(19) DANMARK

(10) DK/EP 1988167 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 61 K 47/64 (2017.01)* *C 07 K 14/47 (2006.01)* *C 07 K 14/82 (2006.01)* *C 12 N 15/62 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2020-07-13**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-04-01**

(86) Europæisk ansøgning nr.: **08102597.5**

(86) Europæisk indleveringsdag: **2005-02-17**

(87) Den europæiske ansøgnings publiceringsdag: **2008-11-05**

(62) Stamansøgningsnr: **05290363.0**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU MC NL PL PT RO SE SI SK TR**

(73) Patenthaver: **Sorbonne Universite, 21 rue de L'École de Médecine, 75006 Paris, Frankrig**
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS), 3, rue Michel-Ange, 75794 Paris Cedex 16, Frankrig

(72) Opfinder: **Caboche, Jocelyne, 33, boulevard Lefebvre, 75015 Paris, Frankrig**
Vanhoutte, Peter, 6, rue Esserts, 94140 Alfortville, Frankrig

(74) Fuldmægtig i Danmark: **Plougmann Vingtoft A/S, Strandvejen 70, 2900 Hellerup, Danmark**

(54) Benævnelse: **Intracellulære inhiberende peptider**

(56) Fremdragne publikationer:
WO-A-00/62067
WO-A-01/90197
WO-A-02/39947
WO-A-94/04686
WO-A-98/52614
WO-A-03/012068
WO-A-2004/068139
BONNY C ET AL: "Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death" DIABETES, vol. 50, no. 1, janvier 2001 (2001-01), pages 77-82, XP002172261 ISSN: 0012-1797
WATANABE NOBUO ET AL: "Bio-effectiveness of Tat-catalase conjugate: a potential tool for the identification of H2O2-dependent cellular signal transduction pathways." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. 28 MAR 2003, vol. 303, no. 1, 28 mars 2003 (2003-03-28), pages 287-293, XP002366348 ISSN: 0006-291X
HALL D J ET AL: "Transduction of a dominant-negative H-Ras into human eosinophils attenuates extracellular signal-regulated kinase activation and interleukin-5-mediated cell viability." BLOOD, vol. 98, no. 7, 1 octobre 2001 (2001-10-01), pages 2014-2021, XP002366349 ISSN: 0006-4971
MYOU SHIGEHARU ET AL: "Blockade of focal clustering and active conformation in beta 2-integrin-mediated adhesion of eosinophils to intercellular adhesion molecule-1 caused by transduction of HIV TAT-dominant negative Ras." JOURNAL OF IMMUNOLOGY, vol. 169, no. 5, 1 septembre 2002 (2002-09-01), pages 2670-2676,

Fortsættes ...

XP002366350 ISSN: 0022-1767
FISCHER P M ET AL: "STRUCTURE-ACTIVITY RELATIONSHIP OF TRUNCATED AND SUBSTITUTED ANALOGUES OF THE INTRACELLULAR DELIVERY VECTOR PENETRATIN" JOURNAL OF PEPTIDE RESEARCH, vol. 55, no. 2, février 2000 (2000-02), pages 163-172, XP000899124 ISSN: 1397-002X
MANN D A ET AL: "ENDOCYTOSIS AND TARGETING OF EXOGENOUS HIV-1 TAT PROTEIN" EMBO JOURNAL, vol. 10, no. 7, 1991, pages 1733-1739, XP001205687 ISSN: 0261-4189
NORI A ET AL: "Tat-conjugated synthetic macromolecules facilitate cytoplasmic drug delivery to human ovarian carcinoma cells" BIOCONJUGATE CHEMISTRY, vol. 14, no. 1, 2003, pages 44-50, XP002347558 ISSN: 1043-1802
SCHWARZE S ET AL: "In vivo protein transduction: delivery of a biologically active protein into the mouse" SCIENCE, vol. 285, no. 5433, 3 septembre 1999 (1999-09-03), pages 1569-1572, XP002140133 ISSN: 0036-8075
POUYSSEGUR J ET AL: "Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling" BIOCHEMICAL PHARMACOLOGY, vol. 64, no. 5-6, 1 septembre 2002 (2002-09-01), pages 755-763, XP002366351 ISSN: 0006-2952
BARDWELL A J ET AL: "Docking sites on mitogen-activated protein kinase (MAPK) kinases, MAPK phosphatases and the Elk-1 transcription factor compete for MAPK binding and are crucial for enzymic activity" BIOCHEMICAL JOURNAL, vol. 370, no. 3, 15 mars 2003 (2003-03-15), pages 1077-1085, XP002366352 ISSN: 0264-6021
KELEMEN, BRADLEY R. ET AL: "Selective in vivo inhibition of mitogen-activated protein kinase activation using cell-permeable peptides" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 177, no. 10, 26 décembre 2001 (2001-12-26), pages 8741-8748, XP002497901 ISSN: 0021-9258 -& "SUPLEMETARY MATERIAL"[Online] 26 décembre 2001 (2001-12-26), XP002497902 Extrait de l'Internet: URL:http://www.jbc.org/cgi/content/full/277/10/8741/DC1> [extrait le 2008-09-17]
WENDER P A ET AL: "The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 97, no. 24, 21 novembre 2000 (2000-11-21), pages 13003-13008, XP002347555 ISSN: 0027-8424
DEROSSI D ET AL: "TROJAN PEPTIDES: THE PENETRATIN SYSTEM FOR INTRACELLULAR DELIVERY" TRENDS IN CELL BIOLOGY, vol. 8, no. 2, 1 février 1998 (1998-02-01), pages 84-87, XP002940006 ISSN: 0962-8924
GANJU R K ET AL: "Human immunodeficiency virus Tat modulates the Flk-1/KDR receptor, mitogen-activated protein kinases, and components of focal adhesion in Kaposi's sarcoma cells" JOURNAL OF VIROLOGY, vol. 72, no. 7, juillet 1998 (1998-07), pages 6131-6137, XP002497903 ISSN: 0022-538X
J. LAVAUR ET AL: "A TAT DEF Elk-1 Peptide Regulates the Cytonuclear Trafficking of Elk-1 and Controls Cytoskeleton Dynamics", JOURNAL OF NEUROSCIENCE, vol. 27, no. 52, 26 December 2007 (2007-12-26), pages 14448-14458, XP55000748, ISSN: 0270-6474, DOI: 10.1523/JNEUROSCI.2279-07.2007

Cellular inhibiting peptides

[0001] The present invention relates to peptides for the selective inhibition of the ERK-type MAP kinase pathway relative to a given substrate and in a given cell compartment.

MAP kinases (mitogen activated protein kinases) are ubiquitous proteins involved in varied cell functions. These proteins ensure intracellular signal transduction: from the surface of the cell to the nucleus. Three major families of MAP kinases (ERK, p38, JNK) have been identified, which correspond to cascade signaling pathways. These signaling pathways play important roles in cell functions: from apoptosis to proliferation, differentiation, or even neuronal plasticity. These functions depend strictly on, firstly, the type of MAP kinase and, for each type of MAP kinase, on its cellular localization.

[0002] In order to elucidate the molecular mechanisms governed by ERK signaling pathways and to be able to interfere with this signaling cascade at a given level, it is useful to have specific inhibitors. The compounds currently available: PD98059 (2'-amino-3'-methoxyflavone, a nitrogenous polycyclic inhibitor) and U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenyl-thio)butadiene), are inhibitors specific for MEKs, the kinases upstream of ERKs. However, their action is located upstream of ERKs, thereby resulting in complete inhibition of the activation of the latter and, consequently, of all the downstream substrates, without discrimination among them and without distinction with respect to their cellular localization. The patent application WO 2004/068139 describes the inhibition of ERK from peptides consisting solely of the anchoring domains of an Elk-1 ERK substrate in acellular *in vitro* experiments and also the fusion of penetration sequences, an anchoring domain and an enzyme substrate, to detect the intracellular enzyme activity but not to influence it by, for example, inhibiting it.

It would therefore be useful to have inhibitors which are highly selective for ERKs and which act downstream, on one or more specific substrate(s) that is (are) cytoplasmic or nuclear, in order to minimize, preferably completely avoid, any related, or even pleiotropic, effect.

[0003] The present invention provides peptides which are useful as highly selective inhibitors of ERK-type MAP kinases with respect to their nuclear or cytoplasmic substrates.

[0004] According to the present invention, the term "ERK-type MAP kinase" or "ERK" denotes any ERK MAP kinase. In particular, said ERK-type MAP kinase can be a mammalian, in particular human, primate or murine, MAP kinase. It can also be non-mammalian (lamprey, zebrafish, C. elegans, drosophila, xenopus).
According to the present invention, the term "ERK inhibitor" or "ERK-type MAP kinase inhibitor" denotes any compound which makes it possible to inhibit the kinase function of ERK on at least one given substrate.
According to the present invention, the term "peptide" or "peptide chain" denotes any chain of amino acids. Said chain of amino acids generally contains from 2 to 100 residues, preferably from 5 to 75 residues, more preferably from 10 to 50 residues (see IUPAC definition, http://www.chem.qmul.ac.uk/iupac/AminoAcid/A1113.html#AA11).
Preferably, said chain contains 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, …, 50, …, 100 amino acid residues.
According to the present invention, the term "amino acid" or "amino acid residue" denotes any amino acid residue known to those skilled in the art (see, for example: N. Sewald, H.-D. Jakubke, Peptides: Chemistry and Biology 2002, Wiley-VCH Verlag GmbH, Weinheim; IUPAC nomenclature http://www.chem.qmul.ac.uk/iupac/AminoAcid/).

[0005] This comprises the natural amino acids (including, for example, according to the three-letter code, Ala, bAla, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val), and also rare and/or synthetic amino acids and their derivatives (including, for example, Aad, Abu, Acp, Ahe, Aib, Apm, Dbu, Des, Dpm, Hyl, MeLys, MeVal, Nva, HAO, NCap, Abu, Aib, MeXaa and the like (see, for example: J. S. Nowick, J.O. Brower, J. Am. Chem. Soc. 2003, 125, 876-877; R. Aurora, G. D. Rose, Protein Science 1998, 7, 21-38; W. Maison, E. Arce, P. Renold, R. J. Kennedy, D. S. Kemp, J. Am. Chem. Soc. 2001, 123, 10245-10254; D. Obrecht, M. Altorfer, J. A. Robinson, Adv. Med. Chem. 1999, 4, 1-68; K. Müller, D. Obrecht, A. Knierzinger, C. Stankovic, C. Spiegler, W. Bannwarth, A. Trzeciak, G. Englert, A. M. Labhard, P. Schönholzer in Perspectives in Medicinal

Chemistry, (Eds.: B. Testa, E. Kyburz, W. Fuhrer, R. Giger), Verlag Helv. Chim. Acta, Basel, 1993, pp. 513-533; F. Formaggio, A. Bettio, V. Moretto, M. Crisma, C. Toniolo, Q. B. Broxterman, J. Peptide Sci. 2003, 9, 461-466).

Said amino acid residue or its derivative can be any isomer thereof, in particular any chiral isomer, for example the L- or D-isoform, and mixtures thereof. The D-isoform has the advantage of better stability.

The term "amino acid derivative" here denotes any amino acid derivative, in particular any derivative known to those skilled in the art (see, for example: N. Sewald, H.-D. Jakubke, Peptides: Chemistry and Biology 2002, Wiley-VCH Verlag GmbH, Weinheim; IUPAC nomenclature http://www.chem.qmul.ac.uk/iupac/AminoAcid/).

For example, the amino acid derivatives include residues that can be derived from natural amino acids bearing additional side chains, for example alkyl side chains, and/or substitutions of heteroatoms.

The notion of an "amino acid sequence" is known to those skilled in the art. An amino acid sequence comprises at least two residues covalently bound by means of at least one peptide bond.

The amino acid sequences will subsequently be given using the one-letter code.

Said peptide can be obtained by methods known to those skilled in the art, for example said peptide can be obtained by synthetic methods, such as solid-support synthesis or synthesis in solution (synthetic peptides), or techniques derived from molecular biology (recombinant peptide).

[0006] The present invention relates to the following propositions:

1. A peptide comprising:
   - at least one amino acid sequence which allows said peptide to penetrate into a cell;
   - optionally, an intracellular targeting amino acid sequence chosen from NESs;
   - optionally, an intracellular targeting sequence chosen from NLSs;
   - an amino acid sequence corresponding to a docking domain sequence of a substrate of an ERK-type MAP kinase;
   - optionally, at least one spacer sequence;

- optionally, an enzymatic cleavage sequence possibly surrounded by spacer sequences.

2. The peptide according to proposition 1, such that said docking domain sequence is chosen from the D or FXFP domains of the substrates of ERK-type MAP kinases.

3. The peptide according to any of propositions 1 and 2, such that said sequence which allows said peptide to penetrate into a cell is chosen from the sequences of an HIV-TAT penetrating peptide, of penetratin, and the 7/11R or X7/11R sequences.

4. The peptide according to any one of propositions 1 to 3, which is coupled to a fluorophore, preferably covalently, or to an enzyme such as beta-galactosidase, or which is biotinylated.

5. A nucleic acid encoding a peptide according to any one of propositions 1 to 3.

6. An expression vector comprising a nucleic acid according to proposition 5.

7. A kit containing at least one peptide according to any one of propositions 1 to 4 and/or at least one expression vector according to proposition 6.

8. The use of a peptide according to any one of propositions 1 to 4 as an inhibitor, *in vivo* or *in vitro*, of the activity of said ERK-type MAP kinase relative to a given substrate in a given cell compartment.

[0007] The present invention concerns a peptide comprising:

- At least one amino acid sequence which allows said peptide to penetrate into a cell;
- Optionally, an intracellular targeting amino acid sequence chosen from NES;
- Optionally, an intracellular targeting sequence chosen from NLS;
- An amino acid sequence corresponding to a docking domain sequence of a substrate of an ERK-type MAP kinase;
- Optionally, at least one spacer sequence;
- Optionally, an enzymatic cleavage sequence possibly surrounded by spacer sequences.

[0008] The expression "amino acid sequence which allows said peptide to penetrate into a cell" denotes, according to the present invention, any amino acid sequence that facilitates and/or mediates the transport of said peptide from the outside of a cell to its inside. Such sequences are known to those skilled in the art. Said sequence which allows said peptide to penetrate into a cell can be chosen according to the cell type of said cell, in order to optimize the penetration efficiency.

According to one embodiment, said sequence which allows said peptide to penetrate into a cell is from 2 to 20 residues in length, in particular 6, 7, 8, 9, ..., 17, 18, 19 or 20 residues.

According to one embodiment, said sequence which allows said peptide to penetrate into a cell is chosen from: the sequence of the HIV-TAT penetrating peptide, penetratin, a sequence of seven to eleven arginines, a sequence referred to as "X7/11R sequence".

The term "X7/11R sequence" is intended to mean any peptide sequence of 7 to 25, preferably 7 to 20 amino acids containing between seven and eleven arginine residues (7/11R), in which the arginine residues (R) can be placed randomly within said sequence. Examples are given below, but those skilled in the art are able to give other possibilities.

[0009] According to one embodiment, said sequence which allows said peptide to penetrate into a cell is chosen from:

SEQ ID No.	Sequence → penetration	Origin
1	GRKKRRQRRR	HIV-TAT
2	RQIKIWFQNRRMKWKK	Penetratin
3	RRRRRRR	7R
4	XRRRRRRRX	X7R (example)
5	XRRRXRRRR	X7R (other example)
6	RRRXRRRRX	X7R (other example)
7	RRRRRRRXX	X7R (other example)
8	XXRRRRRRR	X7R (other example)
9	RRRRRRRRRRR	11R
10	XRRRRRXRRRRRR	X11R (other example)
11	RRRRRXRRRRRRX	X11R (other example)

[0010] The notion of NLS (nuclear localization signal) is known to those skilled in the art. It is generally an amino acid sequence which allows the targeting of a given protein to the nucleus, via the phenomenon of nuclear import.

According to one embodiment, said NLS sequence is a sequence rich in basic amino acids (arginine or lysine).

According to one embodiment, said NLS sequence is from 2 to 20 residues in length, in particular 6, 7, 8, 9, …, 17, 18, 19 or 20 residues.

According to one embodiment, said NLS sequence is chosen from:

SEQ ID No.	NLS sequence	Origin
12	P**KKKRK**V	SV40 large T-antigen
13	**KR**PAAI**KK**AGQA**KKKK**	nucleoplasmin
14	**R**QA**RR**N**RR**N**RRRR**W**R**	HIV1Rev
1	GRKKRRQRRR	HIV-TAT
2	RQIKIWFQNRRMKWKK	penetratin
3	RRRRRRR	7R
9	RRRRRRRRRRR	11R

[0011] The notion of NES (nuclear export signal) is known to those skilled in the art. They are generally amino acid sequences which mediate nuclear export, resulting in translocation of a given protein from the nucleus to the cytoplasm. According to one embodiment, said NES sequence is from 2 to 20 residues in length, in particular 6, 7, 8, 9, …, 10, 11, 12, …, 17, 18, 19 or 20 residues. According to one embodiment, said NES sequence is chosen from:

SEQ ID No.	NES sequence	Origin
15	XLXXXLXXLXLX	Elk-1 type consensus
16	XLXXXLXXLXRX	Net type consensus
17	A**L**QKK**L**EE**L**E**L**D	MAPKK
18	T**L**WQF**L**LQ**L**L**L**D	Net (ERK substrate)
19	T**L**WQF**L**LQ**L**LRE	Elk-1 (ERK substrate)

[0012] Said amino acid sequence corresponding to a docking domain sequence of a substrate of an ERK-type MAP kinase can comprise any docking domain of an ERK substrate known to those skilled in the art.
The notion of "docking domain" is known to those skilled in the art. It is generally a portion of the substrate of a MAP kinase which specifically conditions the interaction and/or the recruitment between said substrate and said MAP kinase. It is all or part of a docking site of said substrate for said MAP kinase. The sequence of said docking domain is therefore specific and selective for a given interaction. Thus, advantageously according to the invention, each of these docking domain sequences corresponds to a portion (amino acid sequences) of an ERK MAP kinase substrate, which portion specifically conditions the interaction and/or the recruitment between said substrate and said ERK-type MAP kinase.

According to one embodiment, said amino acid sequence corresponding to a docking domain sequence of a substrate of an ERK-type MAP kinase can comprise only a part of a docking domain of an ERK substrate. Thus, since said amino acid sequence corresponding to a docking domain sequence of a substrate of an ERK-type MAP kinase contains only a portion of the docking domain, it is possible to obtain inhibition of several given substrates of ERK.

According to one embodiment of the present invention, said amino acid sequence corresponding to a docking domain sequence of a substrate of an ERK-type MAP kinase is 12-25 residues in length, preferably 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 residues.

According to one embodiment, said "docking domain" sequence is chosen from "FXFP-type docking domain sequences".

According to one embodiment, said docking domain sequence is 12-25 residues in length, preferably 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 residues.

The expression "FXFP-type docking domain sequence" is here intended to mean any FXFP-type docking domain sequences known to those skilled in the art. This includes in particular the sequences of the "FXFP" domains and the corresponding flanking Nter and Cter sequences of said substrate).

According to one embodiment, said docking domain sequence is chosen from:

SEQ ID No.	Docking domain sequence	Type of the docking domain	Substrate of ERK
20	SPAKLS**FQFP**SGSAQVHI	FXFP	Elk-1
21	SPARLQGANTL**FQFP**SVLN	FXFP	Sap-1
22	SPARLQGPSTL**FQFP**TLLN	FXFP	Sap-2
23	MAVLDRGTSTTTV**FNFPV**	FXFP	MKP-1
24	PNPSPGQRDSR**FSFP**D	FXFP	KSR
25	SLTPTAAHSGSHL**FGFPP**	FXFP	GATA-2
36	PGIMLRRLQKGNLPVRAL	D	MKP-3

[0013] A single substrate can sometimes contain several docking domains for ERK. This is the case, for example, of Elk-1 and MKP-1 with respect to ERK. In this case, one or other of the docking domain sequences may be used in a peptide according to the invention for blocking the ERK/substrate interaction. Alternatively, the joint use of two peptides according to the invention, one of the peptides containing a docking domain sequence, for example an FXFP sequence, and the other peptide containing another docking domain sequence, for example a D sequence, will make it possible to improve the inhibition at subliminal concentrations.

[0014] Advantageously according to the invention, said peptide has the following properties: once brought into contact with a cell, by virtue of said amino acid sequence which allows said peptide to penetrate into a cell, the peptide according to the invention enters said cell. Subsequently, depending on the nature of said intracellular targeting sequence, said peptide becomes localized either in the nucleus (if NLS) or in the cytoplasm (if NES). Alternatively, in the absence of an additional intracellular targeting sequence, given the content rich in basic amino acids of said sequence which allows penetration, the latter also plays the role of an NLS, such that said peptide is localized in the nucleus. Thus, according to the structure of said peptide according to the invention, the latter advantageously adopts a specific intracellular localization. Said docking domain sequence then plays an inhibitory role: it advantageously makes it possible to mimic the presence of said substrate with respect to the ERK-type MAP kinase, thus resulting in a selective and specific inhibition of the interaction between ERK and said substrate, with a specific intracellular localization: depending on the case, the

inhibition is specific for the nuclear interaction, or specific for the cytoplasmic interaction, between ERK and said substrate. Thus, advantageously according to the invention, the resulting inhibition is specific not only for the substrate/ERK couple (due to the docking domain), but also specific in terms of the intracellular localization (selective inhibition of the nuclear interaction, or selective inhibition of the cytoplasmic interaction: specific differential inhibition).

Said cell is a eukaryotic cell, preferably a higher eukaryotic cell, for example a mammalian cell or a human cell. It may be a cell undergoing mitosis or a quiescent (post-mitotic) cell, for example a neuronal cell.

Advantageously according to the invention, said optional spacer sequence makes it possible to ensure a certain conformational flexibility between said sequence which allows said peptide to penetrate into a cell and said docking domain sequence. For example, said spacer sequence can comprise at least one, preferably several proline residues, for example 2, 3 or 4 proline residues.

Moreover, according to one embodiment, said peptide can comprise an enzymatic cleavage site for separating said amino acid sequence for penetration of said peptide into a cell, from the rest of said peptide. Advantageously according to the invention, said peptide can thus comprise two consecutive cysteine residues, thus allowing intracellular cleavage by cytoplasmic glutathione (the disulfide bridges that exist between these two residues are cleaved after penetration into the cell). Any other enzymatic cleavage site, in particular for cleavage by an intracellular protease known to those skilled in the art, can also be used. According to one embodiment of the invention, said cleavage site can be a cleavage site for cysteine proteases of caspase type or for NSE (neuron specific enolase).

According to one embodiment, the peptide according to the invention is coupled to at least one fluorophore, preferably covalently. Said fluorophore can be any fluorophore known to those skilled in the art. In particular, said fluorophore can be chosen from Fam, Hex, Tet, Joe, Rox, Tamra, Max, Edans, Cy dyes such as Cy5, Cy2 or Cy3, fluorescein, coumarin, eosin, rhodamine, bodipy, alexa, cascade blue, Yakima yellow, Lucifer yellow and Texas red AMCA (registered trade marks). Alternatively, said peptide can be biotinylated and visualized, indirectly, with avidin labeled with the fluorophores described above. Said peptide may also be coupled to an enzymatic label, for example of beta-galactosidase type. Advantageously according to the invention, said fluorophores, biotin or enzyme (beta-galactosidase, for example) are located at the C-terminal or N-terminal

region of the docking site of said peptide so as to be able to locate it in the whole animal *in vivo*, on a preparation of cells *in vitro*, as well as on a preparation of fixed cells.

[0015] The present invention also relates to a nucleic acid encoding a peptide as described above. For a given peptide, those skilled in the art will be able to identify which nucleic acid sequence(s) encode(s) such a peptide, on the basis of the genetic code, the degeneracy of said code, and codon adaptation according to species.

The present invention also relates to an expression vector comprising a nucleic acid encoding a peptide as described above.

According to one embodiment, said expression vector is a eukaryotic expression vector.

Said expression vector will advantageously be suitable for a given cell type, depending on the use for which the peptide according to the invention is intended. Thus, those skilled in the art will be able to design such as vector. In particular, those skilled in the art will be able to choose between a constitutive or tissue-specific promoter, allowing the expression of said peptide from said vector. In addition, said expression promoter can be chosen from constitutive promoters, inducible promoters and specific promoters, for example tissue-specific promoters.

According to one embodiment, said expression vector contains a nestin-type promoter, in order to allow the early expression of said peptide during development.

According to another embodiment, said expression vector comprises at least one tissue-specific promoter, in order to allow the expression of said peptide in targeted tissues.

Furthermore, for a given tissue, the expression of said peptide may be restricted to certain regions of said tissue, for example certain regions of the brain:

According to one embodiment, said expression vector contains a CaMKII-type promoter, in order to obtain a preferential expression in the hippocampus (site of spatial memory).

According to one embodiment, said expression vector contains a D1-type dopaminergic receptor promoter, in order to obtain a striatum-specific expression (site of addictive processes).

According to one embodiment, said expression vector contains a tyrosine hydroxylase promoter for expression in the substantia nigra compacta (site of degenerative processes in Parkinson's disease).

According to one embodiment, said expression vector also contains an inducible promoter, for example a promoter induced or repressed by tetracycline (TetOn, TetOff system).

Said expression vector can contain a bacterial origin of replication which allows its replication in bacterial host cells, typically E. coli.

According to one embodiment, said expression vector is designed so that it can be used to generate transgenic animals, for example transgenic mice, which will express said peptide at desired moments in a given tissue, and, within said tissue (for example in the brain), in a given region.

According to one embodiment, said expression vector is a viral vector. Said viral vector can be chosen from the group of retroviral vectors, canine viral vectors and lentiviral vectors. Said viral vector then allows a tissue-specific expression: a retroviral vector makes it possible to preferentially target dividing cells; a canine virus makes it possible to target post-mitotic cells of neuronal type; a lentiviral vector can integrate into the genome of the host cell without discrimination. Said viral vector can also be used in the context of a gene therapy.

[0016] The present invention also relates to a kit containing at least one peptide as described above and/or at least one vector or nucleic acid encoding peptides as described above. In addition, said kit can contain controls (positive or negative) in the form of peptides or of vectors, for carrying out control experiments in parallel with the experiments involving at least one peptide according to the present invention. For example, a negative control peptide can contain a "scrambled" sequence of amino acids. Said kit can, moreover, contain instructions for use.

According to one embodiment, said kit can contain at least two different peptides according to the invention. Said peptides can be intended to inhibit the interaction of an ERK-type MAP kinase with at least two distinct substrates, or just one sole substrate. Indeed, a single substrate can sometimes contain several docking domains for the same MAP kinase. This is the case, for example, of Elk-1 and MKP-1 with respect to ERK. In this case, one or the other of the docking domain sequences may be used in a peptide according to the invention for blocking the ERK/substrate interaction. Alternatively, the joint use of two peptides according to

the invention, one of the peptides containing a docking domain sequence, for example an FXFP sequence, and the other peptide containing another docking domain sequence, for example a D sequence, will make it possible to improve the inhibition at subliminal concentrations.

[0017] The present invention also relates to the use of a peptide as defined above, as an in vitro or in vivo inhibitor of the activity of said ERK-type MAP kinase relative to a given substrate. This type of use covers very varied fields, depending on the nature of the substrate(s) of said ERK-type MAP kinase, the cell type considered, and the type of extracellular stimulation considered.
Advantageously, said peptide may be labeled, for example coupled to a label (for example fluorophore, biotin or beta-galactosidase), and may thus be tested in vivo in the whole animal after systemic or intratissular injection. After systemic injection, said peptide may be located in the various tissues, including in the central nervous system (the presence of said sequence which allows penetration allowing the blood-brain barrier to be crossed), by virtue of the label coupled to the peptide.
Thus, the peptide according to the invention is useful in the study of various types of phenomena, in particular in neurobiology (study of development, of neuronal plasticity, of addictive processes) and cancerology (cell cycle regulation).

Extracellular stimulation	Cell type	ERK substrate	Inhibition	Phenomenon
Neurotransmitter	Neuron	Elk-1 MKP-1	Nuclear	Drug addiction memory learning survival plasticity
Neurotransmitter	Neuron	MKP-3	Cytoplasmic	Neurodegeneration, neuronal death, example: Parkinson's
Neurotransmitter	Neuron	Elk-1	Cytoplasmic	Neurodegeneration, example: Parkinson's
Genetic mutations or stress or cancer or growth factors	Mitotic cells	Elk-1	Nuclear	Cancer, cell cycle

[0018] The peptides according to the present invention can have the following structure:

N-terminal				C-terminal
«penetration sequence »	«C »	«NES targeting »	« docking domain »	
«penetration sequence »	«C »	« docking domain »	«NES targeting »	
«penetration sequence »	«C »	«NES targeting »	« S »	« docking domain »
«penetration sequence »	« S »	« docking domain »	«C »	«NES targeting »
«penetration sequence »	« S »	« docking domain »		
«penetration sequence »	«S »	«NLS targeting»	« docking domain »	
«penetration sequence »	«S »	« docking domain »	«NLS targeting»	
«penetration sequence »	«S »	«NLS targeting»	« S »	« docking domain »
«penetration sequence »	«S »	« docking domain »	« S »	«NLS targeting»
«penetration sequence »	«C »	«NLS targeting»	« docking domain »	
«penetration sequence »	«C »	« docking domain »	«NLS targeting»	
«penetration sequence »	«C »	«NLS targeting»	« S »	«docking domain»
«penetration sequence »	«C »	« docking domain »	« S »	«NLS targeting»
«penetration sequence »	«C »	« docking domain"	«NES targeting »	
«penetration sequence »	«C »	«NES targeting »	« docking domain"	
«penetration sequence »	«C »	« docking domain"	« S »	«NES targeting »
«penetration sequence »	«C »	«NES targeting »	« S »	« docking domain"
«penetration sequence »	«S »	« docking domain »	«NLS targeting»	
«penetration sequence »	«S »	«NLS targeting»	« docking domain »	
«penetration sequence »	«S »	« docking domain »	« S »	«NLS targeting»
«penetration sequence »	«S »	«NLS targeting»	« S »	« docking domain »
«penetration sequence »	« docking domain »	« S »	«NLS targeting»	
«penetration sequence »	«C »	«NLS targeting»	« docking domain »	
«penetration sequence »	«C »	« docking domain »	«NLS targeting»	
«penetration sequence »	«C »	«NLS targeting»	«S »	« docking domain »

«penetration sequence »	«C »	« docking domain »	«S »	«NLS targeting»
« docking domain »	«S »	«penetration sequence »		

Or

«docking domain»	«NES targeting »	«C»	«penetration sequence»	
«NES targeting »	«docking domain»	«C»	«penetration sequence»	
«docking domain»	«S»	«NES targeting »	«C»	«penetration sequence»
«docking domain»	«S»	«penetration sequence»	«C»	«NES targeting »
«docking domain»	«S»	«penetration sequence»		
«docking domain»	«NLS targeting»	«S»	«penetration sequence»	
«NLS targeting»	«docking domain»	«S»	«penetration sequence»	
«docking domain»	«S»	«NLS targeting»	«S»	«penetration sequence»
«NLS targeting»	«S»	«docking domain»	«S»	«penetration sequence»
«docking domain»	«NLS targeting»	«C»	«penetration sequence»	
«NLS targeting»	«docking domain»	«C»	«penetration sequence»	
«docking domain»	«S»	«NLS targeting»	«C»	«penetration sequence»
«NLS targeting»	«S»	«docking domain »	«C»	«penetration sequence»
«docking domain»	«NES targeting »	«C»	«penetration sequence»	
«NES targeting »	«docking domain»	«C»	«penetration sequence»	
«docking domain»	«S»	«NES targeting »	«C»	«penetration sequence»
«NES targeting »	« S»	«docking domain»	«C»	«penetration sequence»
«docking domain»	«NLS targeting»	«S»	«penetration sequence»	
«NLS targeting»	«docking domain»	«S»	«penetration sequence»	
«docking domain»	«S»	«NLS targeting»	«S»	«penetration sequence»
«NLS targeting»	«S»	« docking domain »	«S»	«penetration sequence»
«NLS targeting»	«S»	«docking domain»	«penetration sequence»	

«NLS targeting»	«docking domain»	«C»	«penetration sequence»	
«docking domain»	«NLS targeting»	«C»	«penetration sequence»	
«docking domain»	«S»	«NLS targeting»	«C»	«penetration sequence»
«NLS targeting »	«S»	«docking domain»	«C»	«penetration sequence»
«penetration sequence»	«S»	«docking domain»		

where:

- "penetration sequence" comprises at least one amino acid sequence which allows said peptide to penetrate into a cell;
- "S" comprises or corresponds to an optional sequence of spacer type, for example two prolines, or a gamma-aminobutyric acid, which allows flexibility between the penetrating sequence and the docking peptide;
- "C" comprises or corresponds to an enzymatic cleavage site which makes it possible to release, inside the cell, the docking peptide and its localization sequence from the penetrating sequence; this cleavage site may or may not comprise a spacer "S", placed in C-term, in N-term or on either side of the cleavage site. In other words, C refers to C on its own, bordered by two S or "flanked" by S on the C-ter or N-ter side;
- "targeting" comprises an intracellular targeting sequence of NES or NLS type;
- "docking domain" comprises the FXFP-type or D-type docking domain amino acid sequence of a given substrate of ERK.

[0019] Advantageously according to the invention, the cleavage site makes it possible to separate the sequence which allows penetration, which is at one of the ends of the peptide, from the rest of the peptide. This is particularly advantageous when the targeting sequence is an NES: the localization of the peptide is then still restricted to the cytoplasm.

[0020] The advantages of the peptides according to the invention will be understood more clearly upon reading the following nonlimiting examples.

EXAMPLES

Example 1

Peptides according to the invention

[0021] The following peptides are synthesized by the solid-phase synthesis method: peptides P1, P2 and P3.

Peptides P1 and P2: inhibition of the interaction between Elk-1 and ERK

[0022] Peptide P1 (SEQ ID No. 39)

GRKKRRQRRRCC*TLWQFLLHLLLD*SPAKLSFQFPSGSAQVHI

in which:

- sequence which allows penetration: **GRKKRRQRRR** (HIV-TAT penetrating peptide) (SEQ ID No. 1)
- enzymatic cleavage site: CC
- targeting sequence: *TLWQFLLHLLLD* (NES of Net)(SEQ ID No. 18)
- docking domain sequence: SPAKLSFQFPSGSAQVHI (SEQ ID No. 20)

P1 penetrates into the cells and becomes localized therein in the cytoplasm.

[0023] Peptide P2 (SEQ ID No. 40)

GRKKRRQRRRPPSPAKLSFQFPSGSAQVHI

in which:

- sequence which allows penetration: **GRKKRRQRRR** (SEQ ID No. 1)
- spacer sequence: PP
- docking domain sequence: SPAKLSFQFPSGSAQVHI (SEQ ID No. 20)

P2 penetrates into the cells and adopts a nuclear localization.

Peptide P3: inhibition of the interaction between MKP-3 and ERK

[0024] Peptide P3 (SEQ ID No. 41)

GRKKRRQRRRCC*TLWQFLLHLLLD*PGIMLRRLQKGNLPVRAL (SEQ ID No. 41)

in which:

- sequence which allows penetration: **GRKKRRQRRR** (SEQ ID No. 1)
- enzymatic cleavage site: CC
- targeting sequence: *TLWQFLLHLLLD* (NES of Net) (SEQ ID No. 18)
- docking domain sequence: PGIMLRRLQKGNLPVRAL (SEQ ID No. 36)

P3 penetrates into the cells and adopts a cytoplasmic localization.

Example 2

Example of cell penetration and of nuclear localization of a peptide according to the invention

[0025] The peptide F2 according to the invention ("docking peptide") is used here: it has the same sequence as the peptide P2 (see Example 1), and is coupled to FITC (fluorophore) at its C-terminal end.

[0026] It therefore has the following structure:
[HIV-TAT penetrating peptide]-[spacer of PP type]-[FXFP-type docking domain of the ERK/Elk-1 couple]-[FITC].

[0027] HEK293 cells are placed in the presence of the peptide F2 at various concentrations (1 mM stock solution in distilled water, then dilutions to 25, 50 and 100 μM in DMEM culture medium), for 15, 30 or 60 minutes, continuously. The cell nuclei are labeled using Hoechst dye (left panel), and the peptide F2 is visualized by means of the FITC label (middle panel). Figure 1 shows the results obtained for the 100 μM concentration of peptide F2, as a function of time. The labeled cell nuclei are shown on the left panels, the peptide F2 on the middle panels and the superimposition of these two labelings is represented on the right panels (panels marked fusion).

[0028] The peptide F2 according to the invention rapidly penetrates into the cells, and adopts a nuclear localization after only 30 minutes. In the absence of an additional intracellular targeting sequence, given the content rich in basic amino acids of the HIV-TAT penetration sequence, the latter also plays the role of NLS, and the peptide F2 is thereby advantageously localized in the nucleus.

[0029] The peptide F2 rapidly penetrates the cells and then adopts an exclusively nuclear localization.

Example 3

Example of cell penetration and of cytoplasmic localization of a peptide according to the invention

[0030] The peptide F1 according to the invention ("docking peptide") is used here: it has the same sequence as the peptide P1 (see Example 1), and is coupled to FITC (fluorophore) at its C-terminal end.

[0031] It therefore has the following structure:
[HIV-TAT penetrating peptide]-[C-C cleavage site]-[FXFP-type docking domain of the ERK/Elk-1 couple]-[FITC].

[0032] HEK293 cells are placed in the presence of the peptide F1 at various concentrations (1 mM stock solution in distilled water, then dilutions to 25, 50 and 100 μM in DMEM culture medium) for 15, 30 or 60 minutes, continuously. The cell nuclei are labeled using Hoechst dye, and the peptide F1 is visualized by means of the FITC label. Figure 2 shows the results obtained for the 100 μM concentration of peptide F1, as a function of time.

[0033] The labeled cell nuclei are shown on the left panels, the peptide F2 on the middle panels and the superimposition of these two labelings is represented on the right panels (panels marked fusion).

[0034] The peptide according to the invention rapidly penetrates the cells and adopts a cytoplasmic localization.

Example 4

Biochemical characterization of the inhibitory effects of a peptide according to the invention: P2 inhibits the activation of Elk-1 by serum in mitotic cells

[0035] The peptide P2 according to the invention (see Example 1) is used here.

[0036] HEK cells were treated as indicated in Example 2 (Figure 1) with the peptide P2 (40 minutes), followed by a treatment with serum (10%) for 20 minutes or 5 minutes. The serum activates the MAP kinase/ERK pathway.

[0037] The activation of ERK is characterized by Western blotting using an anti-P-ERK1/2 antibody directed against the phosphorylated (active) form of ERK (anti rabbit Phospho Thr202-Tyr204 ERK, cell signaling, dilution 1/5000) (Figure 3, top panels). The activation of Elk-1 is visualized with an anti-P-Elk-1 antibody directed against the phosphorylated form of Elk-1 (anti mouse Phospho Ser383 Elk-1, Santa-Cruz, dilution 1/200) (Figure 3, bottom panel). The proteins are revealed using anti-rabbit and anti-mouse secondary antibodies respectively coupled to horseradish peroxidase (Amersham, dilutions 1/5000) and visualized by chemiluminescence (Amersham, ELC kit). Dose-response curves were produced in order to determine the lowest concentration of peptide which was effective.

[0038] Advantageously according to the invention, the induction of P-Elk-1 by serum is completely inhibited in the presence of the peptide P2 at 10 μM. This

inhibition is absent at 1 μM of P2 (Figure 3, bottom panel). The higher doses (50;100 μM) of peptide P2 are also found to be effective on the inhibition of Elk-1. Advantageously according to the invention, the induction of P-ERK by serum is not modified by the peptide P2 at 10 μM.

Example 5

Specificity of the inhibition by a peptide according to the invention: The phosphorylation of Elk-1 is inhibited by P2, but not by P1

[0039] The peptides P1 and P2 according to the invention (see Example 1) are used here.

[0040] HEK cells are placed in the presence of the peptide P2 (Figure 4, middle panels) or P1 (Figure 4, right panels), at the concentration of 10 μM for 40 minutes. Nontreated cells (without peptide) are used as controls (Figure 4, left panels).

[0041] The cells are then treated for 20 minutes using fetal calf serum (serum) in order to activate the MAP kinase/ERK pathway.

[0042] The presence of the activated form of the ERK MAP kinase is visualized by immunodetection using an anti-phospho ERK antibody (anti rabbit Phospho Thr202-Tyr204 ERK, cell signaling, dilution 1/500 comment: the dilutions are indeed 10 times weaker for this experiment compared with the Western blotting) and revealed using a fluorescent secondary antibody coupled to Cy3 (anti rabbit Cy3, sigma, 1/2000) (Figure 4, three panels of the second line marked P-ERK). The induction of P-ERK is clearly observed (Figure 4, by way of example, a P-ERK labeling is represented by a white star, the nucleus of this cell is marked with the same star on the top panel corresponding to the Hoechst labeling), whatever the treatment conditions.

[0043] The presence of the activated form of Elk-1 is visualized by immunocytochemistry using an antibody against phospho-Ser383 of Elk-1 (anti mouse Phospho Ser383 Elk-1, Santa-Cruz, dilution 1/200) and revealed using an anti-mouse secondary antibody coupled to Cy3 (anti mouse Cy3, Jackson Immunoresearch, 1/600) (Figure 4, by way of example, a P-Elk-1 labeling is represented by a white star, on the panels of the fourth line left and right). The corresponding nuclei are visualized by means of the same star on the panels of the third line, marked Hoechst.

[0044] The induction of P-Elk-1 is observed in the cytoplasmic and nuclear compartments in response to the serum (Figure 4, panels of the fourth line on the left), and also in the presence of serum and of the peptide P1 (Figure 4, panels of the fourth line on the right).

[0045] The absence of induction of P-Elk-1 is also observed in the cells pretreated with the peptide P2 (Figure 4, panels of the fourth line in the middle).

SEQUENCE LISTING

<110> CNRS
Université Pierre et Marie Curie

<120> Peptides inhibiteurs

<130> GBu/CN/VF - EP05290363

<160> 41

<170> PatentIn version 3.3

<210> 1
<211> 10
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 1

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10

<210> 2
<211> 16
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 2

Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys
1 5 10 15

<210> 3
<211> 7
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 3

Arg Arg Arg Arg Arg Arg Arg
1 5

<210> 4
<211> 9
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa can be any naturally occurring amino acid

<400> 4

Xaa Arg Arg Arg Arg Arg Arg Arg Xaa
1 5

<210> 5
<211> 9
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa can be any naturally occurring amino acid

<400> 5

Xaa Arg Arg Arg Xaa Arg Arg Arg Arg
1 5

<210> 6
<211> 9
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa can be any naturally occurring amino acid

<400> 6

Arg Arg Arg Xaa Arg Arg Arg Arg Xaa
1 5

<210> 7
<211> 9
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (8)..(9)
<223> Xaa can be any naturally occurring amino acid

<400> 7

Arg Arg Arg Arg Arg Arg Arg Xaa Xaa
1 5

<210> 8
<211> 9
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (1)..(2)
<223> Xaa can be any naturally occurring amino acid

<400> 8

Xaa Xaa Arg Arg Arg Arg Arg Arg Arg
1 5

<210> 9
<211> 11
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 9

Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10

<210> 10
<211> 13
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa can be any naturally occurring amino acid

<400> 10

Xaa Arg Arg Arg Arg Arg Xaa Arg Arg Arg Arg Arg Arg
1 5 10

<210> 11
<211> 13
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (13)..(13)
<223> Xaa can be any naturally occurring amino acid

<400> 11

Arg Arg Arg Arg Arg Xaa Arg Arg Arg Arg Arg Arg Xaa
1 5 10

<210> 12
<211> 7

<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 12

Pro Lys Lys Lys Arg Lys Val
1 5

<210> 13
<211> 16
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 13

Lys Arg Pro Ala Ala Ile Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

<210> 14
<211> 15
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 14

Arg Gln Ala Arg Arg Asn Arg Arg Asn Arg Arg Arg Arg Trp Arg
1 5 10 15

<210> 15
<211> 12
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (3)..(5)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (7)..(8)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa can be any naturally occurring amino acid

<400> 15

Xaa Leu Xaa Xaa Xaa Leu Xaa Xaa Leu Xaa Leu Xaa
1 5 10

<210> 16
<211> 12
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (3)..(5)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (7)..(8)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa can be any naturally occurring amino acid

<400> 16

Xaa Leu Xaa Xaa Xaa Leu Xaa Xaa Leu Xaa Arg Xaa
1 5 10

<210> 17
<211> 12
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 17

Ala Leu Gln Lys Lys Leu Glu Glu Leu Glu Leu Asp
1 5 10

<210> 18
<211> 12
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 18

Thr Leu Trp Gln Phe Leu Leu Gln Leu Leu Leu Asp
1 5 10

<210> 19
<211> 12
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 19

Thr Leu Trp Gln Phe Leu Leu Gln Leu Leu Arg Glu
1 5 10

<210> 20
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 20

Ser Pro Ala Lys Leu Ser Phe Gln Phe Pro Ser Gly Ser Ala Gln Val
1 5 10 15

His Ile

<210> 21
<211> 19
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 21

Ser Pro Ala Arg Leu Gln Gly Ala Asn Thr Leu Phe Gln Phe Pro Ser
1 5 10 15

Val Leu Asn

<210> 22
<211> 19
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 22

Ser Pro Ala Arg Leu Gln Gly Pro Ser Thr Leu Phe Gln Phe Pro Thr
1 5 10 15

Leu Leu Asn

<210> 23
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 23

Met Ala Val Leu Asp Arg Gly Thr Ser Thr Thr Thr Val Phe Asn Phe
1 5 10 15

Pro Val

<210> 24
<211> 16

<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 24

Pro Asn Pro Ser Pro Gly Gln Arg Asp Ser Arg Phe Ser Phe Pro Asp
1 5 10 15

<210> 25
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 25

Ser Leu Thr Pro Thr Ala Ala His Ser Gly Ser His Leu Phe Gly Phe
1 5 10 15

Pro Pro

<210> 26
<211> 17
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 26

Lys Gly Arg Lys Pro Arg Asp Leu Glu Leu Pro Leu Ser Pro Ser Leu
1 5 10 15

Leu

<210> 27
<211> 16
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 27

Arg Ser Lys Lys Pro Lys Gly Leu Gly Leu Ala Pro Thr Leu Val Ile

1 5 10 15

<210> 28
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 28

Lys Ala Lys Lys Pro Lys Gly Leu Glu Ile Ser Ala Pro Pro Leu Leu
1 5 10 15

Val Leu

<210> 29
<211> 17
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 29

Ser Ser Ile Leu Ala Gln Arg Arg Val Arg Lys Leu Pro Ser Thr Thr
1 5 10 15

Leu

<210> 30
<211> 19
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 30

Arg Arg Ser Thr Leu Ala Gln Arg Arg Gly Ile Lys Lys Ile Thr Ser
1 5 10 15

Thr Ala Leu

<210> 31
<211> 18

<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 31

Ser Ser Asn Leu Ala Gln Arg Arg Gly Met Lys Arg Leu Thr Ser Thr
1 5 10 15

Arg Leu

<210> 32
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 32

Lys Ser Arg Leu Ala Arg Arg Arg Ala Leu Ala Gln Ala Gly Arg Ser
1 5 10 15

Arg Asp

<210> 33
<211> 19
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 33

Gln Ser Lys Leu Ala Gln Arg Arg Gln Arg Ala Ser Leu Ser Ser Ala
1 5 10 15

Thr Pro Val

<210> 34
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 34

Lys Ala Pro Leu Ala Lys Arg Arg Lys Met Lys Lys Thr Ser Thr Ser
1 5 10 15

Thr Glu

<210> 35
<211> 17
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 35

Arg Phe Ser Thr Ile Val Arg Arg Arg Ala Lys Gly Ala Lys Gly Ala
1 5 10 15

Gly

<210> 36
<211> 18
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 36

Pro Gly Ile Met Leu Arg Arg Leu Gln Lys Gly Asn Leu Pro Val Arg
1 5 10 15

Ala Leu

<210> 37
<211> 17
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 37

Leu Pro Ala Leu Leu Leu Arg Arg Leu Arg Arg Gly Ser Leu Ser Val
1 5 10 15

Arg

<210> 38
<211> 16
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 38

Gly Leu Gln Glu Arg Arg Gly Ser Asn Val Ser Leu Thr Leu Asp Met
1 5 10 15

<210> 39
<211> 42
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 39

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Cys Cys Thr Leu Trp Gln
1 5 10 15

Phe Leu Leu His Leu Leu Leu Asp Ser Pro Ala Lys Leu Ser Phe Gln
20 25 30

Phe Pro Ser Gly Ser Ala Gln Val His Ile
35 40

<210> 40
<211> 30
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 40

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Ser Pro Ala Lys
1 5 10 15

Leu Ser Phe Gln Phe Pro Ser Gly Ser Ala Gln Val His Ile
20 25 30

<210> 41
<211> 42
<212> PRT
<213> artificial

<220>
<223> artificial sequence

<400> 41

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Cys Cys Thr Leu Trp Gln
1 5 10 15

Phe Leu Leu His Leu Leu Leu Asp Pro Gly Ile Met Leu Arg Arg Leu
20 25 30

Gln Lys Gly Asn Leu Pro Val Arg Ala Leu
35 40

Patentkrav

1. Peptid med følgende struktur:

N-terminal C-terminal

« penetrationssekvens » « S » « docking domæne »

hvor

« **penetrationssekvens** » omfatter mindst en aminosyresekvens, som lader nævnte peptid trænge ind i en celle, valgt fra sekvenserne af et indtrængende HIV-TAT-peptid, Penetratin og sekvenserne 7/11R eller X7/11R ;

« S » svarer til en valgfri sekvens af afstandssekvens-typen; og

« **docking domæne** » omfatter aminosyresekvensen svarende til en forankringssekvens af et substrat af en MAP-kinase af typen ERK valgt fra FXFP-domænerne af MAP-kinasesubstraterne af typen ERK.

2. Peptid ifølge krav 1, hvor nævnte aminosyresekvens, som lader nævnte peptid trænge ind i cellen, er valgt fra SEQ ID No: 1, SEQ ID No: 2, SEQ ID No: 3, SEQ ID No: 4, SEQ ID No: 5, SEQ ID No: 6, SEQ ID No: 7, SEQ ID No: 8, SEQ ID No: 9, SEQ ID No: 10 og SEQ ID No: 11.

3. Peptid ifølge et hvilket som helst af kravene 1 og 2, hvor nævnte forankringssekvens er valgt fra SEQ ID No : 20, SEQ ID No : 21, SEQ ID No : 22, SEQ ID No : 23, SEQ ID No : 24, SEQ ID No : 25.

4. Peptid ifølge et hvilket som helst af kravene 1 til 3, forbundet med en fluorofor, fortrinsvis på en kovalent måde eller med et enzym såsom beta-galaktosidase, eller biotinyleret.

5. Kit indeholdende mindst et peptid ifølge et hvilket som helst af kravene 1 til 4.

6. Anvendelse af et peptid ifølge et hvilket som helst af kravene 1 til 3 som en *in vitro* inhibitor af aktiviteten af nævnte MAP-kinase af typen ERK mod et givet substrat i et givet cellulært rum.

7. Peptid ifølge et hvilket som helst af kravene 1 til 4 til anvendelse deraf i terapi, idet nævnte peptid er en inhibitor af aktiviteten af MAP-kinase af typen ERK mod et givet substrat i et givet cellulært rum.

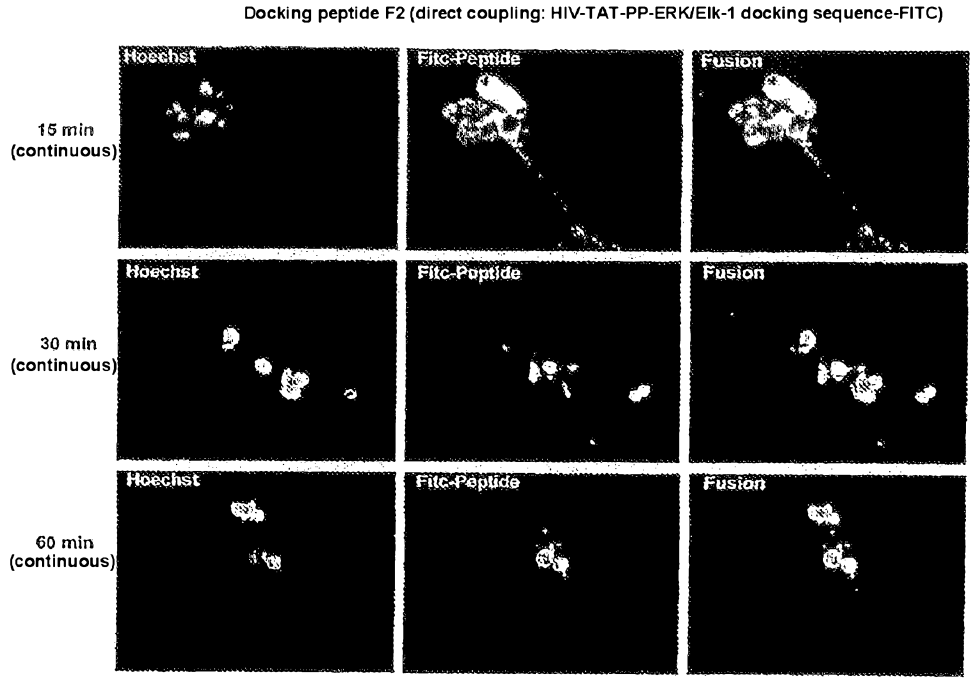
Docking peptide F2 (direct coupling: HIV-TAT-PP-ERK/Elk-1 docking sequence-FITC)
Hoechst
Fitc-Peptide
Fusion
15 min (continuous)
Hoechst
Fitc-Peptide
Fusion
30 min (continuous)
Hoechst
Fitc-Peptide
Fusion
60 min (continuous)

FIGURE 1

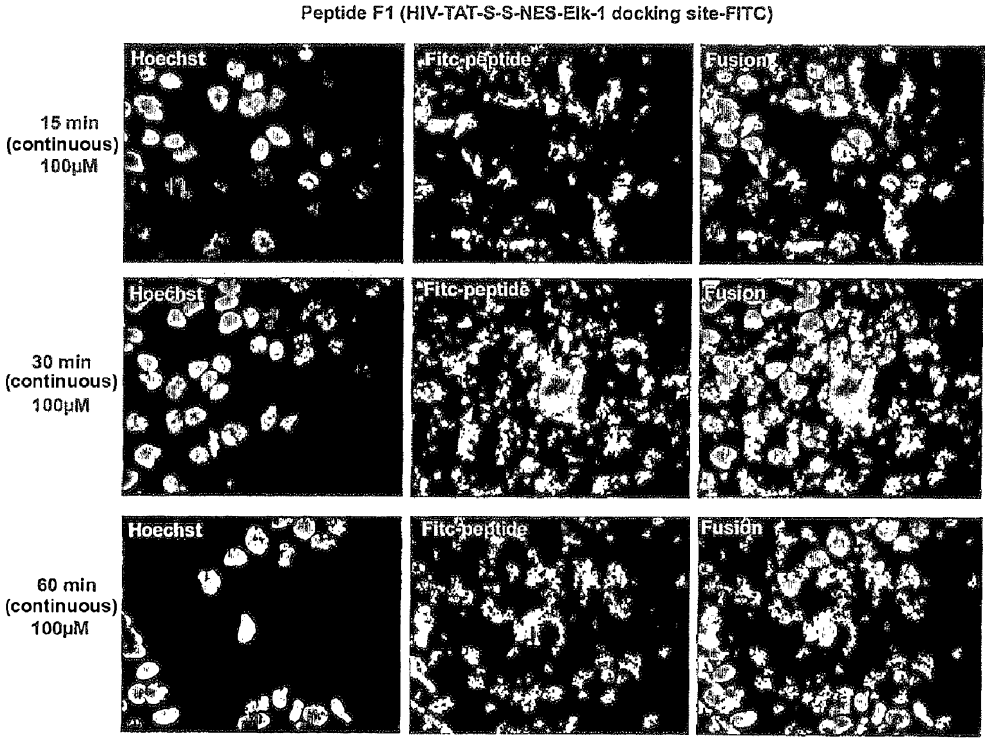
Peptide F1 (HIV-TAT-S-S-NES-Elk-1 docking site-FITC)
Hoechst
Fitc-peptide
Fusion
15 min
(continuous)
100µM
Hoechst
Fitc-peptide
Fusion
30 min
(continuous)
100µM
Hoechst
Fitc-peptide
Fusion
60 min
(continuous)
100µM

FIGURE 2

5

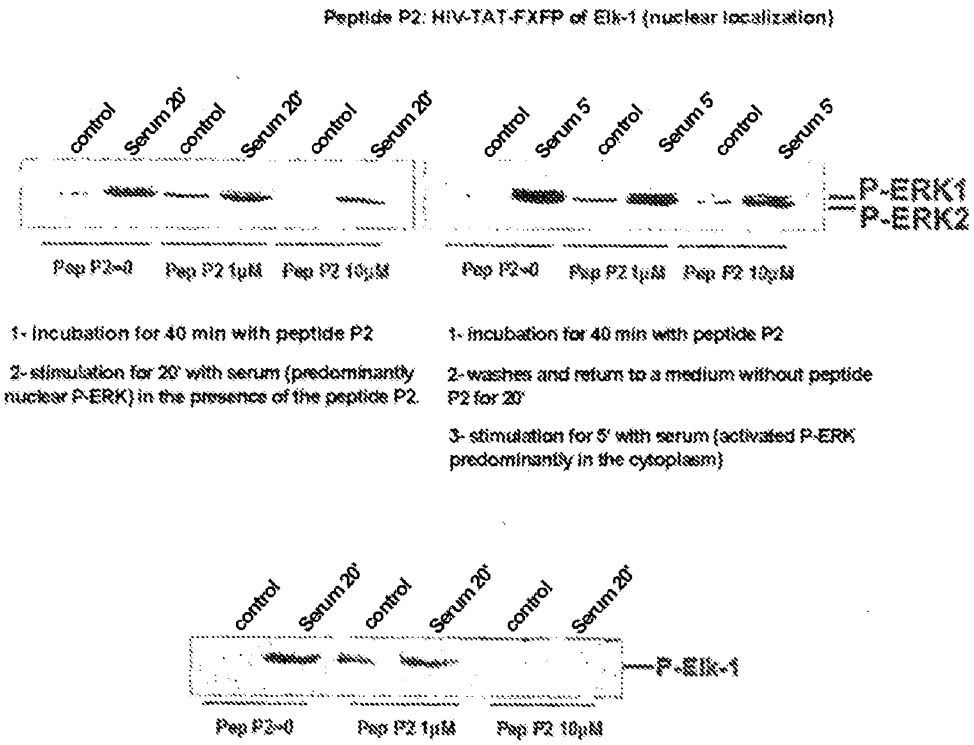
Peptide P2: HIV-TAT-FXFP of Elk-1 (nuclear localization)
control
Serum 20'
control
Serum 20'
control
Serum 20'
control
Serum 5'
control
Serum 5
control
Serum 5
P-ERK1
P-ERK2
Pep P2=0
Pep P2 1µM
Pep P2 10µM
Pep P2=0
Pep P2 1µM
Pep P2 10µM
1- incubation for 40 min with peptide P2
2- stimulation for 20' with serum (predominantly nuclear P-ERK) in the presence of the peptide P2.
1- incubation for 40 min with peptide P2
2- washes and return to a medium without peptide P2 for 20'
3- stimulation for 5' with serum (activated P-ERK predominantly in the cytoplasm)
control
Serum 20'
control
Serum 20'
control
Serum 20'
P-Elk-1
Pep P2=0
Pep P2 1µM
Pep P2 10µM

FIGURE 3

5

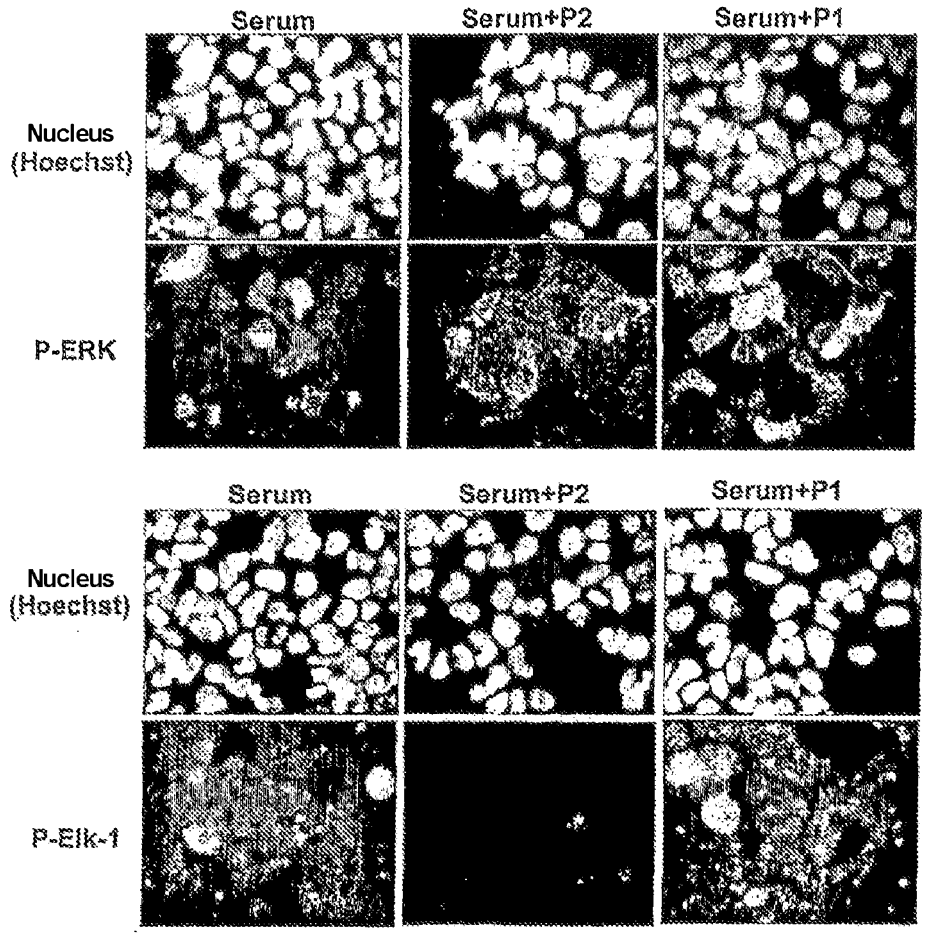
Serum
Serum+P2
Serum+P1
Nucleus (Hoechst)
P-ERK
Serum
Serum+P2
Serum+P1
Nucleus (Hoechst)
P-Elk-1

FIGURE 4