

NOVEL POLYPEPTIDE, POLYPEPTIDE DERIVATIVE AND USE THEREOF

TECHNICAL FIELD

The present application belongs to the technical field of biomedicine, specifically relates to a novel polypeptide, a polypeptide derivative and uses thereof, and more specifically relates to a novel polypeptide, a polypeptide derivative and uses thereof in tumor prevention or treatment.

BACKGROUND

Open reading frames (ORFs) for encoding proteins are composed of a string of sense codons, starting from a start codon and ending at a stop codon. Due to the limitations of traditional computer algorithms and detection methods, most algorithms for predicting ORFs have a minimum lower limit of 100 amino acids. With the development of ribosome profiling sequencing technology, computational biology, and high-throughput omics technology, an increasing number of studies have shown that many non-coding RNAs actually contain small open reading frames (sORFs) with a length of less than 300 nt, which can encode functional polypeptides with a length of less than 100 amino acids, known as micropeptides. Transcript sources of micropeptides mainly include non-coding RNAs (lncRNAs, circRNAs, pri-miRNAs), ribosomal RNAs (rRNA), antisense transcripts, 5'UTR, 3'UTR and intergenic ORFs.

The earliest research on micropeptides began in 1996, when German scientists discovered a 679 nt RNA transcribed from an ENOD40 (early nodulin 40) gene in leguminous plants, and the RNA exerts non-coding functions. Based on the length greater than 200 nt, the RNA was classified as a lncRNA at the time and can actually encode proteins and regulate plant growth. The specific results were published in the journal Science. In 2002, German researchers conducted a more detailed study of the RNA and found that the RNA contained two sORFs which indeed encoded micropeptides (one encoding a 12-amino acid polypeptide and the other encoding a 24-amino acid polypeptide), and confirmed the ability of the RNA to interact with a sucrose synthase. In the following 20 years, with the development of computational biology and multi-omics deep sequencing technologies (e.g., ribosomal imprinting sequencing and polypeptide omics sequencing), scientists have discovered a large number of previously unannotated sORFs hidden in non-coding RNAs in a plurality of species such as nematodes, fruit flies, zebrafish, plants, mice, and humans. These sORFs can translate completely new

polypeptides involved in biological functions such as muscle development, mRNA modification, immune regulation and tumor development, opening up a new perspective for proteomics research.

In recent years, some studies have reported that micropeptides (e.g., SMIM22, HOXB-AS3, SMIM30, MP31, and PINT87aa) encoded by ncRNAs can regulate occurrence and development of different tumors (including breast cancer, colorectal cancer, glioma, liver cancer, melanoma and esophageal squamous cell carcinoma), and can specifically play a role in promoting or inhibiting proliferation, metastasis, energy metabolism, drug resistance, and the like of tumors, suggesting that micropeptides can be in-depth developed as potential tumor diagnostic markers or therapeutic targets.

The Chinese invention patent with the publication number CN112442116A records a micropeptide HMMW encoded by a lncRNA and a use thereof in detection and treatment of malignant tumors such as head and neck cancer, thyroid cancer, and kidney cancer. HMMW is a completely new endogenous polypeptide sequence, specifically consisting of 51 amino acid residues, and a direct binding receptor is an AQP2 protein.

SUMMARY

1. Objectives of the invention

Regarding the new micropeptide HMMW first identified by the applicant (refer to the applicant's previous patent with a granted publication number CN112442116B for details),

a first objective of the present application is to verify the function of polypeptides that have homology with the micropeptide HMMW;

a second objective of the present application is to study key active amino acids of the micropeptide HMMW and provide a homologous functional fragment of the micropeptide HMMW that is shorter in length but has the same or substantially the same function, namely the novel polypeptide in the present application;

a third objective of the present application is to provide a derivative of the novel polypeptide, such as a derivative of the novel polypeptide obtained by long-acting modification, to better preserve the in vivo activity of the novel polypeptide, improve stability, and prolong the half-life; and

a fourth objective of the present application is to further provide uses of the novel polypeptide or the derivative thereof in preparation of tumor prevention or treatment drugs, and specifically the uses comprise prevention or treatment effects on human glioma,

neuroblastoma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer, uterine cancer, ovarian cancer, colon cancer, small intestine cancer, ileocecal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, sarcoma, myeloma, lymphoma, leukemia and the like.

2. Technical solution

To achieve the objectives of the invention, the technical solution adopted in the present application is as follows:

The present application provides a novel polypeptide, and the novel polypeptide is based on a completely new micropeptide HMMW (SEQ ID NO.1) discovered by the inventor, has been determined to have the same or substantially the same function as the micropeptide HMMW by means of sequence modification, structural analysis, amino acid mutation and the like, and is specifically selected from any one of the following:

(1): a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1;

(2): a polypeptide with the same or substantially the same function formed by deleting 1 to 34 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1; and

(3): a polypeptide with the same or substantially the same function formed by substituting one or more amino acids of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1, or (1), or (2).

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids at positions 3N-2, 3N-1, and 3N from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1, wherein N=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.2 to SEQ ID NO.18.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids at positions 3N-2, 3N-1, and 3N from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1, wherein N=1, 2, 3, 5,

6, 7, 8, 9, 12, 14, 15, or 16.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.2 to SEQ ID NO.4, SEQ ID NO.6 to SEQ ID NO.10, SEQ ID NO.13, or SEQ ID NO.15 to SEQ ID NO.17.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids at positions 3N-2, 3N-1, and 3N from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1, wherein N=1, 2, 3, 5, 6, 7, 8, 9, 12, or 16.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.2 to SEQ ID NO.4, SEQ ID NO.6 to SEQ ID NO.10, SEQ ID NO.13, or SEQ ID NO.17.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 1-34 amino acids at N-terminus of the polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 4, 9, 14, 19, 24, 29, or 34 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.19 to SEQ ID NO.25.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 1-19 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 4, 9, 14, or 19 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.19 to SEQ ID NO.22.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 1-14 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by deleting 4, 9, or 14 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.19 to SEQ ID NO.21.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by substituting one or more amino acids of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the novel polypeptide is a polypeptide with the same or substantially the same function formed by substituting one amino acid of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1.

Further, the substituting one amino acid refers to substituting the amino acid at the position 2, 3, 8, 9, 10, 11, 12, 13, 17, 19, 20, 21, 22, 25, 27, 28, 30, 31, 32, 40, 41, 42, 43, 44, 45, 48, 49, or 50 in the amino acid sequence set forth in SEQ ID NO.1, to form the polypeptide with the same or substantially the same function.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.26 to SEQ ID NO.53.

Further, the substituting one amino acid refers to substituting the amino acid at the position 2, 3, 8, 9, 10, 11, 12, 13, 17, 19, 20, 21, 22, 25, 27, 28, 30, 31, 32, 40, 41, 42, 43, 44, 45, 48, or 50 in the amino acid sequence set forth in SEQ ID NO.1, to form the polypeptide with the same or substantially the same function.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.26 to SEQ ID NO.51, and SEQ ID NO.53.

Further, the substituting one amino acid refers to substituting the amino acid at the position 2, 3, 8, 9, 10, 11, 12, 13, 17, 19, 20, 21, 22, 25, 27, 28, 30, 31, 32, 40, 41, 42, 44, 45, or 50 in the amino acid sequence set forth in SEQ ID NO.1, to form the polypeptide with the same or substantially the same function.

Further, the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID

NO.26 to SEQ ID NO.47, SEQ ID NO.49 to SEQ ID NO.50, and SEQ ID NO.53.

The present application further provides a nucleotide, and the nucleotide encodes one of the novel polypeptide.

The present application further provides a recombinant vector, and the recombinant vector comprises the nucleotide sequence for encoding the novel polypeptide, and may be a plasmid or viral vector or any other vector known to those skilled in the art.

The present application further provides a derivative of the novel polypeptide, and the derivative is a derivative with the same or substantially the same function of the novel polypeptide obtained by modification.

Further, the modification comprises one or more of N-terminus modification, C-terminus modification, side-chain modification, amino acid modification, or backbone modification.

Further, the modification comprises one or more long-acting modifications of polyethylene glycol modification, fatty acid linkage, fusion with Fc (igg1, igg4) polypeptide, binding with human serum albumin, non-natural amino acid substitution, polypeptide cyclization, or high-end formulations .

Furthermore, the polyethylene glycol modification mainly modifies an N-terminus, a C-terminus, a Lys side chain, and a thiol group of Cys of a polypeptide.

Further, the molecular weight range of single molecules of polyethylene glycol used for the polyethylene glycol modification is PEG5 to PEG40.

Further, the polyethylene glycol modification may use a single polyethylene glycol molecule or a plurality of polyethylene glycol molecules.

Further, the fatty acid linkage exerts a binding effect with serum albumin in vivo, and the linkage comprises direct N-terminus linkage and linkage with an 18-carbon fatty acid and polypeptide using a linker, with a small peptide Ala-Ala-Asn as the linker.

Further, the fatty acid linkage comprises N-terminus linkage.

Further, the N-terminus linkage comprises N-terminus linkage with a 12- to 20-carbon fatty acid. Still further, the N-terminus linkage comprises N-terminus linkage with an 18-carbon fatty acid.

Further, the fatty acid linkage comprises linkage with a fatty acid using a linker.

Further, the linkage using a linker comprises linkage with a 12- to 20-carbon fatty acid using a linker. Still further, the linkage using a linker comprises linkage with an 18-carbon fatty acid using a linker.

Further, the linker is a peptide less than 5 amino acids in length.

Further, the linker is a peptide less than 3 amino acids in length. Still further, the linker is

the peptide Ala-Ala-Asn.

The present application further provides uses of the novel polypeptide or the derivative thereof, the nucleotide for encoding the novel polypeptide, or the recombinant vector in preparation of tumor prevention or treatment drugs.

The present application further provides uses of the polypeptide and pharmaceutically acceptable salts and esters thereof, or the polypeptide derivative and pharmaceutically acceptable salts and esters thereof in preparation of tumor prevention or treatment drugs.

Further, the tumors comprise one or more of glioma, neuroblastoma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer, uterine cancer, ovarian cancer, colon cancer, small intestine cancer, ileocecal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, sarcoma, myeloma, lymphoma, and leukemia.

Further, the tumor prevention or treatment comprises inhibiting proliferation and/or metastasis of tumor cells.

Further, the tumor prevention or treatment comprises inhibiting proliferation of tumor cells.

Further, the tumor prevention or treatment comprises inhibiting metastasis of tumor cells.

Further, the tumor cells in the inhibiting proliferation of tumor cells comprise:

cancer cells of the glioblastoma, comprising: U87-MG;

cancer cells of the neuroblastoma, comprising: SH-SY5Y;

cancer cells of the head and neck cancer, comprising: any one of oral epidermoid carcinoma cell KB, laryngeal epidermoid carcinoma cell HEP2, tongue squamous cell carcinoma cell CAL27, and nasopharyngeal carcinoma cell CNE-2Z;

cancer cells of the esophageal cancer, comprising: Eca-109 and/or TE-13;

cancer cells of the thyroid cancer, comprising: NPPA87-1;

cancer cells of the lung cancer, comprising: any one or combination of 95-D, SK-MES-1, SPC-A1, and A549;

cancer cells of the liver cancer, comprising: HCCLM3 and/or HepG2;

cancer cells of the kidney cancer, comprising: Ketr-3 and/or 786-O;

cancer cells of the breast cancer, comprising: SK-BR-3 and/or MDA-MB-231;

cancer cells of the cervical cancer, comprising: CaSki and/or Hela;

cancer cells of the uterine cancer, comprising: RL-952 and/or MS751;

cancer cells of the ovarian cancer, comprising: ES-2 and/or SKOV3;

cancer cells of the colon cancer, comprising: HT29 and/or SW480;

cancer cells of the small intestine cancer, comprising: HIC;

cancer cells of the ileocecal cancer, comprising: HCT-8;
cancer cells of the gastric cancer, comprising: MGC-803 and/or BGC-823;
cancer cells of the bladder cancer, comprising: T24 and/or EJ;
cancer cells of the pancreatic cancer, comprising: ASPC-1 and/or PANC-1;
cancer cells of the prostate cancer, comprising: 22RV1 and/or PC-3;
cancer cells of the cholangiocarcinoma, comprising: QBC939;
cancer cells of the melanoma, comprising: SK-mel-2 and/or A375;
cancer cells of the sarcoma, comprising: MG63 and/or HT-1080;
cancer cells of the myeloma, comprising: LP-1;
cancer cells of the lymphoma, comprising: any one or combination of Raji, RPMI 8226, U937, and WSU-DLCL2; and
cancer cells of the leukemia, comprising: any one or combination of CEM/C1, NB4, KG-1, K562, HL60, Jurkat, and Clone E6-1.

Further, tumor cells in the inhibiting metastasis of tumor cells comprise:

cancer cells of the melanoma, comprising: A375;
cancer cells of the cervical cancer, comprising: Hela;
cancer cells of the ovarian cancer, comprising: Skvo3;
cancer cells of the liver cancer, comprising: Hep3B; and
cancer cells of the lung cancer, comprising: A549.

The present application further provides a pharmaceutical composition for preventing or treating tumors, and the pharmaceutical composition comprises at least the novel polypeptide or the derivative thereof and a pharmaceutically acceptable carrier. The tumors comprise one or more of glioma, neuroblastoma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer, uterine cancer, ovarian cancer, colon cancer, small intestine cancer, ileocecal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, sarcoma, myeloma, lymphoma, and leukemia.

3. Beneficial effects

Compared with the related art, the beneficial effects of the present application are as follows:

(1) The novel polypeptide provided in the present application is a polypeptide obtained by performing deletion or truncation on the micropeptide HMMW, consists of 17-50, especially 37-50 amino acids, and is shorter in length than the micropeptide HMMW. On the one hand, the shorter polypeptide has a lower molecular weight and is more conducive to absorption. As

in Examples, at the same dose, the novel polypeptide HMMW15-51 has a significantly better inhibitory effects on proliferation of cells of human glioma, neuroblastoma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer, uterine cancer, ovarian cancer, colon cancer, small intestine cancer, ileocecal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, sarcoma, myeloma, lymphoma, and leukemia than those of the micropeptide HMMW, a significantly better inhibitory effect on metastasis of human melanoma cells than that of the micropeptide HMMW, and a comparable inhibitory effect on metastasis of cells of human ovarian cancer, liver cancer, and lung cancer to that of the micropeptide HMMW. On the other hand, a synthesis process can be simplified and synthesis costs are saved.

(2) The novel polypeptide provided in the present application is formed by substituting amino acids of the micropeptide HMMW or the polypeptide obtained by performing deletion or truncation on the micropeptide HMMW. The present application confirms that L43, R48, and R49 are key active sites in the micropeptide HMMW sequence by attempting to combine different screening methods and activity evaluation systems, providing a basis for secondary development of the micropeptide HMMW.

(3) The derivative of the novel polypeptide provided in the present application, comprising polyethylene glycol modification, fatty acid modification, or any other long-acting modification, can better preserve in vivo activity of the novel polypeptide, improve stability, and prolong the half-life. The present application determines long-acting modification schemes through experimentation and in vivo activity evaluation. The half-life and in vivo anti-tumor time of the novel polypeptide are significantly prolonged, and the novel polypeptide has potential applications in development of tumor drugs. At the same dose, the in vivo anti-tumor effect of the novel polypeptide obtained by long-acting modification is significantly better than that of the novel polypeptide, and the frequency of administration is significantly shortened.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the effects of different deletants of a micropeptide HMMW on proliferation of tongue squamous cell carcinoma CAL27 cells;

FIG. 2 shows the effects of different truncated fragments of the micropeptide HMMW on proliferation of tongue squamous cell carcinoma CAL27 cells;

FIG. 3 shows sequence information of alanine scan mutations in the micropeptide HMMW;

FIG. 4 shows the effects of single amino acid mutation sequences of the micropeptide HMMW on proliferation of tongue squamous cell carcinoma CAL27 cells;

FIG. 5 shows statistical results of metastatic activity of melanoma A375 cells inhibited with the novel polypeptide and the micropeptide HMMW;

FIG. 6 shows statistical results of metastatic activity of cervical cancer Hela cells inhibited with the novel polypeptide and the micropeptide HMMW;

FIG. 7 shows statistical results of metastatic activity of ovarian cancer Skvo3 cells inhibited with the novel polypeptide and the micropeptide HMMW;

FIG. 8 shows statistical results of metastatic activity of liver cancer Hep3B cells inhibited with the novel polypeptide and the micropeptide HMMW;

FIG. 9 shows statistical results of metastatic activity of lung cancer A549 cells inhibited with the novel polypeptide and the micropeptide HMMW;

FIG. 10 shows inhibition results of the novel polypeptide and the micropeptide HMMW on the in vivo tumorigenicity of human tongue squamous cell carcinoma CAL27 cells;

FIG. 11 shows the effects of the novel polypeptide and a peptide obtained by long-acting modification on proliferation of human tongue squamous cell carcinoma CAL27 cells;

FIG. 12 shows the effects of the novel polypeptide and the peptide obtained by long-acting modification on proliferation of human ovarian cancer SKOV3 cells;

FIG. 13 shows the effects of the novel polypeptide and the peptide obtained by long-acting modification on proliferation of human liver cancer Hep3B cells; and

FIG. 14 shows inhibition results of the novel polypeptide and the peptide obtained by long-acting modification on the in vivo tumorigenicity of human tongue squamous cell carcinoma CAL27 cells.

DETAILED DESCRIPTION

The present application is further described below with reference to specific examples.

Example 1

This example provides the effects of different deletion mutants of a micropeptide HMMW on proliferation of human tongue squamous cell carcinoma CAL27 cells.

Due to the fact that the micropeptide HMMW (SEQ ID NO.1) is a completely new sequence, further development of smaller functional fragments thereof may reduce the cost of later development. Firstly, three amino acids in a group were sequentially deleted from an N-terminus (1-51aa) of the polypeptide, resulting in 17 different deletant polypeptides,

respectively named HMMW-1 (1-3aa deleted, SEQ ID NO.2), HMMW-2 (4-6aa deleted, SEQ ID NO.3), HMMW-3 (7-9aa deleted, SEQ ID NO.4), HMMW-4 (10-12aa deleted, SEQ ID NO.5), HMMW-5 (13-15aa deleted, SEQ ID NO.6), HMMW-6 (16-18aa deleted, SEQ ID NO.7), HMMW-7 (19-21aa deleted, SEQ ID NO.8), HMMW-8 (22-24aa deleted, SEQ ID NO.9), HMMW-9 (25-27aa deleted, SEQ ID NO.10), HMMW-10 (28-30aa deleted, SEQ ID NO.11), HMMW-11 (31-33aa deleted, SEQ ID NO.12), HMMW-12 (34-36aa deleted, SEQ ID NO.13), HMMW-13 (37-39aa deleted, SEQ ID NO.14), HMMW-14 (40-42aa deleted, SEQ ID NO.15), HMMW-15 (43-45aa deleted, SEQ ID NO.16), HMMW-16 (46-48aa deleted, SEQ ID NO.17), HMMW-17 (49-51aa deleted, SEQ ID NO.18). In the deletant polypeptides obtained by chemical solid-phase synthesis, the five polypeptides HMMW-4, HMMW-10, HMMW-11, HMMW-13, and HMMW-17 may have undergone changes in amino acid composition and have poor solubility, and thus cannot conduct subsequent activity experiments for evaluation. Therefore, the other 12 deletant polypeptides with good solubility were selected to compare the effects thereof on proliferation of human tongue squamous cell carcinoma CAL27 cells.

When human tongue squamous cell carcinoma CAL27 cells were cultured to a density of 90% in a 37°C, 5% CO₂ incubator, the cells were digested with trypsin and collected, resuspended in a culture solution, and counted under a microscope. The cell concentration was adjusted to approximately 5×10^4 cells/mL, and a cell suspension was seeded into a 96-well plate with 100 μ L per well, and cultured overnight in a 37°C, 5% CO₂ incubator. After the cells fully adhered to the wall, 100 μ L of the 12 deletant polypeptides and HMMW having a concentration of 80 μ M were added separately, and a culture solution without any drugs was used as a negative control group. After incubation was continued for 48 h, add 100 μ L of CCK8 solution to each well of the 96-well plate. An absorbance OD was measured at a detection wavelength of 450 nm using a microplate reader, and a proliferation inhibition (PI) was calculated using a formula: $PI(\%) = 1 - \text{Treatment group} / \text{Negative control group}$. The experiment was independently repeated 3 times, and the results were expressed as Mean $\pm$ SD. A statistical T-test was performed, and the results are shown in Table 1 and FIG. 1.

Table 1 Effects of different deletion mutants of micropeptide HMMW on proliferation of human tongue squamous cell carcinoma CAL27 cells

Group	48 h		Solubility
	OD	Proliferation inhibition (%)	PBS/0.1% DMSO
Negative control group	2.048 $\pm$ 0.241	-	

HMMW	0.260±0.098	87.302±4.785	Soluble
HMMW-1	0.390±0.087	80.965±4.248	Soluble
HMMW-2	0.404±0.076	80.251±3.711	Soluble
HMMW-3	0.415±0.091	79.742±4.443	Soluble
HMMW-5	0.410±0.079	79.992±3.857	Soluble
HMMW-6	0.352±0.068	82.830±3.320	Soluble
HMMW-7	0.392±0.059	80.840±2.881	Soluble
HMMW-8	0.365±0.047	82.201±2.295	Soluble
HMMW-9	0.405±0.061	80.234±2.979	Soluble
HMMW-12	0.307±0.073	85.016±3.564	Soluble
HMMW-14	1.007±0.052	50.816±2.539***	Soluble
HMMW-15	1.208±0.038	41.022±1.855***	Soluble
HMMW-16	0.403±0.075	80.314±3.662	Soluble

Note: Compared with the HMMW treatment group, * represents $P<0.05$, ** represents $P<0.01$, and *** represents $P<0.001$.

According to Table 1 and FIG. 1, in case of a normal solubility, compared with the HMMW treatment group, at the same dose, 10 deletant polypeptides HMMW-1, HMMW-2, HMMW-3, HMMW-5, HMMW-6, HMMW-7, HMMW-8, HMMW-9, HMMW-12, and HMMW-16 have comparable inhibitory effects to that of the HMMW on proliferation of the human tongue squamous cell carcinoma CAL27 cells, and do not have significantly decreased activity; and HMMW-14 and HMMW-15 have significantly decreased inhibitory effects on proliferation of the human tongue squamous cell carcinoma CAL27 cells compared to the HMMW, but still have activity.

Example 2

This example provides the effects of different truncated fragments of the micropeptide HMMW on proliferation of human tongue squamous cell carcinoma CAL27 cells.

Based on the results of Example 1, truncation was performed from the N-terminus of the micropeptide HMMW. Since HMMW-1 in Example 1 was obtained by deletion of the amino acid at the position 1-3aa, and it was found that an anti-tumor effect of the polypeptide was not affected, truncation was performed from the N-terminus, the amino acids at the positions 5-51, 10-51, 15-51, 20-51, 25-51, 30-51, and 35-51 were retained respectively, and the resulting

polypeptides were named HMMW5-51 (SEQ ID NO.19), HMMW10-51 (SEQ ID NO.20), HMMW15-51 (SEQ ID NO.21), HMMW20-51 (SEQ ID NO.22), HMMW25-51 (SEQ ID NO.23), HMMW30-51 (SEQ ID NO.24), and HMMW35-51 (SEQ ID NO.25) respectively. In the seven truncated polypeptides obtained by chemical solid-phase synthesis, the three polypeptides HMMW25-51, HMMW30-51, and HMMW35-51 may have undergone significant changes in amino acid composition and have poor solubility, and thus cannot conduct subsequent activity experiments for evaluation. Therefore, the four truncated polypeptides HMMW5-51, HMMW10-51, HMMW15-51, and HMMW20-51 with good solubility were selected to compare the effects thereof on proliferation of the human tongue squamous cell carcinoma CAL27 cells.

Similarly, when human tongue squamous cell carcinoma CAL27 cells were cultured to a density of 90% in a 37°C, 5% CO₂ incubator, the cells were digested with trypsin and collected, resuspended in a culture solution, and counted under a microscope. The cell concentration was adjusted to approximately 5×10^4 cells/mL, and a cell suspension was seeded into a 96-well plate with 100 μ L per well, and cultured overnight in a 37°C, 5% CO₂ incubator. After the cells fully adhered to the wall, 100 μ L of the HMMW micropeptide and the four truncated polypeptides HMMW5-51, HMMW10-51, HMMW15-51, and HMMW20-51 having a concentration of 80 μ M were added separately, and a culture solution without any drugs was used as a negative control group. After incubation was continued for 48 h, 100 μ L of CCK8 was added per well in a 96-well plate for dissolution. An absorbance OD was measured at a detection wavelength of 450 nm using a microplate reader, and a proliferation inhibition (PI) was calculated using a formula: $PI(\%) = 1 - \text{Treatment group} / \text{Negative control group}$. The experiment was independently repeated 3 times, and the results were expressed as Mean $\pm$ SD. A statistical T-test was performed, and the results are shown in Table 2 and FIG. 2.

Table 2 Effects of different truncated fragments of micropeptide HMMW on proliferation of human tongue squamous cell carcinoma CAL27 cells

Group	48 h	
	OD	Proliferation inhibition (%)
Negative control group	1.832 $\pm$ 0.158	-
HMMW	0.351 $\pm$ 0.078	80.841 $\pm$ 4.258
HMMW5-51	0.384 $\pm$ 0.085	79.000 $\pm$ 3.891
HMMW10-51	0.426 $\pm$ 0.093	76.747 $\pm$ 5.076
HMMW15-51	0.521 $\pm$ 0.087	71.561 $\pm$ 4.749

HMMW20-51	0.798±0.076	56.441±4.148**
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Note: Compared with the HMMW treatment group, * represents $P<0.05$, and ** represents $P<0.01$.

According to Table 2 and FIG. 2, compared with the HMMW treatment group, at the same dose, the three truncated polypeptides HMMW5-51, HMMW10-51, and HMMW15-51 have comparable inhibitory effects to that of the HMMW on proliferation of the human tongue squamous cell carcinoma CAL27 cells; and the truncated peptide HMMW20-51 has a significantly decreased inhibitory effect on proliferation of the human tongue squamous cell carcinoma CAL27 cells, but still has activity. Based on the results of Example 1, it is predicted that HMMW15-51 is the smallest functional fragment of the HMMW.

Example 3

This example provides alanine scan mutations of single amino acids in the micropeptide HMMW and affinity analysis.

Molecular docking simulation was performed on the micropeptide HMMW and a binding protein AQP2 thereof using MOE software, to identify predicted potential interaction sites. The docking simulation was divided into the following two groups: (1) random docking of an AQP2 homotetramer with the HMMW, without limiting a binding region; and (2) random docking of an AQP2 monomer with the HMMW, without limiting a binding region. Interaction sites obtained from the above two docking methods were integrated and analyzed, and all possible binding amino acids were selected as potential key sites for alanine (Ala) mutations. Due to alanine being the smallest chiral amino acid, the impact of a specific amino acid on an overall protein biological activity can be distinguished by systematically and sequentially substituting each amino acid in a sequence with Ala. The predicted polypeptides were obtained by solid-phase synthesis, and the affinity of different mutated polypeptides to the AQP2 protein was detected by microscale thermophoresis (MST) assay. The sequence information of the alanine scan mutations in the micropeptide HMMW is shown in FIG. 3, and the specific predicted key sites and the binding condition to the AQP2 are shown in Table 3.

Table 3 Prediction of key binding sites between micropeptide HMMW and AQP2 protein

Polypeptide name	SEQ ID NO	Affinity (μ M)
HMMW	SEQ ID NO.1	3.07
HMMW-2A	SEQ ID NO.26	2.5
HMMW-3A	SEQ ID NO.27	2.8

HMMW-8A	SEQ ID NO.28	14.76
HMMW-9A	SEQ ID NO.29	3.95
HMMW-10A	SEQ ID NO.30	4.03
HMMW-11A	SEQ ID NO.31	2.33
HMMW-12A	SEQ ID NO.32	1.12
HMMW-13A	SEQ ID NO.33	6.61
HMMW-17A	SEQ ID NO.34	3.93
HMMW-19A	SEQ ID NO.35	5.11
HMMW-20A	SEQ ID NO.36	2.59
HMMW-21A	SEQ ID NO.37	7.64
HMMW-22A	SEQ ID NO.38	3.21
HMMW-25A	SEQ ID NO.39	1.97
HMMW-27A	SEQ ID NO.40	2.39
HMMW-28A	SEQ ID NO.41	2.27
HMMW-30A	SEQ ID NO.42	2.23
HMMW-31A	SEQ ID NO.43	3.06
HMMW-32A	SEQ ID NO.44	2.01
HMMW-40A	SEQ ID NO.45	1.19
HMMW-41A	SEQ ID NO.46	4.32
HMMW-42A	SEQ ID NO.47	0.25
HMMW-43A	SEQ ID NO.48	0.36
HMMW-44A	SEQ ID NO.49	5.23
HMMW-45A	SEQ ID NO.50	3.24
HMMW-48A	SEQ ID NO.51	11.64
HMMW-49A	SEQ ID NO.52	20.76
HMMW-50A	SEQ ID NO.53	0.55

According to Table 3, compared with the HMMW, the affinity of the polypeptide to the protein AQP2 significantly decreased following mutations of the amino acids at the positions 8, 48, and 49, preliminarily indicating that the key sites of the HMMW are the amino acids at the positions 8, 48, and 49.

Example 4

This example provides the effects of single amino acid mutation sequences of the micropeptide HMMW on proliferation of tongue squamous cell carcinoma CAL27 cells.

When human tongue squamous cell carcinoma CAL27 cells were cultured to a density of 90% in a 37°C, 5% CO₂ incubator, the cells were digested with trypsin and collected, resuspended in a culture solution, and counted under a microscope. The cell concentration was adjusted to approximately 5×10^4 cells/mL, and a cell suspension was seeded into a 96-well plate with 100 μ L per well, and cultured overnight in a 37°C, 5% CO₂ incubator. After the cells fully adhered to the wall, 100 μ L of the HMMW micropeptide and the single amino acid mutated polypeptide synthesized in Example 3 having a concentration of 80 μ M were added separately, and a culture solution without any drugs was used as a negative control group. After incubation was continued for 48 h, 100 μ L of CCK8 was added per well in a 96-well plate for dissolution. An absorbance OD was measured at a detection wavelength of 450 nm using a microplate reader, and a proliferation inhibition (PI) was calculated using a formula: $PI(\%) = 1 - \text{Treatment group} / \text{Negative control group}$. The experiment was independently repeated 3 times, and the results were expressed as Mean $\pm$ SD. A statistical T-test was performed, and the results are shown in FIG. 4, where * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$. At a concentration of 80 μ M, except the two peptides HMMW-3A and HMMW-11A which have poor solubility and cannot conduct activity comparison, compared with the HMMW treatment group, the inhibitory effects of the polypeptides with the amino acids at the positions 43, 48, and 49 mutated on proliferation of the human tongue squamous cell carcinoma CAL27 cells were significantly decreased. The polypeptide with the amino acid at the position 8 mutated shows no difference in anti-tumor activity compared to the HMMW. Therefore, it is determined that the amino acids at the positions 43, 48, and 49 are the key active sites of the micropeptide HMMW.

Example 5

This example provides detection of inhibitory effects of the novel polypeptide HMMW15-51 (SEQ ID NO.21) and the micropeptide HMMW on proliferation of different tumor cells.

When human neuroblastoma cells SH-SY5Y, brain astrocytoma cells U87MG, oral epidermoid carcinoma cells KB, laryngeal epidermoid carcinoma cells HEp2, tongue squamous cell carcinoma cells CAL27, nasopharyngeal carcinoma cells CNE-2Z, esophageal cancer cells Eca-109 and TE-13, papillary thyroid carcinoma cells NPPA87-1, giant cell lung cancer cells 95-D, lung squamous cell carcinoma cells SK-MES-1, lung adenocarcinoma cells SPC-A1, non-small cell lung cancer cells A549, liver cancer cells HCCLM3 and HepG2, kidney cancer cells Ketr-3 and 786-O, breast cancer cells SK-BR-3 and MDA-MB-231, cervical cancer cells CaSki and Hela, uterine cancer cells RL-952 and MS751, ovarian cancer cells ES-2 and SKOV3, colon cancer cells HT29 and SW480, small intestine cancer cells HIC,

ileocecal cancer cells HCT-8, gastric cancer cells MGC-803 and BGC-823, bladder cancer cells T24 and EJ, pancreatic cancer cells ASPC-1 and PANC-1, prostate cancer cells 22RV1 and PC-3, cholangiocarcinoma cells QBC939, malignant melanoma cells SK-mel-2 and A375, osteosarcoma cells MG63, fibrosarcoma cells HT-1080, multiple myeloma cells LP-1, lymphoma cells Raji, peripheral blood B lymphocytes RPMI 8226 in multiple myeloma, histiocytic lymphoma cells U937, diffuse large B-cell lymphoma (DLBCL) cells WSU-DLCL2, acute lymphoblastic leukemia cells CEM/C1, acute promyelocytic leukemia cells NB4, acute myeloid leukemia cells KG-1, chronic myeloid leukemia cells K562, promyelocytic leukemia cells HL60, and T lymphoblastic leukemia cells (Jurkat, Clone E6-1) were cultured to a density of 90% in a 37°C, 5% CO₂ incubator, the cells were digested with trypsin and collected, resuspended in a culture solution, and counted under a microscope. The cell concentration was adjusted to approximately 1.0×10^5 cells/mL, and a cell suspension was seeded into a 96-well plate with 100 μ L per well, and cultured overnight in a 37°C, 5% CO₂ incubator. After the cells fully adhered to the wall, 100 μ L of the HMMW micropeptide having a concentration of 50 μ M and the novel polypeptide HMMW15-51 having concentrations of 50 μ M/75 μ M/100 μ M were added separately as a treatment group; and a culture solution without any drugs was used as a negative control group. After incubation was continued for 48 h, 10 μ L of CCK8 was added per well in a 96-well plate for dissolution. Incubation was continued for 4 h in a cell incubator, an absorbance was measured at 450 nm, and a proliferation inhibition was calculated. Data was presented as mean $\pm$ SD, and GraphPad Prism 5.0 statistical software was used. A *t*-test was used for comparison between two groups, while One-way Anova (Dunnett) was used for comparison among a plurality of groups. The results are shown in Tables 4-56, where * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

Table 4. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human brain astrocytoma cells U87MG

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.965 $\pm$ 0.026	-
Novel polypeptide HMMW15-51	100 μ M	0.112 $\pm$ 0.024**	88.359 $\pm$ 2.440**
	75 μ M	0.169 $\pm$ 0.023**	82.487 $\pm$ 2.383**
	50 μ M	0.197 $\pm$ 0.042**	79.585 $\pm$ 4.324**
HMMW	50 μ M	0.365 $\pm$ 0.019**	62.176 $\pm$ 2.001**

Table 5. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on

proliferation of human neuroblastoma cells SH-SY5Y

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.983±0.015	-
Novel polypeptide HMMW15-51	100 μM	0.162±0.019	83.525±1.948**
	75 μM	0.191±0.037	80.576±3.787**
	50 μM	0.263±0.034	73.220±3.443**
HMMW	50 μM	0.486±0.049	50.542±4.982**

Table 6. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human oral epidermoid carcinoma cells KB

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.010±0.021	-
Novel polypeptide HMMW15-51	100 μM	0.122±0.026**	87.888±2.575**
	75 μM	0.167±0.005**	83.432±0.508**
	50 μM	0.218±0.012**	78.383±1.148**
HMMW	50 μM	0.471±0.040**	53.399±3.971**

Table 7. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human laryngeal epidermoid carcinoma cells HEp2

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.875±0.011	-
Novel polypeptide HMMW15-51	100 μM	0.127±0.007**	85.518±0.744**
	75 μM	0.169±0.027**	80.716±3.030**
	50 μM	0.210±0.024**	76.029±2.728**
HMMW	50 μM	0.504±0.020**	42.416±2.236**

Table 8. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human tongue squamous cell carcinoma cells CAL27

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.165±0.012	-

Novel polypeptide HMMW15-51	100 μ M	0.151 $\pm$ 0.010**	87.035 $\pm$ 0.828**
	75 μ M	0.211 $\pm$ 0.039**	81.912 $\pm$ 3.381**
	50 μ M	0.261 $\pm$ 0.021**	77.619 $\pm$ 1.804**
HMMW	50 μ M	0.383 $\pm$ 0.064**	67.115 $\pm$ 5.457**

Table 9. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human nasopharyngeal carcinoma cells CNE-2Z

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.963 $\pm$ 0.023	-
Novel polypeptide HMMW15-51	100 μ M	0.112 $\pm$ 0.012**	88.404 $\pm$ 1.252**
	75 μ M	0.154 $\pm$ 0.017**	84.043 $\pm$ 1.806**
	50 μ M	0.244 $\pm$ 0.044**	74.697 $\pm$ 4.553**
HMMW	50 μ M	0.472 $\pm$ 0.042**	50.952 $\pm$ 4.312**

Table 10. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human esophageal cancer cells Eca-109

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.949 $\pm$ 0.041	-
Novel polypeptide HMMW15-51	100 μ M	0.076 $\pm$ 0.017**	92.024 $\pm$ 1.793**
	75 μ M	0.109 $\pm$ 0.025**	88.510 $\pm$ 2.588**
	50 μ M	0.168 $\pm$ 0.034**	82.256 $\pm$ 3.531**
HMMW	50 μ M	0.350 $\pm$ 0.028**	63.071 $\pm$ 2.982**

Table 11. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human esophageal cancer cells TE-13

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.828 $\pm$ 0.017	-
Novel polypeptide HMMW15-51	100 μ M	0.132 $\pm$ 0.012**	84.018 $\pm$ 1.451**
	75 μ M	0.205 $\pm$ 0.013**	75.242 $\pm$ 1.579**
	50 μ M	0.302 $\pm$ 0.029**	63.486 $\pm$ 3.547**
HMMW	50 μ M	0.389 $\pm$ 0.016**	53.060 $\pm$ 1.911**

Table 12. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human papillary thyroid carcinoma cells NPPA87-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.922±0.032	-
Novel polypeptide HMMW15-51	100 µM	0.221±0.019**	76.030±2.061**
	75 µM	0.271±0.044**	70.607±4.793**
	50 µM	0.381±0.026**	58.677±2.767**
HMMW	50 µM	0.478±0.028**	48.192±2.999**

Table 13. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human giant cell lung cancer cells 95-D

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.098±0.012	-
Novel polypeptide HMMW15-51	100 µM	0.150±0.018**	86.343±1.600**
	75 µM	0.211±0.020**	80.789±1.791**
	50 µM	0.259±0.032**	76.449±2.914**
HMMW	50 µM	0.442±0.059**	59.757±5.365**

Table 14. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human lung squamous cell carcinoma cells SK-MES-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.964±0.024	-
Novel polypeptide HMMW15-51	100 µM	0.119±0.024**	87.621±2.501**
	75 µM	0.203±0.030**	78.942±3.061**
	50 µM	0.279±0.018**	71.058±1.844**
HMMW	50 µM	0.435±0.043**	54.910±4.426**

Table 15. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human lung adenocarcinoma cells SPC-A1

Group	48 h
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		OD	Inhibition (%)
Negative control group		1.032±0.038	-
Novel polypeptide HMMW15-51	100 µM	0.151±0.028**	85.368±2.729**
	75 µM	0.259±0.018**	74.935±1.734**
	50 µM	0.358±0.044**	65.278±4.300**
HMMW	50 µM	0.525±0.032**	49.128±3.053**

Table 16. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human non-small cell lung cancer cells A549

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.079±0.022	-
Novel polypeptide HMMW15-51	100 µM	0.229±0.041**	78.808±3.770**
	75 µM	0.354±0.041**	67.161±3.844**
	50 µM	0.469±0.003**	56.534±0.278**
HMMW	50 µM	0.508±0.022**	52.950±2.023**

Table 17. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human liver cancer cells HCCLM3

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.998±0.032	-
Novel polypeptide HMMW15-51	100 µM	0.117±0.025**	88.239±2.474**
	75 µM	0.201±0.037**	79.820±3.700**
	50 µM	0.301±0.039**	69.863±3.920**
HMMW	50 µM	0.460±0.076**	53.926±7.634**

Table 18. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human liver cancer cells HepG2

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.073±0.036	-
Novel polypeptide HMMW15-51	100 µM	0.154±0.022**	85.674±2.069**
	75 µM	0.241±0.019**	77.564±1.730**

	50 μ M	0.311 $\pm$ 0.052**	70.976 $\pm$ 4.848**
HMMW	50 μ M	0.509 $\pm$ 0.027**	52.579 $\pm$ 2.504**

Table 19. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human kidney cancer cells Ketr-3

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.929 $\pm$ 0.049	-
Novel polypeptide HMMW15-51	100 μ M	0.178 $\pm$ 0.016**	80.797 $\pm$ 1.717**
	75 μ M	0.248 $\pm$ 0.026**	73.295 $\pm$ 2.760**
	50 μ M	0.351 $\pm$ 0.020**	62.240 $\pm$ 2.197**
HMMW	50 μ M	0.476 $\pm$ 0.045**	48.708 $\pm$ 4.852**

Table 20. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human kidney clear cell adenocarcinoma cells 786-O

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.105 $\pm$ 0.019	-
Novel polypeptide HMMW15-51	100 μ M	0.205 $\pm$ 0.035**	81.478 $\pm$ 3.122**
	75 μ M	0.312 $\pm$ 0.029**	71.765 $\pm$ 2.634**
	50 μ M	0.341 $\pm$ 0.054**	69.140 $\pm$ 4.893**
HMMW	50 μ M	0.413 $\pm$ 0.078**	62.624 $\pm$ 7.014**

Table 21. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human breast cancer cells SK-BR-3

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.096 $\pm$ 0.039	-
Novel polypeptide HMMW15-51	100 μ M	0.122 $\pm$ 0.019**	88.899 $\pm$ 1.746**
	75 μ M	0.224 $\pm$ 0.049**	79.592 $\pm$ 4.474**
	50 μ M	0.291 $\pm$ 0.022**	73.418 $\pm$ 1.970**
HMMW	50 μ M	0.507 $\pm$ 0.054**	53.710 $\pm$ 4.902**

Table 22. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on

proliferation of human breast cancer cells MDA-MB-231

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.233±0.007	-
Novel polypeptide HMMW15-51	100 µM	0.211±0.027**	82.865±2.194**
	75 µM	0.237±0.032**	80.757±2.620**
	50 µM	0.341±0.042**	72.378±3.406**
HMMW	50 µM	0.429±0.056**	65.216±4.512**

Table 23. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human cervical cancer epithelial cells CaSki

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.981±0.035	-
Novel polypeptide HMMW15-51	100 µM	0.228±0.035**	76.800±3.597**
	75 µM	0.302±0.030**	69.226±3.007**
	50 µM	0.402±0.024**	59.001±2.408**
HMMW	50 µM	0.458±0.038**	53.329±3.884**

Table 24. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human cervical cancer cells Hela

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.964±0.023	-
Novel polypeptide HMMW15-51	100 µM	0.198±0.026**	83.661±1.557**
	75 µM	0.328±0.011**	74.508±2.703**
	50 µM	0.377±0.044**	66.508±2.412**
HMMW	50 µM	0.493±0.049**	52.644±4.018**

Table 25. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human endometrial cancer cells RL-952

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.860±0.027	-

Novel polypeptide HMMW15-51	100 μ M	0.190 $\pm$ 0.038**	77.916 $\pm$ 4.415**
	75 μ M	0.324 $\pm$ 0.022**	62.379 $\pm$ 2.505**
	50 μ M	0.355 $\pm$ 0.044**	58.776 $\pm$ 5.167**
HMMW	50 μ M	0.422 $\pm$ 0.034**	50.988 $\pm$ 3.897**

Table 26. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human uterine epidermal cancer cells MS751

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.934 $\pm$ 0.013	-
Novel polypeptide HMMW15-51	100 μ M	0.259 $\pm$ 0.026**	72.296 $\pm$ 2.763**
	75 μ M	0.355 $\pm$ 0.024**	61.942 $\pm$ 2.517**
	50 μ M	0.430 $\pm$ 0.031**	53.945 $\pm$ 3.268**
HMMW	50 μ M	0.474 $\pm$ 0.024**	49.268 $\pm$ 2.531**

Table 27. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human ovarian clear cell carcinoma cells ES-2

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.966 $\pm$ 0.032	-
Novel polypeptide HMMW15-51	100 μ M	0.183 $\pm$ 0.013**	81.049 $\pm$ 1.298**
	75 μ M	0.270 $\pm$ 0.028**	72.006 $\pm$ 2.904**
	50 μ M	0.410 $\pm$ 0.042**	57.508 $\pm$ 4.384**
HMMW	50 μ M	0.464 $\pm$ 0.007**	51.916 $\pm$ 0.690**

Table 28. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human ovarian cancer cells SKOV3

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.983 $\pm$ 0.042	-
Novel polypeptide HMMW15-51	100 μ M	0.161 $\pm$ 0.015**	83.661 $\pm$ 1.557**
	75 μ M	0.251 $\pm$ 0.027**	74.508 $\pm$ 2.703**
	50 μ M	0.329 $\pm$ 0.024**	66.508 $\pm$ 2.412**
HMMW	50 μ M	0.466 $\pm$ 0.040**	52.644 $\pm$ 4.018**

Table 29. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human colon cancer cells HT29

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.974±0.016	-
Novel polypeptide HMMW15-51	100 µM	0.103±0.019**	89.456±1.928**
	75 µM	0.129±0.014**	86.717±1.402**
	50 µM	0.192±0.006**	80.246±0.584**
HMMW	50 µM	0.479±0.024**	50.805±2.422**

Table 30. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human colon cancer cells SW480

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.941±0.028	-
Novel polypeptide HMMW15-51	100 µM	0.160±0.035**	82.991±3.678**
	75 µM	0.269±0.029**	71.439±3.113**
	50 µM	0.358±0.016**	61.906±1.695**
HMMW	50 µM	0.479±0.025**	49.114±2.626**

Table 31. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human small intestine cancer cells HIC

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.018±0.018	-
Novel polypeptide HMMW15-51	100 µM	0.221±0.040**	78.251±3.911**
	75 µM	0.293±0.022**	71.209±2.133**
	50 µM	0.383±0.031**	62.332±3.021**
HMMW	50 µM	0.507±0.022**	50.213±2.165**

Table 32. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human ileocecal cancer cells HCT-8

Group	48 h
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		OD	Inhibition (%)
Negative control group		0.939±0.029	-
Novel polypeptide HMMW15-51	100 µM	0.205±0.047**	78.211±4.966**
	75 µM	0.297±0.066**	68.346±7.028**
	50 µM	0.445±0.064**	52.590±6.777**
HMMW	50 µM	0.482±0.036**	48.687±3.785**

Table 33. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human gastric cancer cells MGC-803

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.145±0.029	-
Novel polypeptide HMMW15-51	100 µM	0.134±0.010**	88.329±0.861**
	75 µM	0.190±0.020**	83.382±1.747**
	50 µM	0.254±0.010**	77.852±0.861**
HMMW	50 µM	0.675±0.035**	41.094±3.077**

Table 34. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human gastric cancer cells BGC-823

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.976±0.038	-
Novel polypeptide HMMW15-51	100 µM	0.169±0.021**	82.690±2.158**
	75 µM	0.229±0.027**	76.511±2.776**
	50 µM	0.299±0.037**	69.409±3.800**
HMMW	50 µM	0.510±0.046**	47.730±4.739**

Table 35. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human bladder transitional cell carcinoma cells T24

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.950±0.013	-
Novel polypeptide HMMW15-51	100 µM	0.090±0.013**	90.523±1.319**
	75 µM	0.158±0.033**	83.363±3.522**

	50 μ M	0.205 $\pm$ 0.011**	78.378 $\pm$ 1.184**
HMMW	50 μ M	0.460 $\pm$ 0.045**	51.562 $\pm$ 4.780**

Table 36. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human bladder cancer cells EJ

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.223 $\pm$ 0.018	-
Novel polypeptide HMMW15-51	100 μ M	0.169 $\pm$ 0.052**	86.185 $\pm$ 4.258**
	75 μ M	0.345 $\pm$ 0.017**	71.798 $\pm$ 1.375**
	50 μ M	0.439 $\pm$ 0.016**	64.142 $\pm$ 1.270**
HMMW	50 μ M	0.638 $\pm$ 0.018**	47.820 $\pm$ 1.486**

Table 37. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human pancreatic cancer cells ASPC-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.024 $\pm$ 0.020	-
Novel polypeptide HMMW15-51	100 μ M	0.131 $\pm$ 0.027**	87.179 $\pm$ 2.645**
	75 μ M	0.234 $\pm$ 0.035**	77.188 $\pm$ 3.413**
	50 μ M	0.363 $\pm$ 0.063**	64.562 $\pm$ 6.146**
HMMW	50 μ M	0.497 $\pm$ 0.045**	51.448 $\pm$ 4.377**

Table 38. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human pancreatic cancer cells PANC-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.853 $\pm$ 0.034	-
Novel polypeptide HMMW15-51	100 μ M	0.095 $\pm$ 0.013**	88.906 $\pm$ 1.493**
	75 μ M	0.184 $\pm$ 0.023**	78.398 $\pm$ 2.704**
	50 μ M	0.310 $\pm$ 0.047**	63.672 $\pm$ 5.525**
HMMW	50 μ M	0.424 $\pm$ 0.052**	50.273 $\pm$ 6.135**

Table 39. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on

proliferation of human prostate cancer cells 22RV1

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.115±0.030	-
Novel polypeptide HMMW15-51	100 µM	0.193±0.042**	82.720±3.730**
	75 µM	0.305±0.044**	72.646±3.906**
	50 µM	0.426±0.036**	61.794±3.210**
HMMW	50 µM	0.552±0.019**	50.463±1.693**

Table 40. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human prostate cancer cells PC-3

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.025±0.014	-
Novel polypeptide HMMW15-51	100 µM	0.193±0.049**	81.203±4.742**
	75 µM	0.244±0.017**	76.163±1.651**
	50 µM	0.291±0.038**	71.642±3.715**
HMMW	50 µM	0.509±0.040**	50.341±3.864**

Table 41. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human cholangiocarcinoma cells QBC939

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.850±0.032	-
Novel polypeptide HMMW15-51	100 µM	0.156±0.037**	81.608±4.366**
	75 µM	0.253±0.041**	70.196±4.847**
	50 µM	0.344±0.037**	59.529±4.305**
HMMW	50 µM	0.429±0.018**	49.490±2.138**

Table 42. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human malignant melanoma cells SK-mel-2

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.867±0.029	-

Novel polypeptide HMMW15-51	100 μ M	0.133 $\pm$ 0.025**	84.704 $\pm$ 2.846**
	75 μ M	0.174 $\pm$ 0.019**	79.977 $\pm$ 2.225**
	50 μ M	0.233 $\pm$ 0.014**	73.136 $\pm$ 1.614**
HMMW	50 μ M	0.466 $\pm$ 0.042**	46.311 $\pm$ 4.884**

Table 43. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human malignant melanoma cells A375

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.974 $\pm$ 0.035	-
Novel polypeptide HMMW15-51	100 μ M	0.150 $\pm$ 0.045**	84.571 $\pm$ 4.579**
	75 μ M	0.269 $\pm$ 0.058**	72.357 $\pm$ 5.914**
	50 μ M	0.360 $\pm$ 0.015**	63.052 $\pm$ 1.491**
HMMW	50 μ M	0.491 $\pm$ 0.040**	49.572 $\pm$ 4.128**

Table 44. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human osteosarcoma cells MG63

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.917 $\pm$ 0.032	-
Novel polypeptide HMMW15-51	100 μ M	0.126 $\pm$ 0.025**	86.228 $\pm$ 2.728**
	75 μ M	0.200 $\pm$ 0.023**	78.161 $\pm$ 2.520**
	50 μ M	0.270 $\pm$ 0.026**	70.603 $\pm$ 2.845**
HMMW	50 μ M	0.435 $\pm$ 0.024**	52.544 $\pm$ 2.562**

Table 45. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human fibrosarcoma cells HT-1080

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.922 $\pm$ 0.030	-
Novel polypeptide HMMW15-51	100 μ M	0.166 $\pm$ 0.022**	81.953 $\pm$ 2.407**
	75 μ M	0.256 $\pm$ 0.036**	72.260 $\pm$ 3.852**
	50 μ M	0.365 $\pm$ 0.032**	60.434 $\pm$ 3.432**
HMMW	50 μ M	0.432 $\pm$ 0.038**	53.092 $\pm$ 4.138**

Table 46. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human multiple myeloma cells LP-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		0.882±0.037	-
Novel polypeptide HMMW15-51	100 µM	0.146±0.027**	83.440±3.052**
	75 µM	0.200±0.009**	77.278±1.054**
	50 µM	0.299±0.023**	66.125±2.629**
HMMW	50 µM	0.462±0.038**	47.561±4.259**

Table 47. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human lymphoma cells Raji

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.268±0.042	-
Novel polypeptide HMMW15-51	100 µM	0.189±0.017**	85.099±1.317**
	75 µM	0.237±0.020**	81.340±1.546**
	50 µM	0.380±0.036**	70.013±2.862**
HMMW	50 µM	0.576±0.047**	54.560±3.675**

Table 48. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human peripheral blood B lymphocytes RPMI 8226 in multiple myeloma

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.229±0.022	-
Novel polypeptide HMMW15-51	100 µM	0.148±0.025**	87.954±2.000**
	75 µM	0.235±0.011**	80.846±0.908**
	50 µM	0.360±0.034**	70.700±2.799**
HMMW	50 µM	0.578±0.056**	52.930±4.562**

Table 49. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human histiocytic lymphoma cells U937

Group	48 h
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		OD	Inhibition (%)
Negative control group		1.337±0.034	-
Novel polypeptide HMMW15-51	100 µM	0.331±0.031**	75.274±2.328**
	75 µM	0.498±0.050**	62.787±3.714**
	50 µM	0.599±0.044**	55.234±3.264**
HMMW	50 µM	0.855±0.029**	36.042±2.134**

Table 50. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human diffuse large B-cell lymphoma (DLBCL) cells WSU-DLCL2

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.313±0.040	-
Novel polypeptide HMMW15-51	100 µM	0.277±0.028**	78.934±2.143**
	75 µM	0.478±0.032**	63.629±2.463**
	50 µM	0.626±0.048**	52.360±3.672**
HMMW	50 µM	0.731±0.032**	44.365±2.462**

Table 51. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human acute lymphoblastic leukemia cells CEM/C1

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.177±0.035	-
Novel polypeptide HMMW15-51	100 µM	0.251±0.043**	78.709±3.692**
	75 µM	0.443±0.050**	62.344±4.216**
	50 µM	0.579±0.059**	50.849±5.040**
HMMW	50 µM	0.630±0.017**	46.518±1.484**

Table 52. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human acute promyelocytic leukemia cells NB4

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.415±0.037	-
Novel polypeptide HMMW15-51	100 µM	0.354±0.047**	74.988±3.332**
	75 µM	0.504±0.040**	64.390±2.797**

	50 μ M	0.685 $\pm$ 0.019**	51.578 $\pm$ 1.363**
HMMW	50 μ M	0.740 $\pm$ 0.070**	47.692 $\pm$ 4.919**

Table 53. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human acute myeloid leukemia cells KG-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.161 $\pm$ 0.046	-
Novel polypeptide HMMW15-51	100 μ M	0.319 $\pm$ 0.033**	72.532 $\pm$ 2.815**
	75 μ M	0.420 $\pm$ 0.016**	63.863 $\pm$ 1.416**
	50 μ M	0.561 $\pm$ 0.017**	51.665 $\pm$ 1.467**
HMMW	50 μ M	0.725 $\pm$ 0.070**	37.600 $\pm$ 5.989**

Table 54. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human chronic myeloid leukemia cells K562

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.285 $\pm$ 0.026	-
Novel polypeptide HMMW15-51	100 μ M	0.409 $\pm$ 0.052**	68.197 $\pm$ 4.053**
	75 μ M	0.524 $\pm$ 0.038**	59.222 $\pm$ 2.991**
	50 μ M	0.597 $\pm$ 0.046**	53.567 $\pm$ 3.588**
HMMW	50 μ M	0.618 $\pm$ 0.036**	51.933 $\pm$ 2.838**

Table 55. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on proliferation of human promyelocytic leukemia cells HL60

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.097 $\pm$ 0.035	-
Novel polypeptide HMMW15-51	100 μ M	0.310 $\pm$ 0.041**	71.733 $\pm$ 3.732**
	75 μ M	0.427 $\pm$ 0.025**	61.064 $\pm$ 2.269**
	50 μ M	0.501 $\pm$ 0.008**	54.286 $\pm$ 0.696**
HMMW	50 μ M	0.578 $\pm$ 0.062**	47.264 $\pm$ 5.692**

Table 56. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on

proliferation of human T lymphoblastic leukemia cells Jurkat, Clone E6-1

Group		48 h	
		OD	Inhibition (%)
Negative control group		1.080±0.029	-
Novel polypeptide HMMW15-51	100 μM	0.275±0.053**	74.545±4.881**
	75 μM	0.406±0.051**	62.388±4.707**
	50 μM	0.486±0.059**	55.014±5.442**
HMMW	50 μM	0.602±0.037**	44.307±3.404**

According to Tables 4-56 that at the same dose, the novel polypeptide HMMW15-51 has significantly better inhibitory effects on proliferation of neuroblastoma, human glioma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer, uterine cancer, ovarian cancer, intestinal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, osteosarcoma, fibrosarcoma, myeloma, lymphoma, and hematoma cells than the micropeptide HMMW. Moreover, the inhibitory effects of the novel polypeptide HMMW15-51 on proliferation of the tumor cells showed a dose-dependent relationship, indicating that the novel polypeptide HMMW15-51 may be developed into anti-tumor drugs by inhibiting proliferation of the malignant tumor cells.

Example 6

This example provides inhibitory effects of the novel polypeptide HMMW15-51 (SEQ ID NO.21) and the micropeptide HMMW on metastasis of different human tumor cells.

Melanoma, cervical cancer, ovarian cancer, liver cancer, and lung cancer cells were seeded into transwell chambers, with 100 μL per well. Different doses of the HMMW micropeptide and the novel polypeptide HMMW15-51 were added to each chamber as a treatment group, while no drug was added to a blank group. Then, 0.6 mL of a complete culture medium containing 10% FBS was added to the lower chambers of the transwell to stimulate cell metastasis, and the cells were cultured at 5% CO₂ and 37°C for 48 h. The culture solution was discarded from the wells. The cells were fixed with methanol at room temperature for 30 min, stained with 0.1% crystal violet at room temperature for 10 min, and rinsed with clean water. The upper layer nonmetastatic cells were wiped off with a cotton swab. The cells were observed under a microscope, and four fields of view were selected to take photos and count the cells. A metastasis inhibition (MI) was calculated according to a formula:

$$MI(\%) = \left(1 - \frac{N_{test}}{N_{control}}\right) \times 100\%,$$

where N_{test} represents the number of cell metastases in the treatment group, and $N_{control}$ represents the number of cell metastases in the blank control group.

Data was presented as mean $\pm$ SD, and GraphPad Prism 5.0 statistical software was used. A *t*-test was used for comparison between two groups, where * represents $P<0.05$, ** represents $P<0.01$, and *** represents $P<0.001$. The results are shown in Tables 57-61 and FIGS. 5-9.

Table 57. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on metastasis of human melanoma A375 cells

Group	Dose ($\mu\text{mol/L}$)	Number of cell metastases	Inhibition (%)
None	0	590 $\pm$ 44.73	-
Novel polypeptide	50	61 $\pm$ 30.07	89.63***
	25	316 $\pm$ 4.57	46.40***
HMMW	50	371 $\pm$ 13.00	37.13***

Table 58. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on metastasis of human cervical cancer Hela cells

Group	Dose ($\mu\text{mol/L}$)	Number of cell metastases	Inhibition (%)
None	0	1028 $\pm$ 121.65	-
Novel polypeptide	50	441 $\pm$ 22.66	57.13***
	25	448 $\pm$ 7.96	56.40***
HMMW	50	115 $\pm$ 20.32	88.80***

Table 59. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on metastasis of human ovarian cancer Skvo3 cells

Group	Dose ($\mu\text{mol/L}$)	Number of cell metastases	Inhibition (%)
None	0	1138 $\pm$ 202.60	-
Novel polypeptide	50	115 $\pm$ 21.48	89.87***
	25	468 $\pm$ 65.96	58.90***
HMMW	50	118 $\pm$ 26.16	89.60***

Table 60. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on

metastasis of human liver cancer Hep3B cells

Group	Dose ($\mu\text{mol/L}$)	Number of cell metastases	Inhibition (%)
None	0	1238 $\pm$ 83.12	-
Novel polypeptide	50	50 $\pm$ 28.14	89.65***
	25	583 $\pm$ 75.82	48.90***
HMMW	50	70 $\pm$ 75.82	88.13***

Table 61. Inhibitory effects of novel polypeptide HMMW15-51 and micropeptide HMMW on metastasis of human lung cancer A549 cells

Group	Dose ($\mu\text{mol/L}$)	Number of cell metastases	Inhibition (%)
None	0	623 $\pm$ 29.92	-
Novel polypeptide	50	64 $\pm$ 32.44	89.62***
	25	43 $\pm$ 19.22	92.97***
HMMW	50	33 $\pm$ 20.50	94.57***

According to Tables 57-61 and FIGS. 5-9, compared with the blank control, the novel polypeptide HMMW15-51 has significant inhibitory effects on metastasis of human melanoma, cervical cancer, ovarian cancer, liver cancer, and lung cancer cells, indicating that the novel polypeptide HMMW15-51 may be developed into anti-tumor drugs by inhibiting metastasis of the malignant tumor cells.

Example 7

This example provides detection of in vivo effects of the novel polypeptide HMMW15-51 (SEQ ID NO.21) and the micropeptide HMMW (SEQ ID NO.1) on a subcutaneous transplanted tumor model of tongue squamous cell carcinoma cells CAL27.

(1) A large number of human tongue squamous cell carcinoma CAL27 cells were cultured and digested with a 0.25% trypsin solution. After digestion was terminated, a cell suspension was centrifuged at 1000 rpm for 5 min, and the cells were resuspended in a serum-free DMEM culture medium and counted. The cell concentration was adjusted to 5×10^7 cells/mL.

(2) BALB/c nude mice (4-6 week old, female, weighing 14-16 g, adaptively fed in an SPF animal feeding room for 1 week) were inoculated with 100 μL of cell suspensions of corresponding groups under the left axilla, with an injection volume of 5×10^6 cells/mouse;

(3) After inoculation, the tumor growth at the inoculation sites of the nude mice was closely

observed. On the 7th day after inoculation, white nodules appeared at the inoculation sites and could move subcutaneously upon touch. As the tumor tissues grew, hard tumor blocks gradually formed at the inoculation sites, and the average volume of the tumor tissues reached 80 mm³ in about 14 days. The BALB/c nude mice were randomly divided into 3 groups (a normal saline group served as a blank control, and the dose of the micropeptide HMMW and the novel polypeptide HMMW15-51 was 2.7 μM/kg), with 10 mice in the blank group and 6 mice in each other group. At the beginning of administration, the animal weight was about 20 g.

(4) The volume of the transplanted tumor was measured and recorded every three days. The formula for calculating the tumor volume (TV) is as follows:

Tumor volume = $0.5 \times a \times b^2$, where *a* is the length (mm) of the transplanted tumor and *b* is the width (mm) of the transplanted tumor.

(5) After drug administration, the nude mice were euthanized, and the tumor blocks were removed and weighed to calculate the tumor growth inhibition by a calculation formula as follows:

Tumor growth inhibition = $(1 - T/C) \times 100\%$, where *T* is the average tumor weight of the treatment group, and *C* is the average tumor weight of the control group.

Data was presented as mean ± SD, and GraphPad Prism 5.0 statistical software was used. A *t*-test was used for comparison between two groups, while One-way Anova was used for comparison among a plurality of groups. **** represents $P < 0.0001$.

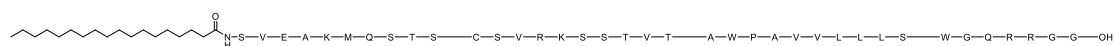
As shown in FIG. 10, compared with the normal saline control group, the micropeptide HMMW and the novel polypeptide HMMW15-51 can significantly inhibit the *in vivo* tumorigenicity of the human tongue squamous cell carcinoma CAL27 cells to varying degrees, and the activity of the HMMW15-51 is not significantly different from that of the HMMW.

Example 8

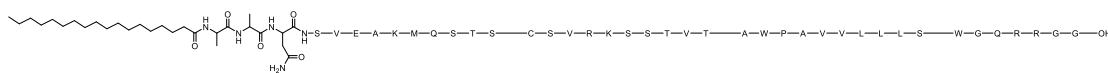
This example provides detection of inhibitory effects of the novel polypeptide HMMW15-51 (SEQ ID NO.21) obtained by long-acting modification on proliferation of different tumor cells.

In this example, the long-acting modification comprises N-terminus linkage with an 18-carbon fatty acid, namely a peptide C18-HMMW15-51 obtained by modification, and linkage with an 18-carbon fatty acid using a small peptide Ala-Ala-Asn as a linker, namely C18-L-HMMW15-51.

The structure of the C18-HMMW15-51 is:



The structure of the C18-L-HMMW15-51 is:



The C18-HMMW15-51 and the C18-L-HMMW15-51 can be coupled with amino acids by solid-phase synthesis. If a fatty acid needs to be coupled, the fatty acid can be coupled to a peptide resin according to an amino acid synthesis scheme (solid-phase synthesis) to obtain a crude product. The crude product is cleaved, subjected to preparative high-performance liquid chromatography, and freeze-dried to obtain a target compound.

When human tongue squamous cell carcinoma cells CAL27, ovarian cancer cells SKOV3, and liver cancer cells Hep3B were cultured to a density of 90% in a 37°C, 5% CO₂ incubator, the cells were digested with trypsin and collected, resuspended in a culture solution, and counted under a microscope. The cell concentration was adjusted to approximately 1.0×10⁵ cells/mL, and a cell suspension was seeded into a 96-well plate with 100 μL per well, and cultured overnight in a 37°C, 5% CO₂ incubator. After the cells fully adhered to the wall, 100 μL of the HMMW15-51, C18-HMMW15-51, and C18-L-HMMW15-51 having a concentration of 100 μM were added separately as a treatment group; and a culture solution without any drugs was used as a negative control group. After incubation was continued for 48 h, 10 μL of CCK8 was added per well in a 96-well plate for dissolution. Incubation was continued for 4 h in a cell incubator, an absorbance was measured at 450 nm, and a proliferation inhibition was calculated. Data was presented as mean±SD, and GraphPad Prism 5.0 statistical software was used. A *t*-test was used for comparison between two groups, while One-way Anova was used for comparison among a plurality of groups.

As shown in FIGS. 11-13, **** represents $P < 0.0001$, and the inhibitory effects of the novel polypeptide HMMW15-51 (SEQ ID NO.21), the peptide C18-HMMW15-51 obtained by modification, and the C18-L-HMMW15-51 obtained by modification on proliferation of different tumor cells have no significant difference.

Example 9

This example provides detection of in vivo effects of the novel polypeptides HMMW15-51 (SEQ ID NO.21), C18-HMMW15-51, and C18-L-HMMW15-51 on a subcutaneous transplanted tumor model of tongue squamous cell carcinoma cells CAL27.

(1) A large number of human tongue squamous cell carcinoma cells CAL27 were cultured and digested with a 0.25% trypsin solution. After digestion was terminated, a cell suspension was centrifuged at 1000 rpm for 5 min, and the cells were resuspended in a serum-free DMEM culture medium and counted. The cell concentration was adjusted to 5×10⁷ cells/mL.

(2) BALB/c nude mice (4-6 week old, female, weighing 14-16 g, adaptively fed in an SPF animal feeding room for 1 week) were inoculated with 100 μ L of cell suspensions of corresponding groups under the left axilla, with an injection volume of 5×10^6 cells/mouse;

(3) After inoculation, the tumor growth at the inoculation sites of the nude mice was closely observed. On the 7th day after inoculation, white nodules appeared at the inoculation sites and could move subcutaneously upon touch. As the tumor tissues grew, hard tumor blocks gradually formed at the inoculation sites, and the average volume of the tumor tissues reached 80 mm³ in about 14 days. The BALB/c nude mice were randomly divided into 4 groups: a normal saline group serving as a blank control, a micropeptide HMMW15-51 group (intravenously injected every day), a C18-HMMW15-51 group (subcutaneously injected on alternate days), and a C18-L-HMMW15-51 group (subcutaneously injected on alternate days), and the dose was 2.7 μ M/kg, with 10 mice in the blank group and 6 mice in each other group. At the beginning of administration, the animal weight was about 20 g.

(4) The volume of the transplanted tumor was measured and recorded every three days. The formula for calculating the tumor volume (TV) is as follows:

Tumor volume = $0.5 \times a \times b^2$, where *a* is the length (mm) of the transplanted tumor and *b* is the width (mm) of the transplanted tumor.

(5) After drug administration for 21 days, the nude mice were euthanized, and the tumor blocks were removed and weighed to calculate the tumor growth inhibition by a calculation formula as follows:

Tumor growth inhibition = $(1 - T/C) \times 100\%$, where *T* is the average tumor weight of the treatment group, and *C* is the average tumor weight of the control group.

Data was presented as mean $\pm$ SD, and GraphPad Prism 5.0 statistical software was used. A *t*-test was used for comparison between two groups, while One-way Anova was used for comparison among a plurality of groups.

As shown in FIG. 14, * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$, compared with the normal saline control group, the micropeptide HMMW15-51 group (intravenously injected every day), the C18-HMMW15-51 group (subcutaneously injected on alternate days), and the C18-L-HMMW15-51 group (subcutaneously injected on alternate days) can significantly inhibit the in vivo tumorigenicity of the human tongue squamous cell carcinoma cells CAL27 to varying degrees, and compared with the HMMW15-51, the C18-L-HMMW15-51 has better inhibitory effects upon reduction in the number of administration times and optimization of an administration route.

CLAIMS

1. Use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors, wherein the novel polypeptide is selected from any one of the following:

(1) a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1;

(2) a polypeptide with the same or substantially the same function formed by deleting 1 to 34 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1; and

(3) a polypeptide with the same or substantially the same function formed by substituting one or more amino acids of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1, or (1), or (2).

2. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 1, wherein

in said (1), a polypeptide with the same or substantially the same function formed by deleting 3 consecutive amino acids at positions 3N-2, 3N-1, and 3N from a polypeptide having the amino acid sequence set forth in SEQ ID NO.1, wherein N=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17; or

in said (2), a polypeptide with the same or substantially the same function formed by deleting 4, 9, 14, 19, 24, 29, or 34 amino acids at N-terminus of a polypeptide having the amino acid sequence set forth in SEQ ID NO.1; or

in said (3), the amino acid at position 2, 3, 8, 9, 10, 11, 12, 13, 17, 19, 20, 21, 22, 25, 27, 28, 30, 31, 32, 40, 41, 42, 43, 44, 45, 48, 49, or 50 in the amino acid sequence set forth in SEQ ID NO.1 is substituted.

3. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 2, wherein the amino acid sequence of the novel polypeptide is shown as any one of SEQ ID NO.2 to SEQ ID NO.53.

4. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 3, wherein the amino acid

sequence of the novel polypeptide is shown as any one of SEQ ID NO.2 to SEQ ID NO.4, SEQ ID NO.6 to SEQ ID NO.10, SEQ ID NO.13, SEQ ID NO.15 to SEQ ID NO.17, SEQ ID NO.19 to SEQ ID NO.21, SEQ ID NO.26 to SEQ ID NO.51, and SEQ ID NO.53.

5. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to any one of claims 1-4, wherein the derivative comprises a derivative with the same or substantially the same function of the novel polypeptide obtained by modification.

6. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 5, wherein the modification comprises one or more of N-terminus modification, C-terminus modification, side-chain modification, amino acid modification, or backbone modification.

7. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 6, wherein the modification comprises one or more long-acting modification of polyethylene glycol modification, fatty acid linkage, fusion with Fc polypeptide, binding with human serum albumin, non-natural amino acid substitution, polypeptide cyclization, or high-end formulations.

8. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 7, wherein the modification comprises fatty acid linkage, wherein the fatty acid linkage comprises N-terminus linkage with a 12- to 20-carbon fatty acid or linkage with a 12- to 20-carbon fatty acid using a linker.

9. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 8, wherein the fatty acid linkage comprises N-terminus linkage with an 18-carbon fatty acid or linkage with an 18-carbon fatty acid using a linker, wherein the linker is a peptide less than 5 amino acids in length.

10. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to any one of claims 1-9, wherein the tumors comprise one or more of glioma, neuroblastoma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer,

uterine cancer, ovarian cancer, colon cancer, small intestine cancer, ileocecal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, sarcoma, myeloma, lymphoma, and leukemia.

11. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 10, wherein the preventing or treating tumors comprises inhibition of proliferation and/or metastasis of tumor cells.

12. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 11, wherein the preventing or treating tumors comprises inhibition of proliferation of tumor cells, wherein the tumors and cells comprise:

wherein the cancer cells of the glioblastoma comprises: U87-MG;

wherein the cancer cells of the neuroblastoma comprises: SH-SY5Y;

wherein the cancer cells of the head and neck cancer comprises: any one of oral epidermoid carcinoma cell KB, laryngeal epidermoid carcinoma cell Hep2, tongue squamous cell carcinoma cell CAL27, and nasopharyngeal carcinoma cell CNE-2Z;

wherein the cancer cells of the esophageal cancer comprises: Eca-109 and/or TE-13;

wherein the cancer cells of the thyroid cancer comprises: NPPA87-1;

wherein the cancer cells of the lung cancer comprises: any one or combination of 95-D, SK-MES-1, SPC-A1, and A549;

wherein the cancer cells of the liver cancer comprises: HCCLM3 and/or HepG2;

wherein the cancer cells of the kidney cancer comprises: Ketr-3 and/or 786-O;

wherein the cancer cells of the breast cancer comprises: SK-BR-3 and/or MDA-MB-231;

wherein the cancer cells of the cervical cancer comprises: CaSki and/or Hela;

wherein the cancer cells of the uterine cancer comprises: RL-952 and/or MS751;

wherein the cancer cells of the ovarian cancer comprises: ES-2 and/or SKOV3;

wherein the cancer cells of the colon cancer comprises: HT29 and/or SW480;

wherein the cancer cells of the small intestine cancer comprises: HIC;

wherein the cancer cells of the ileocecal cancer comprises: HCT-8;

wherein the cancer cells of the gastric cancer comprises: MGC-803 and/or BGC-823;

wherein the cancer cells of the bladder cancer comprises: T24 and/or EJ;

wherein the cancer cells of the pancreatic cancer comprises: ASPC-1 and/or PANC-1;

wherein the cancer cells of the prostate cancer comprises: 22RV1 and/or PC-3;

wherein the cancer cells of the cholangiocarcinoma comprises: QBC939;
wherein the cancer cells of the melanoma comprises: SK-mel-2 and/or A375;
wherein the cancer cells of the sarcoma comprises: MG63 and/or HT-1080;
wherein the cancer cells of the myeloma comprises: LP-1;
wherein the cancer cells of the lymphoma comprises: any one or combination of Raji, RPMI 8226, U937, and WSU-DLCL2; and
wherein the cancer cells of the leukemia comprises: any one or combination of CEM/C1, NB4, KG-1, K562, HL60, Jurkat, and Clone E6-1.

13. The use of a novel polypeptide or a derivative thereof in the preparation of a medicament for preventing or treating tumors according to claim 11, wherein the preventing or treating tumors comprises inhibiting metastasis of tumor cells, wherein the tumors and cells comprise:

wherein the cancer cells of the melanoma comprises: A375;
wherein the cancer cells of the cervical cancer comprises: Hela;
wherein the cancer cells of the ovarian cancer comprises: Skov3;
wherein the cancer cells of the liver cancer comprises: Hep3B; and
wherein the cancer cells of the lung cancer comprises: A549.

14. A pharmaceutical composition for preventing or treating tumors, wherein the pharmaceutical composition comprises at least the novel polypeptide or the derivative thereof according to any one of claims 1-9, and a pharmaceutically acceptable carrier, wherein the tumors comprise one or more of glioma, neuroblastoma, head and neck cancer, esophageal cancer, thyroid cancer, lung cancer, liver cancer, kidney cancer, breast cancer, cervical cancer, uterine cancer, ovarian cancer, colon cancer, small intestine cancer, ileocecal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, cholangiocarcinoma, melanoma, sarcoma, myeloma, lymphoma, and leukemia.