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(72) Inventors: REBOLLO GARCIA, Angelita; 6 rue Gramme, 75015 Paris (FR). BRAVO SICILIA, Jeronimo; C/ Dolores Marqués 33BLQ 3 PTA 6, E-46020 Valencia (ES). FOMINAYA GUTIERREZ, Jesus Maria; Alzuenda a Valverde, 14, 3ºC, 28034 Madrid (ES).

(74) Agent: CABINET BECKER ET ASSOCIES; 25, rue Louis le Grand, 75002 Paris (FR).


(54) Title: PEPTIDES THAT INHIBIT BINDING BETWEEN SET AND CASPASE-9

(57) Abstract: The invention provides a chimeric peptide comprising a cell-penetrating peptide linked to a peptide that inhibits binding between SET protein and Caspase-9 protein, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein consists of, or is derived from, a portion of Caspase-9 that binds a SET protein, or a portion of SET that binds Caspase-9 protein.
PEPTIDES THAT INHIBIT BINDING BETWEEN SET AND CASPASE-9

The invention relates to peptides that inhibit binding between SET and Caspase-9 proteins, and to chimeric peptides linked to a cell-penetrating peptide. These peptides which have a cytotoxic activity are useful, in particular for the treatment of hyperproliferative disorders such as cancer.

**Background of the invention:**

Caspase-9 is required in most scenarios of apoptotic cell death, and consequently impaired caspase-9 activation has profound consequences. The majority of caspase-9 deficient mice die perinatally due to severe morphological deformations of the brain which arise from excess cell numbers that accumulate during embryonic development. Thymocytes isolated from caspase-9 deficient mice exhibit increased resistance to various pro-apoptotic stimuli, including genotoxic stress-inducing anti-cancer drugs and gamma radiation. This apoptotic resistance also suggests that impaired caspase-9 activation or loss of caspase-9 expression might be implicated in cancer development and tumor progression. Indeed, insufficient apoptosome formation and caspase-9 activation were shown to be a key contributor to drug resistance in various cancer models including ovarian cancer, malignant melanoma and leukaemia. Furthermore, polymorphisms in the caspase-9 promoter or the coding regions of the caspase-9 gene, which may affect caspase-9 expression levels or activity, indicate a predisposition to various cancers such as lung, bladder and colorectal cancer. Comparisons of normal and tumor tissue of colorectal cancer patients indicate that caspase-9 expression is frequently decreased in the malignant tissue. It has been shown that the expression level of caspase-9, together with knowledge on the relative abundance of other key proteins involved in cytochrome-c induced apoptosis execution, can be employed to predict whether patients are likely to respond to genotoxic chemotherapeutics that induce mitochondrial outer membrane permeabilisation, such as 5-fluorouracil. Caspase-9 therefore plays an important role in initiating apoptosis execution in cells that need to be eliminated during early developmental stages, and is required for the continuous removal of damaged cells to suppress proliferative diseases during the entire lifetime of multicellular organisms.

The SET protein, also known as (I2PP2A), belongs to a family of multitasking proteins, which is involved in apoptosis, transcription, nucleosome assembly, and histone binding. The SET phosphoprotein is located in the nucleus and cytoplasm and has a critical role in the regulation of normal and cancerous signal transduction. It was originally identified as a translocated gene in acute undifferentiated leukemia. SET is also known as a potent inhibitor of protein phosphatase 2A (PP2A) activity, even though SET has also been described as an inhibitor of the tumor suppressor NM23-H1 that is a granzyme A DNase-activated factor.
Summary of the invention:

The inventors have evaluated a therapeutic approach based on the inhibition of the interaction between Caspase-9 and SET proteins. To do so, they have designed peptides which inhibit the interaction between Caspase-9 factors and SET proteins and chimeric peptides comprising a cell penetrating peptide linked to such peptide inhibitor. Surprisingly, they have shown that small peptides derived from Caspase-9 or SET protein bind domains were able to induce apoptosis of breast cancer cell lines. They have demonstrated the efficacy of this strategy on cellular tumor models of breast cancers.

The inventors have more particularly mapped binding site of Caspase-9 to SET and vice-versa. They have then designed peptides that disturb the Caspase-9/SET interaction, and chimeric peptides wherein a cell penetrating peptide is linked to such peptide. Preferably the peptides that inhibit the binding between SET and Caspase-9 are pro-apoptotic peptides.

The invention provides a chimeric peptide comprising a cell-penetrating peptide linked to a peptide that inhibits binding between SET protein and Caspase-9 protein, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein consists of, or is derived from, a portion of Caspase-9 that binds a SET protein, or a portion of SET that binds Caspase-9 protein.

The invention further provides peptides of 18 to 80 amino acid residues, preferably 18 to 70 amino acid residues, more preferably 18 to 40 amino acid residues wherein said peptide i) inhibits binding between SET protein and Caspase-9 protein, and is preferably pro-apoptotic and ii) comprises or consists of SEQ ID NO: 1, 2, 3, or 4 and a proteolysis-resistant peptide deriving from said peptide by one or more chemical modifications, or a substantially homologous peptide, preferably deriving from SEQ ID NO: 1, 2, 3 or 4, by one or more conservative substitutions.

Another subject of the invention is a nucleic acid that encodes the chimeric peptide construct or the peptide that inhibits binding between SET protein and Caspase-9 protein as defined herein.

A further subject of the invention is a vector comprising said nucleic acid, which is preferably an adenovirus or a lentivirus vector. The peptides, nucleic acid or vector are useful as medicament, preferably for use in treating a hyperproliferative disorder in a patient, more preferably a tumor.

The chimeric peptide construct, or the pro-apoptotic peptide is useful in treating a hyperproliferative disorder, preferably a tumor, in a patient.

In a particular embodiment, the patient is to be administered with a mixture of such various chimeric peptide constructs and/or peptides that inhibit binding between SET protein and Caspase-9 protein.
Legends to the Figures:

Figures 1A and 1B show determination of the binding site of Caspase-9 to SET and vice versa A) Overlapping dodecapeptides with two amino acid shift covering the whole caspase-9 protein were bound to a solid support. The membrane was incubated sequentially with SET protein, and anti-SET antibody, followed by a peroxidase-labeled secondary antibody. The membrane was revealed with ECL system. The sequence corresponding to the identified spots is shown B) Overlapping dodecapeptides with two amino acid shift covering the whole human SET protein were bound to a solid support. The membrane was incubated sequentially with caspase-9 protein, and anti-caspase-9 antibody, followed by a peroxidase-labeled secondary antibody. The membrane was revealed with ECL system. The sequences corresponding to the identified spots are shown.

Figures 2A to 2B show effect of peptides on apoptosis A) BC173 cell line was cultured in the presence of the identified peptides at a concentration of 100 μM for 24h. Apoptosis was detected by Annexin V-FITC staining and analyzed by flow cytometry. B) MDA-MB231 cell line was cultured as above, at a concentration of 100 μM for 24h. Apoptosis was estimated by annexin V- FITC staining.

Figure 3 shows effect of peptide Mut3—SET2h-S4 on apoptosis. Daudi, Raji and Jok 1 cell lines were cultured in the presence or in the absence (control) of the Mut3—SET2h-S4 peptide at a concentration of 100 μM for 24h. Apoptosis was detected by Annexin V-FITC staining and analyzed by flow cytometry.

Figure 4 shows in vitro competition of Caspase 9/PP2A interaction by Mut3—SET2h-S1 peptide. Lysates from MDA-MB321 cell line were immunoprecipitated with anti-caspase 9 antibody and protein A-Sepharose was added. The Caspase 9/PP2A interaction was competed in the presence or in the absence (control) of the Mut3—SET2h-S1 peptide at a concentration of 1.5 mM for 30 min. After washing steps, immunoprecipitates were transferred to nitrocellulose and blotted with anti-PP2A antibody. As internal control, the blot was also hybridized with anti-caspase 9 antibody. Proteins were detected using the ECL system. The blots were scanned and analyzed by densitometry. The densitometric ratio C9/PP2A was determined. Similar ratios of PP2A/Caspase-9 were observed in control and peptide-treated anti-caspase 9 immunoprecipitates.

Detailed description of the invention:

Definitions:

The term "patient" refers to a human or non human animal, preferably a mammal, including male, female, adult and children in need of a treatment wherein a pro-apoptotic effect is desired.
As used herein, the term “treatment” or “therapy” includes curative and/or prophylactic treatment. More particularly, curative treatment refers to any of the alleviation, amelioration and/or elimination, reduction and/or stabilization (e.g., failure to progress to more advanced stages) of a symptom, as well as delay in progression of a symptom of a particular disorder.

Prophylactic treatment refers to any of: halting the onset, reducing the risk of development, reducing the incidence, delaying the onset, reducing the development, as well as increasing the time to onset of symptoms of a particular disorder.

The term “penetrating peptide” or “cell-penetrating peptide” (or “CPP”) or “shuttle peptide”, as used interchangeably, means that the peptide is able to translocate into cells without causing substantial membrane damage, and can be used as a vector of other molecules when linked to them. The terms refer to cationic cell penetrating peptides, also called transport peptides, carrier peptides, or peptide transduction domains. The CPP, as shown herein, have the capability of inducing cell penetration of a peptide fused to the CPP within 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of cells of a given cell culture population, including all integers in between, and allow macromolecular translocation within multiple tissues in vivo upon systemic administration. A cell-penetrating peptide may also refer to a peptide which, when brought into contact with a cell under appropriate conditions, passes from the external environment in the intracellular environment, including the cytoplasm, organelles such as mitochondria, or the nucleus of the cell, in conditions significantly greater than passive diffusion. This property may be assessed by various methods known by the skilled person.

Two amino acid sequences are “homologous”, “substantially homologous” or "substantially similar" when one or more amino acid residue are replaced by a biologically similar residue or when greater than 80 % of the amino acids are identical, or greater than about 90 %, preferably greater than about 95%, are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program, or any of the programs known in the art (BLAST, FASTA, etc.). Preferably, these homologous peptides do not include two cysteine residues, so that cyclization is prevented.

The term “conservative substitution” as used herein denotes the replacement of an amino acid residue by another, without altering the overall conformation and function of the peptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, shape, hydrophobic, aromatic, and the like). Amino acids with similar properties are well known in the art. For example, arginine, histidine and lysine are hydrophilic-basic amino acids and may be interchangeable. Similarly, isoleucine, a hydrophobic amino acid, may be replaced
with leucine, methionine or valine. Neutral hydrophilic amino acids, which can be substituted for one another, include asparagine, glutamine, serine and threonine.

By "substituted" or "modified" the present invention includes those amino acids that have been altered or modified from naturally occurring amino acids.

As such, it should be understood that in the context of the present invention, a conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Examples of conservative substitutions are set out in the Table 1 below:

<table>
<thead>
<tr>
<th>SIDE CHAIN CHARACTERISTIC</th>
<th>AMINO ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-polar</td>
<td>G A P I L V</td>
</tr>
<tr>
<td>Polar-uncharged</td>
<td>C S T M N Q</td>
</tr>
<tr>
<td>Polar-charged</td>
<td>D E K R</td>
</tr>
<tr>
<td>Aromatic</td>
<td>H F W Y</td>
</tr>
<tr>
<td>Other</td>
<td>N Q D E</td>
</tr>
</tbody>
</table>

Alternatively, conservative amino acids can be grouped as described in Lehninger, 1975, as set out in Table 2, immediately below.

**Table 2. Conservative Substitutions II**

<table>
<thead>
<tr>
<th>SIDE CHAIN CHARACTERISTIC</th>
<th>AMINO ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-polar (hydrophobic)</td>
<td></td>
</tr>
<tr>
<td>A. Aliphatic:</td>
<td>A L I V P</td>
</tr>
<tr>
<td>B. Aromatic:</td>
<td>F W</td>
</tr>
<tr>
<td>C. Sulfur-containing:</td>
<td>M</td>
</tr>
<tr>
<td>D. Borderline:</td>
<td>G</td>
</tr>
</tbody>
</table>

| Uncharged-polar           |            |
| A. Hydroxyl:              | S T Y       |
| B. Amides:                | N Q         |
| C. Sulphydryl:            | C           |
| D. Borderline:            | G           |

Positively Charged (Basic): K R H

Negatively Charged (Acidic): D E

As still another alternative, exemplary conservative substitutions are set out in Table 3, immediately below.
Table 3. Conservative Substitutions III

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>Val (V), Leu (L), Ile (I)</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>Lys (K), Gln (Q), Asn (N)</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>Gln (Q), His (H), Lys (K), Arg (R)</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>Glu (E)</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>Ser (S)</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>Asn (N)</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>Asp (D)</td>
</tr>
<tr>
<td>His (H)</td>
<td>Asn (N), Gln (Q), Lys (K), Arg (R)</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>Leu (L), Val (V), Met (M), Ala (A), Phe (F)</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>Ile (I), Val (V), Met (M), Ala (A), Phe (F)</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>Arg (R), Glu (Q), Asn (N)</td>
</tr>
<tr>
<td>Met (M)</td>
<td>Leu (L), Phe (F), Ile (I)</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>Leu (L), Val (V), Ile (I), Ala (A)</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>Gly (G)</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>Thr (T)</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>Ser (S)</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>Tyr (T)</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>Trp (W), Phe (F), Thr (T), Ser (S)</td>
</tr>
<tr>
<td>Val (V)</td>
<td>Ile (I), Leu (L), Met (M), Phe (F), Ala (A)</td>
</tr>
</tbody>
</table>

Peptide that inhibits binding between SET protein and Caspase-9 protein:

The invention provides peptides which consist of or derived of a portion of Caspase-9 that binds to SET or a portion of SET that binds to Caspase-9.

Any SET protein isoform may be used, especially isoform 2. Human isoform 2 SET protein is disclosed as NP_003002.2 (NCBI Reference Sequence). In the context of the present invention, SET and SET2 may be used interchangeably.

Preferably the peptide that inhibits binding between SET protein and Caspase-9 protein is a fragment of human SET (especially isoform 2) that binds Caspase-9 or a fragment of human Caspase-9 protein that binds SET, or derives therefrom. Alternatively, since SET and Caspase-9 proteins are well conserved, portions originating from other animal species can be used, e.g. mouse or rat SET or Caspase-9 proteins.

A sequence that derives from" or "is derived from" a reference sequence is a peptide sequence that is longer that the reference sequence, or is a homologous sequence, as defined herein.
In certain embodiments, the peptide that inhibits binding between SET and Caspase-9 proteins of the invention induces cell toxicity, preferably induces apoptosis, and is useful for inhibiting cell proliferation in vitro and in vivo, in particular for treating a hyperproliferative disorder, such as cancer. One subject of the invention is therefore to provide pro-apoptotic peptides which consist of or derive from a portion of Caspase-9 that binds to SET or a portion of SET that binds to Caspase-9.

The peptide that inhibits binding between SET and Caspase-9 proteins is also useful as in vitro diagnostic reagent, drug screening reagent and research tool to isolate Caspase-9 or SET-associated partners.

The peptide that inhibits binding between SET protein and Caspase-9 protein may preferably have a length comprised between 18 to 80 amino acids, preferably between 18 to 70 amino acids, still preferably between 18 to 40 amino acids, still preferably between 18 and 30 amino acids. Preferably, the invention provides a peptide of 18 to 80 amino acid residues, preferably 18 to 70 amino acid residues, more preferably 18 to 40 amino acid residues wherein said peptide i) inhibits binding between SET protein and Caspase-9 protein, and is preferably pro-apoptotic and ii) comprises or consists of SEQ ID NO: 1, 2, 3 or 4, and a proteolysis-resistant peptide deriving from said peptide by one or more chemical modifications, or a substantially homologous peptide, preferably deriving from SEQ ID NO: 1, 2, 3 or 4 by one or more conservative substitutions.

According to one embodiment, the peptide that inhibits binding between SET protein and Caspase-9 protein consists of or is derived from a portion of Caspase-9 that binds SET protein.

In a preferred embodiment, the peptide that inhibits binding between SET protein and Caspase-9 then comprises or consists of:

QX1PGCFNFLRKKX3FFKTX (SEQ ID NO: 1), wherein:
X1 is methionine or isoleucine;
X2 is leucine or phenylalanine;
X3 is serine or vacant;
or

a proteolysis-resistant peptide deriving from said peptide by one or more chemical modifications, or a substantially homologous peptide, preferably deriving from SEQ ID NO: 1 by one or more conservative substitutions.

More particularly, the peptide that inhibits binding between SET protein and Caspase-9 protein may be QMPGCFNFLRKLFFKTS (SEQ ID NO: 2); or a proteolysis-resistant peptide deriving from said pro-apoptotic peptide by one or more chemical modifications, or a substantially homologous peptide, preferably deriving from SEQ ID NO: 2 by one or more conservative substitutions.
The peptide of SEQ ID NO: 2 corresponds to positions 249 to 266 of human Caspase-9 amino acid sequence BAA87905.1 (GenBank reference).

In another embodiment, the peptide that inhibits binding between SET protein and Caspase-9 protein consists of or is derived from a portion of SET that binds Caspase-9 protein.

In a preferred embodiment, the peptide that inhibits binding between SET protein and Caspase-9 peptide then comprises or consists of a) ILKVEQKYNKLQPFFQKRS (SEQ ID NO: 3); b) RSSQTQNKAQKRQHEEP (SEQ ID NO: 4) or c) a proteolysis-resistant peptide deriving from said pro-apoptotic peptide by one or more chemical modifications, or d) a substantially homologous peptide, preferably deriving from SEQ ID NO: 3 or SEQ ID NO: 4 by one or more conservative substitutions.

The peptide of SEQ ID NO: 3 corresponds to positions 53 to 74 of human SET isoform 2 amino acid sequence NP_003002.2 (NCBI Reference Sequence).

The peptide of SEQ ID NO: 4 corresponds to positions 169 to 186 of human SET isoform 2 amino acid sequence NP_003002.2 (NCBI Reference Sequence).

In an embodiment, the peptide that inhibits binding between SET protein and Caspase-9 protein comprises or consists of an amino acid sequence deriving from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4 by a N- and/or C-terminal deletion of 1 to 4 amino acids.

Preferably, the peptides of the invention, including the proteolysis-resistant or homologous peptides, induce cell apoptosis, in vitro and/or in vivo. Assays for determining if a molecule, for instance a peptide, induces cell apoptosis are well-known in the art and include, for instance, incubating cells with the candidate peptide and determining if apoptosis is induced by said candidate peptide, e.g. by Annexin V and PI labelling of cells and identifying as apoptotic cells, those being Annexin V+ and PI+.

*Chimeric constructs:*

The chimeric peptide comprises an amino acid sequence fused to the N-terminal and/or C-terminal end(s) of the peptide that inhibits binding between SET and Caspase-9. The length of the chimeric peptide is not critical to the invention as long as the peptide is functional.

The peptide that inhibits binding between SET and Caspase-9 is linked to one or more other protein/peptide moieties including those which allow the purification, detection, immobilization, and/or cellular targeting of the protein of the invention, and/or which increase the affinity for Caspase-9 or SET, the bioavailability, the production in expression systems and/or stability of said protein.
These moieties may be selected from: (i) a cell-penetrating moiety, (ii) a labeling moiety such as a fluorescent protein (GFP and its derivatives, BFP and YFP), (iii) a reporter moiety such as an enzyme tag (luciferase, alkaline phosphatase, glutathione-S-transferase (GST), β-galactosidase), (iv) a binding moiety such as an epitope tag (polyHis6, FLAG, HA, myc.), a DNA-binding domain, a hormone-binding domain, a poly-lysine tag for immobilization onto a support, (v) a stabilization moiety, and (vi) a targeting moiety for addressing the chimeric protein to a specific cell type or cell compartment.

In addition, the peptide that inhibits binding between SET and Caspase-9 may be separated from the peptide/protein moiety by a linker which is long enough to avoid inhibiting interactions between the peptide that inhibits binding between SET and Caspase-9 and the protein/peptide moiety. The linker may also comprise a recognition site for a protease, for example, for removing affinity tags and stabilization moieties from the purified chimeric protein according to the present invention.

In one embodiment, the chimeric peptide comprises the peptide that inhibits binding between SET and Caspase-9 linked to at least one cell-penetrating peptide. In particular embodiments, the peptide that inhibits binding between SET and Caspase-9 is linked to two, three or more cell-penetrating peptides. The peptide that inhibits binding between SET and Caspase-9 is advantageously fused to the C-terminus of the cell-penetrating peptide.

In certain embodiments, the chimeric peptide comprises an organelle-specific localization signal, i.e., a sequence that preferentially causes localization of the fusion protein to a specific organelle. In certain embodiments, the localization signal localizes the fusion protein to the nucleus, mitochondria, or endoplasmic reticulum. The localization signal may be within or separate from the peptide that inhibits binding between SET and Caspase-9.

The chimeric peptide construct may preferably have a length comprised between 17 to 80 amino acids, preferably between 20 to 70 amino acids, still preferably between 23 to 40 amino acids.

In a preferred embodiment, the chimeric peptide construct is selected from the group consisting of:

- VKKKKIKAEIKI-QMPGCFNFLRKKLFFKTS (SEQ ID NO: 5), this peptide being designated Mut3-C9h-S4;
- VKKKKIKAEIKI-ILKVEQKYNKLQRPFFQKRSEL (SEQ ID NO: 6), this peptide being designated Mut3-SET2h-S1;
- VKKKKIKAEIKI-RSSQTQNKASRKQHEEP (SEQ ID NO: 7), this peptide being designated Mut3-SET2h-S2,

or homologous or proteolysis-resistant peptides deriving thereof.
Preferably, a chimeric peptide construct according to the invention induces cell apoptosis, in vitro and/or in vivo.

**Cell Penetrating Peptides:**

Preferably, the cell penetrating peptide of the chimeric peptide construct comprises or consists of:

a) X₁-KKKIK-Ψ-EI-X₂-X₃ (SEQ ID NO: 8)

Wherein X₁ is vacant, is a lysine residue, or valine-lysine;

X₂ is vacant, is a lysine residue, or lysine-isoleucine;

X₃ is vacant or is an amino acid sequence of one to 4 amino acids;

and Ψ is any amino-acid;

or a proteolysis-resistant peptide deriving from SEQ ID NO:8 by one or more chemical modifications, or a substantially homologous peptide, especially peptides deriving from SEQ ID NO:8 by one or more conservative substitutions.

b) (RQKRLI)₃ (SEQ ID NO: 9), (RHSRIG)₃ (SEQ ID NO: 10), RHSRIGIIQQRRTRNG (SEQ ID NO: 11), RHSRIGVTRQRRARNG (SEQ ID NO: 12), RRRRRRRRSGRRRRTY (SEQ ID NO: 13), or homologous peptides;

c) Tat peptide, polyarginines peptide, HA2-R₉ peptide, Penetratin peptide (Antennapedia), Transportan peptide, Vectocell® peptide, maurocalcine peptide, decalysine peptide, HIV-Tat derived PTD4 peptide, Hepatitis B virus Translocation Motif (PTM) peptide, mPrP₁-28 peptide, POD, pVEC, EB₁, Rath, CADY, Histatin 5, Antp peptide, Cyt₉₁-₁₀₁ peptide.

In an embodiment, in the cell penetrating peptide of a), X₃ is vacant, i.e. the cell penetrating peptide is X₁-KKKIK-Ψ-EI-X₂ (SEQ ID NO: 14).

In another embodiment, in the cell penetrating peptide of a), X₁ is VK, X₂ is KI and X₃ is vacant, i.e. the cell penetrating peptide is VKKKIK-Ψ-EIKI (SEQ ID NO: 15).

Preferably Ψ is arginine, lysine, asparagine, or alanine.

The cell-penetrating peptide can thus be VKKKIKREIKI (SEQ ID NO:16), VKKKIKAEIKI (SEQ ID NO:17), VKKKIKKEIKI (SEQ ID NO:18) or VKKKKKEIKI (SEQ ID NO:19).

By “Tat peptide”, it is meant a peptide having the sequence RKKRRQRRR (SEQ ID NO: 20, Tat peptide 2) or YGRKKRRQRRR, (SEQ ID NO: 21).

By “polyarginines peptide”, it is meant a peptide consisting of at least 9 arginines. Preferably, a polyarginine peptide is a peptide having the sequence R₉ (SEQ ID NO: 22) or R₁₁ (SEQ ID NO: 23).

By “HA2-R₉ peptide”, it is meant a peptide having the sequence GLFEAIEGFIENGWEGMIDGWYG-R₉ (SEQ ID NO: 24).
By “Penetratin peptide”, it is meant a peptide having the sequence RQIKIWFQNRRMKWKK (SEQ ID NO: 25).

By “Transportan peptide” (also called “Antp peptide”), it is meant a peptide having the sequence GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO: 26).

By “Vectocell® peptide”, it is meant a peptide originating from human heparin binding proteins and/or anti-DNA antibodies.

By “Maurocalcine peptide”, it is meant a peptide having the sequence GDCLPHLKLCKENKDCCKKKCKRGTIEKRCR (SEQ ID NO: 27).

By “decalysine peptide”, it is meant a peptide having the sequence KKKKKKKKKK (K_{10}) (SEQ ID NO: 28).

By “HIV-Tat derived PTD4 peptide”, it is meant a peptide having the sequence YARAAARQARA (SEQ ID NO: 29).

By “Hepatitis B virus Translocation Motif (PTM) peptide”, it is meant a peptide having the sequence PLSSIFSRI (SEQ ID NO: 30).

By “mPrP_{1-28} peptide”, it is meant a peptide having the sequence MANLYWLLALFVTMDTWGKLCKRP (SEQ ID NO: 31).

By “POD peptide”, it is meant a peptide having the sequence GGG(ARKKAADA)_{4} (SEQ ID NO: 32).

By “pVEC peptide”, it is meant a peptide having the sequence LLIIRRRRIRKQAAHHSK (SEQ ID NO: 33).

By “EB1 peptide”, it is meant a peptide having the sequence LIKLWHLIIHFWQTNRLKWKK (SEQ ID NO: 34).

By “Rath peptide”, it is meant a peptide having the sequence TPWWRLWTKWKHRRDLPRKPE (SEQ ID NO: 35).

By “CADI peptide”, it is meant a peptide having the sequence GLWRALWRLRSLWRLLWRA (SEQ ID NO: 36).

By “Histatin 5 peptide”, it is meant a peptide having the sequence DSHAKRHHGYKRFKHEHHKSHRGY (SEQ ID NO: 37).

By “Cyt^{56-101} peptide”, it is meant a peptide having the sequence KKKEERADLIYLKKA (SEQ ID NO: 38).

*Peptide preparation:*

Peptides described herein can be synthesized using standard synthetic methods known to those skilled in the art., for example chemical synthesis or genetic recombination. In a preferred embodiment, peptides are obtained by stepwise condensation of amino acid residues, either by condensation of a preformed fragment already containing an amino acid sequence in appropriate order, or by condensation of several fragments previously prepared,
while protecting the amino acid functional groups except those involved in peptide bond during condensation. In particular, the peptides can be synthesized according to the method originally described by Merrifield.

Examples of chemical synthesis technologies are solid phase synthesis and liquid phase synthesis. As a solid phase synthesis, for example, the amino acid corresponding to the C-terminus of the peptide to be synthesized is bound to a support which is insoluble in organic solvents, and by alternate repetition of reactions, one wherein amino acids with their amino groups and side chain functional groups protected with appropriate protective groups are condensed one by one in order from the C-terminus to the N-terminus, and one where the amino acids bound to the resin or the protective group of the amino groups of the peptides are released, the peptide chain is thus extended in this manner. Solid phase synthesis methods are largely classified by the tBoc method and the Fmoc method, depending on the type of protective group used. Typically used protective groups include tBoc (t-butoxycarbonyl), Cl-Z (2-chlorobenzylexycarbonyl), Br-Z (2-bromobenzylexycarbonyl), Bzl (benzyl), Fmoc (9-fluorenylmethoxycarbonyl), Mbh (4, 4’-dimethoxydibenzhydryl), Mtr (4-methoxy-2, 3, 6-trimethylbenzenesulphonyl), Trt (trityl), Tos (tosyl), Z (benzylxycarbonyl) and Clz-Bzl (2, 6-dichlorobenzyl) for the amino groups; NO2 (nitro) and Pmc (2,2, 5,7, 8-pentamethylchromane-6-sulphonyl) for the guanidino groups); and tBu (t-butyl) for the hydroxyl groups). After synthesis of the desired peptide, it is subjected to the de-protection reaction and cut out from the solid support. Such peptide cutting reaction may be carried with hydrogen fluoride or tri-fluoromethane sulfonic acid for the Boc method, and with TFA for the Fmoc method.

Alternatively, the peptide may be synthesized using recombinant techniques. In this case, a nucleic acid and/or a genetic construct comprising or consisting of a nucleotidic sequence encoding a peptide according to the invention, polynucleotides with nucleotidic sequences complementary to one of the above sequences and sequences hybridizing to said polynucleotides under stringent conditions.

The invention further relates to a genetic construct consisting of or comprising a polynucleotide as defined herein, and regulatory sequences (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) allowing the expression (e.g. transcription and translation) of a peptide according to the invention in a host cell.

Thus, in another aspect, the invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) a peptide of the invention; and/or that contains a polynucleotide of the invention or genetic construct of the invention.

The method of producing the peptide may optionally comprise the steps of purifying said peptide, chemically modifying said peptide, and/or formulating said peptide into a pharmaceutical composition.
Further protection against proteolysis:

The N- and C-termini of the peptides described herein may be optionally protected against proteolysis. For instance, the N-terminus may be in the form of an acetyl group, and/or the C-terminus may be in the form of an amide group. Internal modifications of the peptides to be resistant to proteolysis are also envisioned, e.g. wherein at least a -CONH-peptide bond is modified and replaced by a (CH2NH) reduced bond, a (NHCO) retro-inverso bond, a (CH2-O) methylene-oxo bond, a (CH2-S) thiomethylene bond, a (CH2CH2) carba bond, a (CO-CH2) cetomethylene bond, a (CHOH-CH2) hydroxyethylene bond, a (N-N) bound, a E-alcene bond or also a -CH=CH-bond.

For instance the peptide may be modified by acetylation, acylation, amidation, cross-linking, cyclization, disulfide bond formation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, phosphorylation, and the like.

The peptides of the invention may be composed of amino acid(s) in D configuration, which render the peptides resistant to proteolysis. They may also be stabilized by intramolecular crosslinking, e.g. by modifying at least two amino acid residues with olefinic side chains, preferably C3-C8 alkenyl chains, preferably penten-2-yl chains) followed by chemical crosslinking of the chains, according to the so-called “staple” technology described in Walensky et al, 2004. For instance, amino acids at position i and i+4 to i+7 can be substituted by non-natural amino acids that show reactive olefinic residues. All these proteolysis-resistant chemically-modified peptides are encompassed in the present invention.

In another aspect of the invention, peptides are covalently bound to a polyethylene glycol (PEG) molecule by their C-terminal terminus or a lysine residue, notably a PEG of 1500 or 4000 MW, for a decrease in urinary clearance and in therapeutic doses used and for an increase of the half-life in blood plasma. In yet another embodiment, peptide half-life is increased by including the peptide in a biodegradable and biocompatible polymer material for drug delivery system forming microspheres. Polymers and copolymers are, for instance, poly(D,L-lactide-co-glycolide) (PLGA) (as illustrated in US2007/0184015, SoonKap Hahn et al).

Nucleic acids

The invention also relates to a polynucleotide comprising or consisting of a nucleotide sequence encoding a peptide according to the invention.

The invention further relates to a genetic construct consisting of or comprising a polynucleotide as defined herein, and regulatory sequences (such as a suitable promoter(s),
enhancer(s), terminator(s), etc.) allowing the expression (e.g. transcription and translation) of a peptide according to the invention in a host cell.

The genetic constructs of the invention may be DNA or RNA, preferably cDNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, i.e. a vector that can provide for expression in vitro and/or in vivo (e.g. in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting aspect, a genetic construct of the invention comprises i) at least one nucleic acid of the invention; operably connected to ii) one or more regulatory elements, such as a promoter and optionally a suitable terminator; and optionally also iii) one or more further elements of genetic constructs such as 3'- or 5'-UTR sequences, leader sequences, selection markers, expression markers/reporter genes, and/or elements that may facilitate or increase (the efficiency of) transformation or integration.

In a particular embodiment, the nucleic acid encoding the cell-penetrating peptide of the invention is coupled or fused to a nucleic acid that encodes a peptide or protein of interest. The peptide of interest may be a pro-apoptotic peptide as described herein. More generally it may the peptide or protein of interest may be any peptide or protein to express, such as therapeutic peptide or polypeptide, as well as any antigenic or immunogenic peptide if desired.

The nucleic acid may especially be carried by a viral vector, such as an adenovirus or a lentivirus, for ex vivo or in vivo infection and expression of the chimeric peptide construct or proapoptotic peptide.

Anti-tumor therapy:

Another aspect of the present invention relates to a peptide, polynucleotide, and/or vector as described herein, for use in treating a hyperproliferative disorder, preferably a tumor or cancer, preferably in a human patient.

The peptides, polynucleotides, and/or vectors as described herein are useful for treating a tumor, in particular a malignant tumor and preventing or treating metastasis.

The peptides as defined herein, or nucleic acids that encode said peptides, are useful in anti-tumor therapy, preferably as adjuvants in combination with an anti-tumor agent, preferably a chemotherapeutic agent.
The anti-tumor therapy of the invention is helpful in eradicating any persistent microscopic malignancy, and/or preventing or delaying relapses.

Furthermore, the peptides (or nucleic acids that encode said peptides) may be used for preventing or treating metastases.

It is thus described a method of treatment of a hyperproliferative disorder, preferably a tumor or cancer, in a patient in need thereof, which method comprises administering said patient with a peptide that inhibits binding between SET protein and Caspase-9 protein of the invention, preferably a pro-apoptotic peptide of the invention, or a chimeric peptide construct of the invention, or a nucleic acid encoding said construct, preferably in combination with an anti-tumor agent. Anti-tumor agents include chemotherapeutic agents, including inhibitors of DNA replication such as DNA binding agents in particular alkylating or intercalating drugs, antimetabolite agents such as DNA polymerase inhibitors, or topoisomerase I or II inhibitors, or with anti-mitogenic agents such as alkaloids.

The peptides (or nucleic acids that encode said peptides) described herein are useful for the treatment of a tumor, in particular a cancer tumor, preferably in a human patient.

The tumor may be cancer, such as a haematologic cancer, in particular acute myelogenous leukaemia (AML), chronic lymphocytic leukaemia (CLL), multiple myeloma, Hodgkin’s disease, non-Hodgkin’s lymphoma, B cell, cutaneous T cell lymphoma, or a non-haematologic cancer, for instance brain, epidermoid (in particular lung, breast, ovarian), head and neck (squamous cell), bladder, gastric, pancreatic, head, neck, renal, prostate, colorectal, oesophageal or thyroid cancer, and melanoma. In a preferred embodiment the peptides described herein (or nucleic acids that encode said peptides) are useful for treatment of chronic lymphocytic leukaemia (CLL).

Different types of cancers may include, but are not limited to fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelio-sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, lymphoma, leukemia, B-cell chronic lymphocytic leukemia (CLL), B-cell non-Hodgkin lymphoma (NHL), squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendrogloma, meningioma, melanoma, neuroblastoma, and retinoblastoma, uveal melanoma and breast cancer.
Pharmaceutical compositions:

The peptides of the invention (or nucleic acid that encodes said peptide) may be administered by any convenient route including intravenous, oral, transdermal, subcutaneous, mucosal, intramuscular, intrapulmonary, intranasal, parenteral, rectal, vaginal and topical. Intranasal route is of particular interest.

Advantageously, intra-tumoral administration is also contemplated.

The peptides (or nucleic acid that encodes said peptide) are formulated in association with a pharmaceutically acceptable carrier.

The pharmaceutical composition may also include any other active principle, such as in particular an anti-tumor agent, such as those described above.

In a preferred embodiment, the peptides (or nucleic acid that encodes said peptide) may be administered by electroporation. Electroporation, also known as electroporation or electroinjection, is the permeabilization of cell membranes as a consequence of the application of certain short and intense electric fields across the cell membrane, the cells or the tissues. Typically, electroporation consists of injecting compounds, preferably via intramuscular or intradermal route, followed by applying a series of electric pulses by means of electrodes connected to a generator. The conditions for applying an electric field in the injection zone are now well known to those persons skilled in the art, and are in particular described in the US patent 5468223. Those persons skilled in the art will be able to adapt these conditions according to each case. The electric field may be 50-200 microseconds pulses of high-strength electric fields in the range of 1-5000 V/cm and with a frequency between 0.1 and 1,000 hertz. Typically, a sequence of eight 100 microseconds pulses of 1000-1500 V/cm with a frequency of 1 hertz is applied.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified. In particular, the pharmaceutical compositions may be formulated in solid dosage form, for example capsules, tablets, pills, powders, dragees or granules.

The choice of vehicle and the content of active substance in the vehicle are generally determined in accordance with the solubility and chemical properties of the active compound, the particular mode of administration and the provisions to be observed in pharmaceutical practice. For example, excipients such as lactose, sodium citrate, calcium carbonate, dicalcium phosphate and disintegrating agents such as starch, alginic acids and certain
complex silicates combined with lubricants such as magnesium stearate, sodium lauryl sulphate and talc may be used for preparing tablets. To prepare a capsule, it is advantageous to use lactose and high molecular weight polyethylene glycols. When aqueous suspensions are used they can contain emulsifying agents or agents which facilitate suspension. Diluents such as sucrose, ethanol, polyethylene glycol, propylene glycol, glycerol and chloroform or mixtures thereof may also be used.

Preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes that may provide controlled or sustained release of the product.

The dosing is selected by the skilled person so that a pro-apoptotic effect is achieved, and depends on the route of administration and the dosage form that is used. Total daily dose of peptides (or nucleic acid that encodes said peptide) administered to a subject in single or divided doses may be in amounts, for example, of from about 0.001 to about 100 mg/kg body weight daily and preferably 0.01 to 10 mg/kg/day. Preferably, a total daily dose is from about 5 to 25 mg/kg/day. A daily dosage of about 5mg/kg is still preferred. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

Preferably the peptide construct (or nucleic acid that encodes said peptide) is administered once a day during a period of at least one week, preferably at least two weeks.

**Combinations:**

In a particular embodiment, the patient is to be administered with one or several chimeric peptide constructs or peptides that inhibit binding between SET and Caspase-9 by binding SET, combined with one and several chimeric peptide constructs or peptides that inhibit binding between SET and Caspase-9 by binding Caspase-9. Simultaneous administration (i.e., at the same time, as a single composition or separate compositions), or sequential administration is encompassed.

The invention further provides a pharmaceutical composition comprising a chimeric peptide construct or peptide that inhibits binding between SET and Caspase-9 which binds SET, in combination with a chimeric peptide construct or peptide that inhibits binding between SET and Caspase-9 which binds Caspase-9.

It is also provided a kit comprising a container containing a chimeric peptide construct or peptide that inhibits binding between SET and Caspase-9 which binds SET, and a container
containing a chimeric peptide construct or peptide that inhibits binding between SET and Caspase-9 which binds Capase-9.

Further aspects and advantages of the present invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of the present application.

**Examples:**

**Example 1: Identification of binding site of Caspase-9 to SET and vice versa**

1.1 Material and methods
Peptide synthesis and sequence
Peptides were synthesized in an automated multiple peptide synthesizer with solid phase procedure and standard Fmoc chemistry. The purity and composition of the peptides were confirmed by reverse phase HPLC and by amino acid analysis.

SET2/Caspase-9 binding assay on cellulose-bound peptides containing caspase-9 or SET2 sequences
Overlapping dodecapeptides scanning the whole caspase-9 or SET2 sequence were prepared by automated spot synthesis (Abimed, Langerfeld, Germany) onto an amino-derived cellulose membrane as previously described (Frank and Overwin, 1996; Gaustepohl, et al, 1992). The membrane were blocked using 3% non-fat dry milk/3% BSA, incubated with purified SET2 or Caspase-9 protein and after several washing steps, incubated with anti-SET2 or anti-Caspase-9 antibody, followed by PO-conjugated secondary antibody. Positive spots were visualized using the ECL system.

1.2 Results
Identification of binding site of Caspase-9 to SET and vice versa
To identify peptides containing caspase-9 sequences able to *in vitro* bind to SET, a series of overlapping dodecapeptides from the caspase-9 protein were bound onto a cellulose membrane and incubated with purified SET protein.

The inventors identified four peptides with overlapping sequences that bind to a purified SET protein were identified (Figure 1A). Together these results identified a caspase-9 binding site to SET protein (C9h-S4). The sequence is C9h-S4 QMPGCFNLRKLFKFSQTS (SEQ ID NO:2).

Similarly, to identify peptides containing SET sequences able to *in vitro* bind to Caspase-9, a series of overlapping dodecapeptides from the SET protein were bound onto a cellulose membrane and incubated with purified Caspase-9 protein. A first series of six peptides with
overlapping sequences that bind to a purified Caspase-9 protein were identified (Figure 1B). A second series of four peptides with overlapping sequences that also bind to purified Caspase-9 were identified (Fig 2A). Together these results identified two SET binding site to caspase-9 protein (SET2h-S1 and S2). The two sequences are: SET2h-S1 ILKVEQKYNKLQPFFQKRSSEL (SEQ ID NO:3) and SET2h-S2 RSSQTQNKASRKRQHEEP (SEQ ID NO:4)

Example 2: Design and Effect of Mut3DPT- C9h-S4, Mut3-DPT- SET2h-S1 and Mut3DPT- SET2h-S2

The inventors chemically synthesized the three chimeric peptides Mut3-C9h-S4, Mut3-SET2h-S1, Mut3-SET2h-S2 composed of a cell penetrating peptide, Mut3DPT (VKKKKIKAEIKI) associated to the binding site of Caspase-9 to SET and vice versa (Figures 1A and 1B).

Example 3: Effect of Mut3DPT- C9h-S4, Mut3-DPT- SET2h-S1 and Mut3DPT- SET2h-S2 peptides on apoptosis

3.1 Material and methods

Cells

Human cell-line MDA-MB231 and BC173 breast cancer cell lines were cultured in DMEM medium supplemented with 10% of FCS.

Detection of apoptosis by annexin-V-FITC staining

Apoptotic cells were detected using Annexin-V (-FITC from BD biosciences) as described by the manufacturer. Briefly, the cells were washed in 1x binding buffer, centrifugated and then resuspended in 200 µl of 1x binding buffer containing Annexin V-FITC (0.1 µg/ml) and PI (0.5 µg/ml). After incubation at room temperature in the dark for 10 min, cells were analyzed by flow cytometry. Data acquired by FACSCalibur (BD biosciences) were analyzed with Cellquest Pro software.

3.2. Results

The inventors analyzed the capacity of these peptides to induce apoptosis in apoptosis in the breast cancer cell lines MDA-MB231 and BC173. Using flow cytometry detection of annexin V and PI, we analyzed the capacity of Mut3DPT-C9h-S4 to induce apoptosis. As illustrated on figure 2, Mut3DPT-C9h-S4 strongly induces apoptosis compared to control non treated cells (Figure 2). The new designed peptides Mut3DPT-C9h-S4 and Mut3DPT-SET2h-S1 show a pro-apoptotic effect.
In addition they can also be used as molecular tools to isolate Caspase-9 or SET-associated partners.

**Example 4: Effect of Mut3-C9h-S4**

The inventors assayed cytotoxicity of Mut3-C9h-S4 (VKKKKIAEIKIQMPGCFNFLRRKLFFKTS; SEQ ID NO: 5) on three lymphoma cell lines (Raji, Daudi, Jok-1).

**4.1 Cells**

Human B lymphoblast cell line Daudi, human lymphoblast-like cell line Raji and a human hairy cell leukemia-derived cell line Jok-1 were cultured in DMEM medium supplemented with 10% of FCS.

**4.2 Detection of apoptosis by Annexin-V-FITC staining**

The effect of Mut3-C9h-S4 peptide treatment was compared to untreated cells. The peptides were used at 100 μM for 24h. Apoptotic cells were detected using Annexin-V (-FITC from BD biosciences) as described above.

**4.3 Results**

The inventors analyzed the capacity of Mut3-C9h-S4 peptide to induce apoptosis in human Daudi, Raji and Jok 1 cell lines. Figure 3 shows that Mut3-C9h-S4 peptide has apoptotic effect in Raji, Daudi, Jok-1 cell lines compared to control non treated cells when the peptide is used at 100μM for 24h. Similar results were obtained in three independent experiments.

**Example 5: In vitro competition of Caspase 9/PP2A interaction**

The inventors performed competitive protein-protein interactions to confirm that the interaction between caspase-9 and PP2A is not modified by the peptides of the invention.

The Caspase 9/PP2A interaction was competed using Mut3-SET2h-S1 (VKKKKIAEIKILKVEQKYNKLRQPFPFQKRSEL; SEQ ID NO: 6). Lysates from MDA-MB321 cell line were immunoprecipitated with anti-caspase 9 antibody and protein A-Sepharose was added. The Caspase 9/PP2A interaction was competed with 1.5 mM of peptide Mut3-SET2h-S1 (30 min, room temperature). After washing steps, immunoprecipitates were transferred to nitrocellulose and blotted with anti-PP2A antibody. As internal control, the blot was also hybridized with anti-caspase 9 antibody. Proteins were detected using the ECL system. The blots were scanned and analyzed by densitometry.
As illustrated by Figure 4, similar ratios of PP2A/Caspase-9 were observed in control and peptide-treated anti-caspase 9 immunoprecipitates. The caspase-9/PP2A interaction was not modified by Mut3-SET2h-S1 peptide.
References

CLAIMS

1. A chimeric peptide comprising a cell-penetrating peptide linked to a peptide that inhibits binding between SET protein and Caspase-9 protein, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein consists of, or is derived from, a portion of Caspase-9 that binds a SET protein, or a portion of SET that binds Caspase-9 protein.

2. The chimeric peptide of claim 1, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein is a pro-apoptotic peptide.

3. The chimeric peptide of claim 1 or 2, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein consists of or is derived from a portion of Caspase-9 that binds SET protein.

4. The chimeric peptide of claim 3, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein comprises or consists of:
   \[ QX_1\text{PGCFNLRKKX}_2\text{FFKTX}_3 \] (SEQ ID NO: 1), wherein:
   \[ X_1 \] is methionine or isoleucine;
   \[ X_2 \] is leucine or phenylalanine;
   \[ X_3 \] is serine or vacant;

or

a proteolysis-resistant peptide deriving from said peptide by one or more chemical modifications, or a substantially homologous peptide, preferably deriving from SEQ ID NO: 1 by one or more conservative substitutions.

5. The chimeric peptide of claim 4 wherein the peptide that inhibits binding between SET and Caspase-9 is QMPGCFNLRKKLFFKTS (SEQ ID NO: 2)

6. The chimeric peptide of claim 1 or 2, wherein the peptide that inhibits binding between SET protein and Caspase-9 protein consists of or is derived from a portion of SET that binds Caspase-9 protein.
7. The chimeric peptide of claim 6, wherein the peptide that inhibits binding between SET and Caspase-9 comprises or consists of a) ILKVEQKYNKLRQPFFKRSSEL (SEQ ID NO: 3); b) RSSQTQNASKRKRQHEEP (SEQ ID NO: 4) or c) a proteolysis-resistant peptide deriving from said pro-apoptotic peptide by one or more chemical modifications, or d) a substantially homologous peptide, preferably deriving from (SEQ ID NO: 3) or (SEQ ID NO: 4) by one or more conservative substitutions.

8. The chimeric peptide construct according to claim 1 to 7 wherein said cell-penetrating peptide is selected from:

   a) X1-KKKIK-Ψ-E1-X2-X3 (SEQ ID NO: 8), wherein X1 is vacant, is a lysine residue, or valine-lysine; X2 is vacant, is a lysine residue, or lysine-isoleucine; X3 is vacant or is an amino acid sequence of one to 4 amino acids; and Ψ is any amino-acid; or a proteolysis-resistant peptide deriving from SEQ ID NO: 8 by one or more chemical modifications, or a substantially homologous peptide deriving from SEQ ID NO: 8 by one or more conservative substitutions;

   b) (RQKRL)₃ (SEQ ID NO: 9), (RHSRIG)₃ (SEQ ID NO: 10), RHSRIGIIQQRRTRNG (SEQ ID NO: 11), RHSRIGVTRQRRARNG (SEQ ID NO: 12), RRRRRRRSRGRRRTY (SEQ ID NO: 13), or

   c) Tat peptide, polyarginines peptide, HA2-R9 peptide, Penetratin peptide, Transportan peptide, Vectocell peptide, maurocalcine peptide, decalysine peptide, HIV-Tat derived PTD4 peptide, Hepatitis B virus Translocation Motif (PTM) peptide, mPrP1-28 peptide, POD, pVEC, EB1, Rath, CADY, Histatin, Antp peptide, or Cyt86-101peptide.

9. The chimeric peptide construct of claim 8, wherein said cell-penetrating peptide is X1-KKKIK-Ψ-E1-X2-X3 (SEQ ID NO: 14), wherein Ψ is arginine, alanine, lysine, or asparagine, and X1 is valine-lysine; X2 is lysine-isoleucine; and X3 is vacant, wherein said cell-penetrating peptide preferably is VKKKIKKREIKI (SEQ ID NO: 16), VKKKIKIAEKII (SEQ ID NO: 17), VKKKIKKKEIKI (SEQ ID NO: 18) or VKKKKIKNEIKI (SEQ ID NO: 19), still preferably wherein the chimeric peptide is selected from the group consisting of:

   VKKKKIKAEIKI-QMPSGFNLRKLFKKTSL (SEQ ID NO: 5);
   VKKKKIKAEIKI-ILKVEQKYNLORQPFKRSSEL (SEQ ID NO: 6);
   VKKKKIKAEIKI-RSSQTQNASKRKRQHEEP (SEQ ID NO: 7).
10. A peptide of 18 to 80 amino acid residues, preferably 18 to 70 amino acid residues, more preferably 18 to 40 amino acid residues wherein said peptide i) inhibits binding between SET protein and Caspase-9 protein, and is preferably pro-apoptotic and ii) comprises or consists of SEQ ID NO: 1, 2, 3 or 4, as defined in claims 5 or 7, and a proteolysis-resistant peptide deriving from said peptide by one or more chemical modifications, or a substantially homologous peptide, preferably deriving from SEQ ID NO: 1, 2, 3 or 4 by one or more conservative substitutions.

11. A nucleic acid encoding the chimeric peptide or the peptide that inhibits binding between SET and Caspase-9 as defined in any of claims 1 to 9.

12. A vector comprising the nucleic acid of claim 11.

13. The chimeric peptide of any of claims 1 to 9, or the peptide that inhibits binding between SET protein and Caspase-9 protein of claim 10, a nucleic acid of claim 11 or a vector of claim 12 as a medicament.

14. The chimeric peptide of any of claims 1 to 9, or the peptide that inhibits binding between SET protein and Caspase-9 protein of claim 10, a nucleic acid of claim 11 or a vector of claim 12 for use in treating a hyperproliferative disorder in a patient, preferably a tumor.

15. The chimeric peptide, or the peptide that inhibits binding between SET protein and Caspase-9 protein, for use in treating a hyperproliferative disorder according to claim 14, wherein the patient is to be administered with a combination of chimeric peptides as defined in any of claims 1 to 9 and/or peptides that inhibit binding between SET protein and Caspase-9 protein as defined in claim 10.
FIG. 3

FIG. 4
# INTERNATIONAL SEARCH REPORT

**PCT/EP2016/057148**

## A. CLASSIFICATION OF SUBJECT MATTER

INVENTOR: C07K14/47 A61K38/16

ADDITIONAL:

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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**Further documents are listed in the continuation of Box C.**

**See patent family annex.**

* Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another application or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

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

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

* "Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

* "Z" document member of the same patent family

**Date of the actual completion of the international search**

11 May 2016

**Date of mailing of the international search report**

23/05/2016

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

Authorized officer

Pinheiro Vieira, E
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<td>X</td>
<td>WO 2013/098339 A1 (UNIV PARIS CURIE [FR]; INST CURIE [FR]) 4 July 2013 (2013-07-04) claims 1-18; examples 1,2</td>
<td>1-4,8-15</td>
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<td>A</td>
<td>WO 2012/042038 A2 (INST NAT SANTE RECH MED [FR]; REBOLLO GARCIA ANGELITA [FR]; DECAUDDIN D) 5 April 2012 (2012-04-05) page 3; examples 1-3; table 4</td>
<td>1-15</td>
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