of present invention concern pharmacogenomic methods for classifying patient groups and assisting the physician to adapt/improve his treatment of individual patients, such as:

A method for analyzing the pain (especially neuropathic pain) threshold in an individual comprising analyzing the amount of Cathepsin H in a taken sample of said individual in comparison to one or more reference samples as to whether the amount of Cathepsin H mRNA and/or protein present in said sample is different from that of one or more reference samples, wherein the presence of a higher amount indicates an increased pain (especially neuropathic pain) sensitivity and the presence of a lower amount indicates a decreased pain (especially neuropathic pain) sensitivity in said individual.

A method for adapting the dosage of a pharmaceutical for the prevention and/or treatment of pain in an individual, which method comprises examining a taken sample of an individual as to whether the amount of Cathepsin H mRNA and/or protein present in said sample is different from that of one or more reference samples, said dosage being adapted depending on whether the amount of protein and/or mRNA in the taken sample of the individual is different from that of the one or more reference samples, wherein a higher amount of Cathepsin H in the taken sample of the individual is indicative of a need for a higher dose and a lower amount of Cathepsin H in the sample of the individual is indicative of a need for a lower dose.

The term "taken sample" as used herein, refers to a biological sample taken/separated from the body of one or more individual beings (humans or non-human animals). Biological material and biological samples comprise, e.g. cells, preparations or parts of tissue or organs (e.g. brain, blood, liver, spleen, kidney, heart, blood vessels, etc.), preferably if derived from a vertebrate, and more preferably from a mammal including a human. Comprised are also cells from a cell culture, preferably a eukaryotic cell culture comprising cells derived either from multicellular organisms and tissue (such as HeLA, CHO, COS, SF9 or 3T3 cells) or single cell organisms such as yeast (e.g. *s. pombe* or *s. cerevisiae*), or a procaryotic cell culture, preferably *Pichia* or *E.coli*. Cells and samples derived from tissue can be gained by well-known techniques, such as taking of blood, tissue puncture or surgical techniques. The preparation of recombinant molecules and the purification of naturally occurring molecules from cells or tissue, as well as the preparation of cell- or tissue extracts is well known to the person of skill in the art (see e.g. also the standard literature listed below).

The term "reference sample" refers to a biological sample taken from one or more individuals with a known given pain phenotype or to an *in vitro* biological sample (e.g. a sample stemming from *in vitro* cell or tissue culture (e.g. cultivated cells)) and
5 corresponding in certain characteristics (e.g. its level of Cathepsin H activity, amount or expression) to a given pain phenotype (e.g. high pain sensitivity or low pain sensitivity).

Yet another aspect of present invention concerns the use of a means for the
10 detection of Cathepsin H for determining enhanced pain sensitivity (especially neuropathic pain sensitivity) in an individual by analyzing a biological sample taken from the body of an individual to be examined.

The means for the detection of Cathepsin H can be any means able to specifically detect Cathepsin H polypeptide/protein or nucleic acid present in a biological sample.

15 A means to detect Cathepsin H protein or polypeptide can be any means able to specifically detect either wildtype Cathepsin H protein/polypeptide and can also be a means to detect specifically Cathepsin H protein/polypeptide harbouring one or more mutations regarding the size or the amino acid sequence in comparison to a wild type polypeptide/protein. A preferred example of such a means is an antibody able to
20 specifically detect Cathepsin H protein, e.g. for use in immunohistological or immunohistochemical techniques (e.g. detection of Cathepsin H protein or certain mutations thereof in histological tissue sections or Cathepsin H protein immobilized on suitable carriers like membranes, chips, ELISA plates etc.).

The means to detect Cathepsin H nucleic acid can e.g. be a means to detect
25 Cathepsin H mRNA /cDNA or genomic DNA, either wildtype or also harbouring one or more mutations regarding their length or their nucleic acid sequence in comparison to a wild type Cathepsin H nucleic acid. The means can e.g. be a means to specifically detect and/or quantify Cathepsin H mRNA and preferably comprises or is a specific Cathepsin H nucleic acid probe or a primer set capable of amplifying
30 Cathepsin H DNA or, e.g. for use in PCR sequencing (for the detection of Mutations in the nucleotide sequence) or capable of amplifying Cathepsin H cDNA, e.g. for use in RT PCR (for the detection and/or quantification of Cathepsin H mRNA expression).

Another means can e.g. be a nucleic acid probe able to specifically hybridise to Cathepsin H mRNA or cDNA under standard conditions, e.g. for use in Northern Blot or Chip hybridisation techniques.

5 The term wild type refers to the genotype or phenotype that is found in nature or in the standard laboratory stock for a given organism. According to one preferred embodiment, the wildtype sequences of Cathepsin H are the sequences according to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8 and/or 9.

The design and synthesis of suitable primers is well known in the art (see also above). Primer sets for the detection of Cathepsin H could for example be the
10 following:

Set 1: (Product length for detection of human CTSH transcript variant 1 = 154 nucleotides, product length for detection of human CTSH transcript variant 2=118 nucleotides):

Forward primer 5'-GCGCTCCCAGTTGACGCTCT-3' (SEQ ID NO.10)

15 Reverse primer 5'-CACGCACAGTTCGGCGGC-3' (SEQ ID NO.11)

Set 2: (Product length for detection of human CTSH transcript variant 1 = 153 nucleotides, product length for detection of human CTSH transcript variant 2=117 nucleotides):

Forward primer 5'-CGCTCCCAGTTGACGCTCTGG-3' (SEQ ID NO.12)

20 Reverse primer 5'-CACGCACAGTTCGGCGGC-3' (SEQ ID NO.13).

According to one embodiment of present invention, the means is a primer set for the amplification of Cathepsin H nucleic acid, and preferably a set of primers comprising at least one of the primers according to SEQ ID NOs. 10, 11 (together set 1), 12 and/or 13 (together set 2).

25 According to a further preferred embodiment of present invention, the means is a probe for the detection of Cathepsin H nucleic acid. The design and synthesis of suitable probes is well known in the art (see also standard literature below).

According to yet another preferred embodiment of present invention, the means is an antibody for the specific detection of Cathepsin H protein or polypeptide.

The preparation of suitable antibodies or functional fragments thereof is well known in the art as well, e.g. by immunizing a mammal, for example a rabbit, with Cathepsin H protein or a fragment thereof, 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). Suitable procedures to produce monoclonal antibodies are well known in the art as well (see e.g. literature for standard methods listed below). In the context of present invention, the term antibody or antibody fragment comprises also 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). Cathepsin H antibodies are also commercially available, such as Goat Anti-Mouse Cathepsin H, Catalog# BAF1013, R&D Systems (Minneapolis, USA), Rat Anti-Mouse Cathepsin H, Catalog# MAB1013, R&D Systems (Minneapolis, USA), Goat Anti-Mouse Cathepsin H, Catalog# AF1013 or Rabbit Anti-Human Cathepsin H, Catalog# ABIN285430 (antibodies-online GmbH, Germany, see <http://www.antikoerper-online.de>) or Mouse Anti-Human Cathepsin H, Catalog#ABIN165388 (antibodies-online GmbH, Germany).

The production of Cathepsin H antibodies and the detection of human Cathepsin H from human tissue cytosols and sera is e.g. described in detail in Schweiger et al., Journal of Immunological Methods, 2001, p. 165-172(see e.g. p. 166-167 for the materials and methods).

Another aspect of present invention concerns a diagnostic kit for determining the pain and especially neuropathic pain sensitivity in an individual, which test kit comprises at

least one means for the detection of Cathepsin H in a biological sample and its use.

In the context of the present invention, a test kit is understood to be any combination of the components identified in this application, which are combined, coexisting
5 spatially, to a functional unit, and which can contain further components.

In the context of present invention, a test kit comprises at least a means for detection of Cathepsin H (e.g. amount/or mutation) in a biological sample, suitably together with suitable buffers and/or reagents for performing a detection reaction (e.g.

10 immunological detection of Cathepsin H by means of an antibody, an enzymatic reaction for assaying Cathepsin H activity or the like). and/or sample preparation, and optionally a handling manual for performing the respective detection technique.

Other aspects of present invention concern methods of treatment, such as:

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A method for treating pain in a subject that is experiencing pain comprising administering to said subject a therapeutically effective amount of a composition lowering the amount or activity of Cathepsin H in said subject. This can be the amount or activity of Cathepsin H altogether or in a certain tissue, e.g. in neural
20 tissue, in lymphatic tissue or cells of the immune system such as mast cells, macrophages, neutrophils, T-cells (such as CD8+ T-cells), etc., wherein a therapeutically effective amount comprises an amount sufficient to ameliorate the pain sensation or sensitivity (especially with respect to neuropathic pain) in the individual.

25

A method for lowering the pain (and especially neuropathic pain) sensitivity in a subject comprising administering to said subject a therapeutically effective amount of a composition lowering the amount (e.g. expression, half life) or activity of Cathepsin H in said subject (e.g. in lymphatic or neural tissue or cells of the immune system),
30 concerns yet another aspect of present invention.

Moreover, present invention concerns a method for modulating the pain (and especially neuropathic pain) sensitivity in an offspring from a non-human female subject comprising transferring (e.g. electroporating) a nucleic acid conferring a

modulated Cathepsin H expression into Zygotes, transferring the Zygotes into a non-human foster mother and electing offspring according to its Cathepsin H expression characteristics (lowered or abolished Cathepsin H expression in comparison with wild type subjects, such as mice).

5

Another aspect of present invention concerns a compound that is able to lower Cathepsin H activity and/or expression for the treatment of pain and especially neuropathic pain.

- 10 Inhibitors of Cathepsin H are known in the art, such as E-64d (see e.g. Rüttger et al., BioTechniques, 41: 469-473, 2006) and Kirschke, H et al, 1995, Protein Profile 2: 1581-1643).

- For the production of the medicament the modulators of Cathepsin H of the present invention can be formulated with suitable additives or auxiliary substances, such as
15 physiological buffer solution, e.g. sodium chloride solution, demineralized water, stabilizers, such as protease or nuclease inhibitors, 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.
20 depending on the kind of administration.

- Suitable further additives are, for example, detergents, such as, for example, Triton X-100 or sodium deoxycholate, but also polyols, such as, for example, polyethylene glycol or glycerol, sugars, such as, for example, sucrose or glucose, zwitterionic
25 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, especially a
30 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.

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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

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dispositives 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

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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.

20

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.

25

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.

30

According to one preferred embodiment of the different aspects of present invention, Cathepsin H, the derivative or fragment thereof can be used as an isolated molecule.

In the context of this invention, the term "isolated molecule", especially with respect to Cathepsin H, refers to Cathepsin H polynucleotides or polypeptides purified from natural sources as well as purified recombinant molecules (wherein the term purified comprises a partial purification as well as a complete purification).

The preparation of recombinant polypeptide or polynucleotide molecules and the purification of naturally occurring molecules from cells or tissue, as well as the preparation of cell- or tissue extracts is well known to the person of skill in the art (see e.g. also the standard literature listed below).

These comprise e.g. amplifying polynucleotides of desired length via the polymerase chain reaction (PCR) on the basis of the published genomic or coding polynucleotide sequences and the subsequent cloning of the produced polynucleotides in host cells (see e.g. standard literature listed below).

In the context of present invention, the term „polypeptide“ refers to a molecule comprising amino acids bound to each other by peptide bonds containing at least 50 amino acids coupled to each other in a linear mode to form a polypeptide chain.

Shorter molecules of this kind are referred to as peptides. The term „protein“ refers to molecules comprising at least one polypeptide chain but can refer also to molecules comprising more than one polypeptide chains associated or bound to each other.

Thus, the term „protein“ comprises the term „polypeptide“ as well.

Examples:

Materials and Methods:

5 Mouse strains used:

Five different inbred mouse strains were used: AKR/J (AKR), CBA/J (CBA), C3H/HeJ (C3H), C57BL/6J (B6) and C58/J (C58). Mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). For these mice strains it has been shown that
10 they differ significantly concerning several in vivo measures of pain (Mogil et al 1999)

Total RNA Isolation:

Total RNA from DRGs (dorsal root ganglia) was isolated with the PicoPureTM RNA Isolation Kit (Arcturus) following the manufacturer's instructions. RNA quality was
15 assessed using the 2100 Bioanalyzer and RNA 6000 Nano LabChipTM kit (Agilent).

Affymetrix GeneChipTM Microarrays:

First-strand cDNA synthesis was performed using 500ng total RNA with a 100pM T7-(dT)₂₄ oligomer (GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-dT₂₄
20 SEQ ID NO:14) according to Baugh, L.R, Hill, A.A., Brown, E.L. and Hunter, C.P. (2001) *Nucleic Acids Res.* 29, e29 and SuperScript II Reverse Transcriptase following the manufacturer's instructions. Double-stranded cDNA was synthesized and then extracted using phenol-chloroform followed by an ethanol precipitation step. An *in vitro* transcription reaction was performed with the doublestranded cDNA
25 sample using the BioArray High Yield RNA Transcription Labeling kit (Enzo) according to the manufacturer's instructions. Transcription reactions were incubated at 37°C for 16h. cRNA was purified using the RNeasyTM Mini kit (Qiagen) protocol for RNA cleanup and quantified by a spectrophotometer. The biotin-labeled cRNA was fragmented using a RNA fragmentation buffer (200mM Tris-acetate, 500mM KOAc,
30 150mM MgOAc, pH 8.1). Hybridization and staining of Mouse Genome 430 2.0 GeneChipsTM (Affymetrix) was performed according to the manufacturer's instructions. The microarrays were scanned using a GeneChipTM 3000 Scanner, and the scanned data were imported and analyzed using Resolver v5.1 expression data analysis software (Rosetta Biosoftware).

L5 spinal nerve transection and sham surgical procedures:

In anesthetized mice, the left L5 spinal nerve was exposed and the transverse process was then partially removed. After separation from the L4 spinal nerve, the L5 spinal nerve was transected. Sham surgery was identical to the L5 spinal nerve transection surgery, however, the L5 spinal nerve was not transected (see DeLeo et al. 2000)..

Determination of paw withdrawal threshold:

Paw withdrawal thresholds (PWTs) were assessed using a dynamic plantar aesthesiometer (see Szabo et al. 2005). After acclimation in a compartment with metal mesh floor, the stimulator was positioned under the animal's hindpaw, a straight metal filament driven by an electrodynamic actuator touched the plantar surface and exerted an increasing upward force until the animal removed the paw (paw withdrawal threshold, PWT). PWTs were assessed for hindpaws of the ipsilateral, operated side and of the contralateral side. Each animal was used at one occasion only. In all animal experiments the ethics guidelines for investigations in conscious animals were obeyed, and the procedures were approved by the local Ethics Committee

Correlational analysis:

For correlational analysis, the "pain phenotype" was defined for each nerve-transsected animal (Chung animal) as $C1 - S1$, where

$C1 = \ln(\text{ipsilateral PWT} / \text{contralateral PWT})$ and

$S1 = \text{mean}_{\text{all sham animals within same strain}} \ln(\text{ipsilateral PWT} / \text{contralateral PWT})$.

Two measures of differential transcriptional regulation were defined for each Chung animal and each measured gene based on its intensity expression data. The "raw intensity measure" was taken as the intensity measure computed by the Resolver expression data analysis software (v5.1) for the respective gene and animal. The "log ratio measure" was computed for a specific gene and Chung animal as $\ln(C2 / S2)$, where $C2 = \text{Chung expression intensity}$ and $S2 = \text{mean}_{\text{all sham animals within same strain}} \text{Sham expression intensity}$.

Before correlations were computed, the set of genes was filtered to exclude genes that were expressed below noise level and without significant Chung vs. sham regulation. Eligible genes must be regulated in at least 60% of Chung animals with

an absolute fold-change $\Rightarrow$ 1.5 or in at least 20% of Chung animals with an absolute fold-change $\Rightarrow$ 2.0. Also, corresponding gene expression had to be detectable ("present") in at least five animals as defined by a respective intensity p -value < 0.001 . Pearson correlation coefficients for each gene between the pain phenotypic scores and one of the defined measures of transcriptional regulation were computed using the R software package (<http://www.r-project.org/>). Based on these, p -values of statistical significance and corresponding false-discovery rates (FDRs) were generated following the method of Storey et al. (2002). Genes with $FDR < 0.05$ under "log ratio measure" or "intensity measure" were considered significantly correlated.

Legend to the Figures:

Figure 1: Cathepsin H – Correlation Plot

Figure 1 shows for every individual mouse its neuropathic pain phenotype (mechanical hypersensitivity, X-axis) and the corresponding gene regulation of Cathepsin H (log ratio(Chung vs. Sham control), Y-axis) in the L5 DRG. Mouse data are colour-coded depending on the used strain. A Pearson correlation analysis has been performed and revealed a significant positive correlation of the two parameters pain phenotype and Cathepsin H gene regulation. This means for individual mice that the higher the L5 DRG expression of Cathepsin H in Chung-operated neuropathic mice was, the more pronounced the mechanical hyperalgesia as exhibited in the behavioral test.

This significant correlation indicates a causal relationship of Cathepsin H gene expression for the induction of the neuropathic pain phenotype.

Figure 2: Cathepsin H – Intensity data

Absolute values of Cathepsin H expression in L5 ganglia of the individual mice of the strains AKR, CBA and C57 after chung or sham surgery.

Figure 3: Genomic DNA sequence of homo sapiens Cathepsin H on chromosome 15 according to NCBI Reference Sequence: NG_009614.1 (SEQ ID NO. 1).

Figure 4: Coding sequence of homo sapiens Cathepsin H transcript variant 1 (SEQ ID NO:2) according to NM_004390.3 having a length of 1494 bp and encoding the longer isoform A of Cathepsin H.

- 5 Figure 5: Coding sequence of homo sapiens Cathepsin H transcript variant 2 (SEQ ID NO:3) according to NM_148979.2 having a length of 1458 bp and encoding the shorter isoform B of Cathepsin H. According to the above NCBI entry, this transcript variant lacks an alternative in-frame segment compared to variant 1 resulting in a shorter protein (isoform B) when compared to isoform A encoded by transcript variant
- 10 1. This may result in a protein (isoform B) that may more likely be a secreted than a lysosomal protein

Figure 6: coding sequence of mus musculus Cathepsin H (SEQ ID NO.4) according to NCBI entry BC006878.1.

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- Figure 7: human Cathepsin H protein sequence according to UniProtKB/Swiss-Prot P09668 (SEQ ID NO.5) comprising 335 amino acids and constituting the preproform of Human Cathepsin H (isoform A). This sequence is further processed into a mature form; it is cleaved into the following 3 chains: Cathepsin H mini chain Cathepsin H
- 20 heavy chain, Cathepsin H light chain (light and heavy chain together may be referred to as large chain); all chains are held together by disulfide bonds. Amino Acids 1-22 constitute the signal peptide (22 aa long), amino acids 23-97 constitute the activation peptide (75 aa long), amino acids 98-105 constitute the Cathepsin H mini chain (8 aa long), amino acids 106-115 constitute the propeptide (10 aa long), amino acids 116-
- 25 335 constitute the Cathepsin H long chain (220 aa long) consisting of the heavy and light chain held together by disulfide bonds, amino acids 116-292 constitute the Cathepsin H heavy chain (177 aa long), amino acids 293-335 constitute the Cathepsin H light chain (43 aa long).

- 30 Figure 8: Human isoform A prepro protein according to NM_004390.3 (SEQ ID NO:6; translated amino acid sequence of SEQ ID NO.2).

Figure 9: Human isoform B prepro protein according to NM_148979.2 (SEQ ID NO:7; translated amino acid sequence of SEQ ID NO:3).

Figure 10: protein sequence of human Cathepsin H isoform a preproprotein according to NP_004381.2 (SEQ ID NO.8).

- 5 Figure 11: protein sequence of human Cathepsin H isoform b precursor protein according to NP_683880.1 (SEQ ID NO.9).

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Short Protocols in Molecular Biology, 5th edition, by Frederick M. Ausubel (Editor), Roger Brent (Editor), Robert E. Kingston (Editor), David D. Moore (Editor), J.G.

10 Seidman (Editor), John A. Smith (Editor), Kevin Struhl (Editor), October 2002, John Wiley & Sons, Inc., New York“

Transgenic Animal Technology A Laboratory Handbook. C.A. Pinkert, editor; Academic Press Inc., San Diego, California, 1994 (ISBN: 0125571658)

Gene targeting: A Practical Approach, 2nd Ed., Joyner AL, ed. 2000. IRL Press at Oxford University Press, New York;

20 Manipulating the Mouse Embryo: A Laboratory Manual. Nagy, A, Gertsenstein, M., Vintersten, K., Behringer, R., 2003, Cold Spring Harbor Press, New York;

Remington's Pharmaceutical Sciences, 17th Edition, 1985 (for physiologically tolerable salts (anorganic or organic), see esp. p. 1418)

Standard Literature for Laboratory Methods:

If not indicated otherwise, laboratory methods were or can be performed according to standard methods listed in the below standard literature:

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Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 545 pp or Current Protocols in Molecular Biology;

Current Protocols in Molecular Biology; regularly updated, e.g. Volume 2000; John Wiley & Sons, Inc; Editors: Fred M. Ausubel, Roger Brent, Robert Eg. Kingston, David D. Moore, J.G. Seidman, John A. Smith, Kevin Struhl.

Current Protocols in Human Genetics; regularly updtated, e.g. Volume 2003; John Wiley & Sons, Inc; Editors: Nicholas C. Dracopoli, Honathan L. Haines, Bruce R. Korf, Cynthia C. Morton, Christine E. Seidman, J.G. Seigman, Douglas R. Smith.

10 Current Protocols in Protein Science; regularly updated, e.g. Volume 2003; John Wiley & Sons, Inc; Editors: John E. Coligan, Ben M. Dunn, Hidde L. Ploegh, David W. Speicher, Paul T. Wingfield.

Molecular Biology of the Cell; third edition; Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D.; Garland Publishing, Inc. New York & London, 1994;

Gene Targeting: a practical approach (1995), Editor: A.L. Joyner, IRL Press

Remington's Pharmaceutical Sciences, Edition 17, 1985.

Claims

1. The use of Cathepsin H for identifying compounds that modulate neuropathic pain
2. The use of a non-human transgenic animal heterologously expressing Cathepsin H for identifying or analyzing compounds that modulate neuropathic pain.
3. The use of a non-human transgenic animal heterologously expressing Cathepsin H as a model system for enhanced neuropathic pain sensitivity.
- 10 4. The use of a non-human Cathepsin H knock-out animal for identifying or analyzing compounds that modulate neuropathic pain.
5. The use of a non-human Cathepsin H knock-out animal as a model system for lowered neuropathic pain sensitivity.
6. The use of a cell heterologously expressing Cathepsin H or a functional fragment thereof for identifying compounds that modulate neuropathic pain.
7. The use of a cell heterologously expressing Cathepsin H or a functional fragment thereof as a model system for enhanced neuropathic pain sensitivity.
8. The use of a Cathepsin knock-out cell for identifying or analyzing compounds that modulate neuropathic pain.
- 20 9. The use of a Cathepsin knock-out cell as a model system for lowered neuropathic pain sensitivity.
10. The use of a cell heterologously expressing a reporter gene expressibly linked to the Cathepsin H promoter and/or enhancer or a functional fragment thereof for identifying or analyzing compounds that modulate neuropathic pain.
11. A method of identifying or analyzing compounds modulating and/or preventing neuropathic pain, comprising the steps
 - f. Providing at least two samples;
 - g. Contacting one sample containing Cathepsin H or a functional fragment or derivative thereof with a compound,
 - 30 h. determining the activity of Cathepsin H in the presence of compound,
 - i. determining the activity of Cathepsin H in the absence of compound, and
 - j. comparing the activity of Cathepsin H according to c) with that according to d).
12. A method for identifying or analyzing compounds that modulate and/or prevent

neuropathic pain comprising:

- c. Contacting a Cathepsin H protein or functional fragment or derivative thereof with a test compound; and
- d. Determining whether the test compound modulates the activity of the Cathepsin H protein or functional fragment or derivative thereof.

10 13.A Method for identifying or analyzing compounds that modulate and/or prevent neuropathic pain comprising:

- a. Contacting a cell, which has a detectable amount or activity of Cathepsin H or of a functional fragment or derivative thereof, with a test compound;
- b. Determining whether the test compound is able to modulate the amount or activity of Cathepsin H or the functional fragment or derivative thereof present in the cell.

14.A method for identifying or analyzing compounds that modulate and/or prevent neuropathic pain comprising:

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- a. Contacting a nucleic acid coding for a Cathepsin H protein or a functional fragment or derivative thereof with a test compound in a transcriptionally active system, and
- b. Determining the amount of mRNA coding for the Cathepsin H protein or the functional fragment or derivative thereof present in said system in presence of said compound, and
- c. Determining whether the compound is capable of modulating the amount of mRNA coding for the Cathepsin H protein or functional fragment or derivative present in said system.

15.A method for identifying compounds or analyzing compounds that modulate and/or prevent neuropathic pain comprising:

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- a. Providing a cell transfected with a nucleic acid vector comprising the promoter of a Cathepsin H gene or a functional fragment thereof operationally coupled to a reporter gene or a functional fragment thereof:

b. Providing a cell transfected with a control vector which comprises a reporter gene or a functional fragment thereof not being operationally coupled do a functional Cathepsin H promoter;

c. Determining the reporter gene activity of the cell according to a) and b) in the presence of a test compound;

d. Determining the reporter gene activity of the cell according to a) and b) in absence of the test compound.

16. A method for identifying or analyzing a compound that modulates neuropathic pain comprising

10 c. Selecting a compound that modulates the activity of Cathepsin H as a test compound, and

d. Administering said test compound to a subject in sensation of pain to determine whether the pain is modulated.

17. A method of identifying or analyzing a compound that modulates and/or prevents neuropathic pain in a subject comprising:

a. Assaying a biological activity of Cathepsin H or a functional fragment or derivative thereof in the presence of one or more test compounds to identify one or more modulating compounds that modulate the biological activity of Cathepsin H, and

20 b. Testing one or more of the modulating compounds for their ability to reduce pain, pain sensation or pain sensitivity in a subject.