REVIEW

From amino acid sequence to bioactivity: The biomedical potential of antitumor peptides

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Abstract: Chemoprevention is the use of natural and/or synthetic substances to block, reverse, or retard the process of carcinogenesis. In this field, the use of antitumor peptides is of interest as, (i) these molecules are small in size, (ii) they show good cell diffusion and permeability, (iii) they affect one or more specific molecular pathways involved in carcinogenesis, and (iv) they are not usually genotoxic. We have checked the Web of Science Database (23/11/2015) in order to collect papers reporting on bioactive peptide (1691 registers), which was further filtered searching terms such as "antiproliferative," "antitumoral," or "apoptosis" among others. Works reporting the amino acid sequence of an antiproliferative peptide were kept (60 registers), and this was complemented with the peptides included in CancerPPD, an extensive resource for antiproliferative peptides and proteins. Peptides were grouped according to one of the following mechanism of action: inhibition of cell migration, inhibition of tumor angiogenesis, antioxidative mechanisms, inhibition of gene transcription/cell proliferation, induction of apoptosis, disorganization of tubulin structure, cytotoxicity, or unknown mechanisms. The main mechanisms of action of those antiproliferative peptides with known amino acid sequences are presented and finally, their potential clinical usefulness and future challenges on their application is discussed.

Keywords: bioactive peptide; antitumoral; antiproliferative; cancer; tumor

Additional Supporting Information may be found in the online version of this article.

Aitor Blanco-Míguez and Alberto Gutiérrez-Jácome contributed equally to this work.

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Introduction

According to the American Cancer Society, cancer accounts for one in every eight deaths worldwide; this means a mortality rate higher than the combination of fatalities resulting from HIV/AIDS, tuberculosis, and malaria (http://www.cancer.org). Cancer is caused by one or more cumulated environmental or genetic changes promoting normal cells to grow and develop uncontrolledly, which end in the development of a solid mass, denominated tumor. This tumor will first spread into the surrounding tissues and, if not treated, it will finally spread to other parts of the human body using the lymphatic system and the bloodstream.

The main difference between a cancer cell and a normal cell is that the former loses the so-called self-destructing signals, growing and proliferating infinitely and in a disorganized way. A cancer cell does not know when it has to trigger apoptosis, mainly because they do not respond to the chemical signals of surrounding cells.

Mutations in genes are ultimately responsible for the beginning of cancer. Genes that may be affected or mutated during cancer include (i) oncogenes, responsible for the promotion of cell growth and division, (ii) tumor suppressor genes, which are in charge of halting cell division, and (iii) DNA repair genes that fix mutations in damaged genes. Specific genes are involved in the development of a certain cancer type, such as DNA repair genes, which are usually damaged in bowel cancer.[1] According to the GLOBOCAN project (http://globocan.iarc.fr/Default.aspx), the most frequent cancers (ordered by decreasing mortality rate) worldwide are lung, liver, stomach, bowel, breast, esophagus, pancreas, prostate, cervix, and leukemia, and prevention appears to be the most powerful tool in decreasing cancer mortality.[2]

The aim of this review was to supply the research community with a list of peptide sequences linked to antitumor bioactivity, as defined and shown in scientific publications. The data compiled in this work concerns ribosomal peptides, which have not been modified, or present minor post-translational modifications. Antitumor activity of peptide-like compounds from marine origin, are covered in the works of Zheng *et al*.[3] and the review of Suarez-Jimenez *et al*.[4]

Methodological Approach

To collect the empiric data of peptides with antitumor activity, as well as the molecular mechanism of action, we conducted an extensive search of the Web of Science Database on 23/11/2015. First of all, we retrieved all the records containing the terms "peptide and bioactivity" in the combined title/keywords/abstract search fields. Initially, this led to the selection of about 1691 records, spanning the years 1985–2015. This database was separated into three data subsets, and further filtered by three researchers searching for records containing the following terms: "antiproliferative," "anti-proliferative," "antitumor," "anti-tumor," "antitumoral," "anti-tumoral," "apoptosis," "cancer," "or tumor." Only research works reporting the amino acid sequence of the antiproliferative peptide were kept, which left the number of registers in just 60. We also crossed this information with the peptides included in CancerPPD.[5] To our knowledge, CancerPPD is the sole public database providing curated information on experimentally verified anticancer peptides. After crossing the data with CancerPPD, an additional number of 465 peptides where added, many of them coming from one of the 30 patent data contained in the database. A list of these peptides is provided as Supporting Information, and can be browsed and website (http://crdd.osdd.net/raghava/cancerppd/).

It should be highlighted that not all the selected amino acid sequences had antitumor effects; indeed some of them promote tumor cell growth. Proteins such as survivine, a member of the family of Inhibitor of Apoptosis Proteins (IAPs), promoted cell proliferation and it is overexpressed in many cases in which a tumor develops drug resistance.[6] Secreted phosphoprotein 1, which is expressed in many human cell types, is known to participate in cell survival and in the process of metastasis.[7] Another example is the vasoactive intestinal peptide, which modulates cell proliferation and inhibits apoptosis in ovarian follicles through a mechanism dependent on cyclic AMP.[8] However, even these types of proteins can be manipulated, for instance by modifying specific residues, in order to reverse their effect and induce apoptosis. In addition, proteins promoting tumor growth are a therapeutic target for the development of new drugs that aim to block such activity.

In this review, we have chosen some of the sequences to explain the different mechanisms of action (Table I). Peptides sequences have been included in the Supporting Information Table I.

Definition of antitumor peptides

The research community has devoted significant effort to the investigation of bioactive peptides, which are defined as "naturally occurring or enzymatically generated peptides that exert a physiological effect in the body."[46] As stated in the definition, the enzymatic action is sometimes crucial to bioactive peptide generation, as many of them are encrypted in large amino acid sequences, or released in the gut after host enzymatic digestion. In our review, we have considered a peptide to have antitumor bioactivity based on its ability to decrease, or halt cell proliferation, *in vitro* or *in vivo*.

Table I. *Summary of the Peptides Discussed Throughout This Work*

Peptide	Key points	Molecular mechanism found	Ref
Cilengitide	Cyclic RGD pentapeptide. Superactive α(V)β(3) integrin (1000-fold with respect to linear peptides)	Antiangiogenic	9
-[a]	Synthesis, characterization, and *in vitro* antiangiogenic effect on human umbilical vein endothelial cells	Antiangiogenic	10
Peptides derived from the beta(1) chain of laminin-1	Blocks invasion of basement membranes by tumor cells	Antimetastatic activity	11
AChE-peptide	Inhibition of endocytosis, a key component of the metastatic cascade, in breast cancer cells	Antimetastatic activity	12
HYD1	Composed of D-amino acids. Inhibits adhesion and migration of human prostate tumor cells and blocks the activation of ERK signaling.	Antimetastatic activity	13
-	Isolated from croaker muscle after pepsin, trypsin and alfa-chymotrypsin action. Scavenging of DPPH and other hydroxyl radicals. Inhibition of polyunsaturated fatty acid peroxidation	Antioxidant/cytotoxic activity	4
-	Isolated from croaker muscle after pepsin, trypsin and alfa-chymotrypsin action. Scavenging of DPPH and other hydroxyl radicals. Inhibition of polyunsaturated fatty acid peroxidation	Antioxidant/cytotoxic activity	4
-	Antiproliferative on MCF7 cells. Isolated from tuna dark muscle after papain and protease XXIII action	Anti-proliferative	4
Pigment epithelium-derived factor (PEDF) synthetic peptide (positions 78–102)	Inhibits tumor cell proliferation	Anti-proliferative	14
LyP-1	Peptide showing high specificity for tumor lymphatics. Administered as a self-microemulsifying drug delivery system. Assayed in MDA-MB-231 breast cancer cell lines	Anti-proliferative	15
-	N-terminal acylation of the somatostatin analog RC-160	Anti-proliferative	16
Chimeric peptides consisting on growth hormone releasing peptide linked to somatostatin	High affinity for the somatostatin receptors present in the rat pituitary adenoma cell line GH3	Anti-proliferative, cytotoxic, and GH-inhibitory activities	17
BmK AGAP-SYPU2	Antitumor activity against Ehrlich ascites tumor and S-180 fibrosarcoma models *in vivo*	Antitumoral	18
FIP-fve	Increases interleukin-2 secretion and interferon-γ release from the murine lymphocytes	Antitumoral via immunomodulation	19
Beta Casein Phosphopeptide	Bioactive peptide known for its ability to bind calcium. Apoptosis induction in HT-29 and PC12 cells	Apoptosis	20
Bovine Lactoferricin	Corresponds to lactoferrin fragment 17–41. Apoptosis in Jurkat T-leukemia cells through cell membrane permeabilization	Apoptosis	21
C-terminal flanking peptide of progastrin (CTFP)	Bioactivity of the peptide assayed *in vivo* using colon and stomach tissues in gastrin deficient mice and in two mouse models of cancer	Apoptosis	22
PT	Human IL-6 antagonist	Apoptosis	23
Microcin E492	Ion-forming peptide. Produces release of calcium from intracellular stores inducing apoptosis. *In vitro* effect on HeLa, Jurkat and RJ2.25 cells.	Apoptosis	24,25
N-terminal sequence from Pinellia ternata agglutinin	Mannose-binding lectin	Apoptosis	26
Human IL-24 - RGD	IL-24 is a tumor-suppressor/cytokine, which was combined with the RGD motif, targeting the integrin α(V)β(3) and α(V)β(5) present in tumor vasculature.	Apoptosis	27,28
		Apoptosis	29,30

Table I. *Continued*

Peptide	Key points	Molecular mechanism found	Ref
TNF-related apoptosis-inducing ligand (TRAIL)	Loaded in nanoparticles to extend its biological half-life. Assayed into HCT-116 tumor-bearing BALB/c athymic mice		
p160 peptide	Specific binding to human breast cancer shown in MDA-MB-435 cells	Carrier for chemotherapeutic drugs	31
KLAK peptide	Cationic alpha-helical able to induce cancer cell death by membrane disruption	Cytotoxic	32
Phakellistatin 2	Cyclic octapeptide. Inhibition of the murine P-388 lymphocytic leukemia and human cancer cell lines	Cytotoxic	33
Trunkamide A	Cytotoxic effect on several human cancer cell lines such as HeLa, AGS, and DLD-1	Cytotoxic	4
Patellamide A	Peptide with unique post-translational modifications resulting in potent bioactivity	Cytotoxic	34
Phakellistatin 4-9	Cytostatic activity on mouse leukemia P388 cells	Cytotoxic	35
Phakellistatin 14		Cytotoxic	35
Phakellistatin 10	Cyclic octapeptide. Inhibition of the murine P-388 lymphocytic leukemia and human cancer cell lines	Cytotoxic	33
Phakellistatin 11		Cytotoxic	33
Dolastatin	Cytotoxic effect on several human cancer cell lines such as HeLa, AGS, and DLD-1	Cytotoxic	4
Mollamide B		Cytotoxic	4
Mollamide C		Cytotoxic	4
Human Interleukin-2 (IL-2)	Conjugated to octreoide binds to somatostatin receptors expressed in neoplastic cells, bridging them to immune cells. Assayed in a cytotoxic $CD8^+$ cell line	Enhancing of the cytotoxicity of $CD8^+$ T cells and NK cells	36
Synthetic peptide (RS-83277) derived from human C-reactive protein (CRP)	Increases alveolar macrophage production of tumor necrosis factor alpha (TNF-alpha) and monocyte chemoattractant bioactivity. Administered encapsulated in multi lamellar vesicles	Increases anti-tumor activity of immune cells	37
Pigment epithelium-derived factor (PEDF) synthetic peptide (positions 90–114)	Increases cellular adhesion to collagen type-1. Suppressed the expression of the pro-angiogenic factors vascular endothelial growth factor	Inhibition of tumor angiogenesis	14
Pigment epithelium-derived factor (PEDF) synthetic peptide (positions 40–64)	Decreased Matrigel invasion by tumor cells. Suppressed the expression of the pro-angiogenic factors vascular endothelial growth factor	Inhibition of tumor angiogenesis, antimetastatic	14
Human Endostatin	Endostatin is a fragment of collagen XVIII that acts as an endogenous inhibitor of tumor angiogenesis and tumor growth	Inhibitor of tumor angiogenesis and antiproliferative	38
(PEDF) (aa 387–411)	Inhibits tumor cell adhesion and migration	Inhibits Matrigel invasion	14
Peptide derived from the alfa(5) chain of laminin-1	Binds to the CD44 receptor of B16-F10 melanoma cells via the glycosaminoglycans of CD44	Inhibits tumor cell migration, invasion, and angiogenesis	39
Lan-7m a sandostatin analog	Composed of D-aminoacids; the five central amino acids are cyclic. Strong antitumor effects both *in vitro* and *in vivo*	Inhibits tumor cell proliferation	40
Human Thymosin-alpha 1	Human serum albumin-thymosin alpha 1-fusion protein, produced in Pichia pastoris. Human Thymosin-alpha 1 is indicated in the treatment of several cancers, such as melanoma	Inhibits tumor cell proliferation	41
Lunasin	Antitumoral effect *in vitro* and *in vivo*. Peptide present in soybean and other legumes	Triggers caspase pathway	42
RGD4C	Targets the integrin $\alpha(V)\beta(3)$ and $\alpha(V)\beta(5)$ present in tumor vasculature	Tumor targeting; drug delivery	43
Adrenomedullin	Anti-proliferative effect in animal studies.	Unknown	44
Agglutinin derived peptides	Decrease tumor volumes in Ehrlich's ascites carcinoma and B16 melanoma bearing mice models	Unknown	45

The precise amino acid sequence can be found in the Supporting Information Table I.
[a] No peptide name is provided.

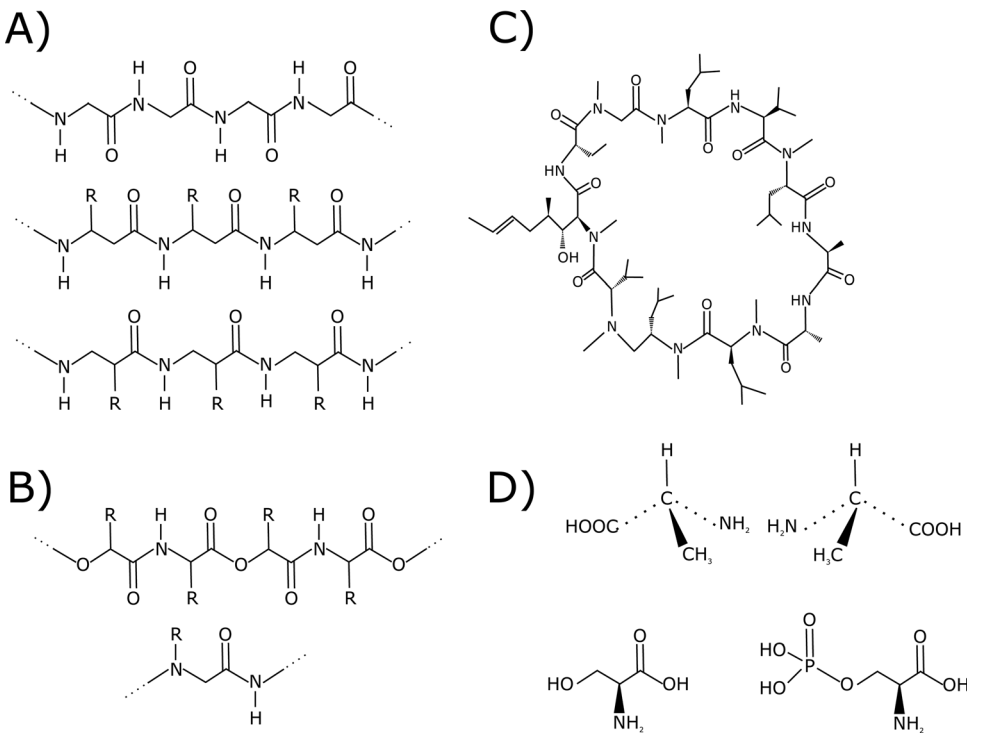

Figure 1. Molecular structure of different types of peptides and peptide-like compounds. A) From the top to the bottom: α-peptide, β^3-peptide, and β^2-peptide, B) depsipeptide and peptoid, C) Cyclic peptide, D) D-versus L-alanine and on the bottom serine versus phosphoserine, a frequent posttranslational modification of the first.

Types of antitumor peptides

Peptides can be generated by different processes such as ribosomal synthesis using the 20 naturally occurring amino acids, non-ribosomal synthesis or enzymatic digestion of larger proteins. In addition, peptides may be linear or cyclic, and contain L-amino acids, D-amino acids, non-natural amino acids, β-amino acids, or a mixture of all of them, which creates an almost infinite catalog of possibilities.

An interesting class of peptide-like compounds is the so-called depsipeptides, in which one or more of the amide bounds are replaced by ester groups (Fig. 1). A well-known cyclodepsipeptide is valinomycin, which depolarizes mitochondria, and, therefore, induces apoptosis after the release of mitochondria compounds to the cytoplasm.[47] Another type of peptide-like molecules are peptoids.[48]

Although many of the peptides are structurally linear, many others are cyclic as a result of the linkage between the N-terminus and the C-terminus of one of the side chains. Naturally occurring, or laboratory synthesized cyclic peptides are of great interest in medicine due to their antitumoral biological activities, as well as for their high resistance to the gastrointestinal digestion.[49]

It is noteworthy that, often, post-translational modifications or conjugation of peptides to other molecules result in better antitumor bioactivity, in better bioavailability, or in improved stability and efficiency, mainly by limiting or avoiding the action of proteases.[16,50]

Sources of antitumor peptides

Almost any protein, or any source of protein, can be used for the isolation or generation of potential antitumor peptides.[51] For instance, bioactive peptides encrypted into large food proteins are normally released into the host by the action of digestive enzymes, and include proteins of vegetable or animal origin, such as milk, soy, eggs, cheese, and so forth.[52,53] Nowadays, the exploration of these food-derived bioactive peptides represents an almost infinite source for researchers interested in discovering new antitumor peptides. Among the compounds present in vegetable-based foods, some peptides have been shown to exert beneficial health effects in the framework of cancer, such as the soy-derived peptide lunasin.[42,54] Particularly interesting is the marine environment, with some of the animals being a source of antitumor peptides and depsipeptides.[4] It is known that several marine protein hydrolysates contain peptides in the range of 2–20 amino acids with antitumor activity. It is quite interesting that these are encrypted peptides, and that their bioactivity is only displayed when they are released from the whole protein; otherwise no effect is exerted.[55] A huge array of small and linear or cyclic peptides, as well as depsipeptides and N-terminal or C-terminal modified peptides coming from diverse marine

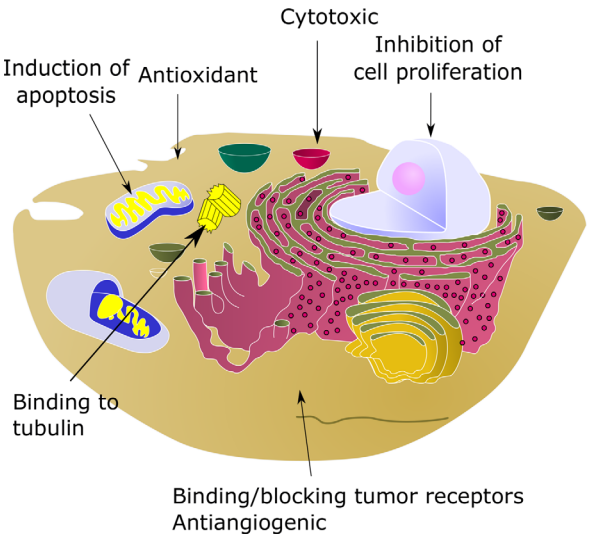

Figure 2. Main molecular mechanism of actions deployed by the antitumor peptides presented in this review.

animals, all with antitumor activity, have been described to date.[3] Other sources of antitumor peptides are human proteins, structural proteins from animals/fungi, industry by-products, or even snake venom. Using peptides rather than the whole protein is a way to improve the delivery of the peptide into the organisms if the bioactivity is kept, as peptides are smaller in size, show better diffusion rates, and can be linked to other molecules such as protectants.[56]

Mechanisms of action

The mechanisms of action and the molecular signalization of many of the antitumor peptides that can be found in scientific literature are partially or completely described. After binding to the target cell, the peptide may elicit different mechanisms, from triggering the apoptotic pathway, to inhibiting the angiogenesis in developing tumors. It is interesting that some peptides can specifically bind to cancer cells, but do not have antitumor effects themselves, making them attractive as selective carriers for other peptides or antitumor drugs. This is the case of the peptide p160, a 12-mer peptide that, both *in vitro* (breast-cancer cell-line MDA-MB-435) and *in vivo* (mice) has been shown to bind to breast-cancer cells and be successfully internalized.[31] Sometimes carrier peptides bind to certain receptors present only on the endothelial cells of the tumor vascular system, rather than cancer cell receptors, constituting effective vehicles for targeting solid tumors. For instance, peptides containing the Arg-Gly-Asp (RGD) motif target the αvβ3 integrin expressed on the endothelial cell surface, as is the case of peptide RGD4C.[43] For this reason, peptides containing the RGD motif are used as carriers for chemotherapeutic drugs/peptides.

We have used a selection of peptides (Table I) to explain the different mechanisms of action, as supported by experimental data. In the case of the peptides not used in this work, and which are listed in Supporting Information Table II, the reader is prompt to http://crdd.osdd.net/raghava/cancerppd/to retrieve additional data.

Inhibition of cell migration

Antitumor peptides inhibiting cell migration might be an important means of targeting tumor metastasis. During cancer development, some cancer cells are able to migrate through the walls of blood/lymphatic vessels, entering the bloodstream and spreading to new body localizations and causing new tumors. This molecular mechanism is used by the synthetic peptide HYD1, composed of D-amino acids, which has been shown to be capable of inhibiting metastatic prostate cell adhesion migration (cell line PC3N).[13] The peptide tridimensional structure is set by three overlapping motifs, each of them necessary to inhibit both cell adhesion and migration. The three motifs are also necessary for the activation of the ERK signaling pathway, triggered after the interaction of HYD1 with laminin-5.[13]

Inhibition of tumor angiogenesis

Angiogenesis is the process where new blood vessels are originated during tumor development, and this step is essential for both tumor growth and further tumoral cells spread throughout the body, in the process denominated metastasis. Tumor angiogenesis involves several signaling molecules and receptors, their inhibitors/ligands are currently being explored as a promising strategy for interfering with cancer progression.[57] There are several peptides with a capacity of inhibiting tumor angiogenesis. Synthetic peptides based on different fragments of the sequence of the pigment epithelium-derived factor (PEDF), which participates in the differentiation of the retinal pigmented epithelium, displayed antiangiogenic and antitumor bioactivities in a human osteosarcoma model.[14] The synthetic amino acid sequence WHLPFKC is a peptide designed for blocking the action of the vascular endothelial growth factor (VEGF), a crucial molecule in the formation of the tumor vascular system that shows high antiangiogenic activity.[10] This peptide is a highly effective αvβ3 integrin inhibitor, that is, an extracellular matrix receptor which is specific for glioblastoma and melanoma cells, showing a bioactivity up to 1000-fold higher than other linear peptides.[9]

Antioxidative mechanisms

Antioxidative peptides fight against the action of reactive oxygen species (ROS), which induce deleterious effects in all biological molecules, including DNA. Continuous exposure to an oxidative environment, or

to oxidative compounds, makes normal cells accumulate genetic mutations, some of them leading to their transformation into a cancer cell.[58] Within this scope, it has been experimentally established that the use of antioxidants prevents not only punctual genetic mutations, but also major chromosomal rearrangements resulting from ROS injuries.[59] Adrenomedullin is a 52 amino acid peptide whose gene is expressed in almost all human tissues.[44] Although its main bioactivity is related to its ability to decrease blood pressure, it also shows anti-proliferative properties on the cardiovascular system through an antioxidative mechanism, which involves a direct decrease on the levels of ROS.[60]

Inhibition of gene transcription/cell proliferation

Stopping uncontrolled division, or inhibiting the expression of certain genes promoting cell proliferation, is yet another molecular mechanism used by certain antitumor peptides. Lunasin is a 43-mer peptide present in the seeds of different plants, most notably it can be isolated from soybean. This peptide acts preventively at different cancer stages, mainly by internalizing inside cells and inhibiting histone acetylation in the nucleus.[54] As histone acetylation is a mechanism involved in chromosome unfolding and gene transcription, the peptide interferes directly with a central pathway of the carcinogenic process. In addition, lunasin has the ability to promote the activation of several genes involved in tumor suppression.[42]

A wide array of linear, cyclic peptide and peptide-like antitumor molecules are isolated from marine animals, in particular from sponges, tunicates, ascidians, and mollusks, such as phakellistatins.[3,4] Another noteworthy example is an encrypted 14-mer peptide in the C-terminal side of the human protein acetylcholinesterase, which is able to inhibit the formation of endocytic membranes, and, therefore, to halt the division *in vitro*.[12]

Induction of apoptosis

On average, human cells reproduce themselves up to 60 times. After this, they die in an ordinated and self-destructing way, denominated apoptosis. This a process which ends with the recycling of the cell without sending signals of inflammation to the surrounding tissues.[61] However, gene abnormalities or molecular damages induced by, for instance, UV radiation or ROS, may trigger apoptosis signals before those 60 divisions, relieving the cell from perpetuating harmful mutations. Apoptotic cells are replaced by new cells differentiated from stem cells. One of the main differences between a cancer cell and a normal cell is that the former loses those self-destructing signals, growing and proliferating infinitely, and in a disorganized way. In addition, cancer cells do not respond to the chemical signals of surrounding cells and do not stick to each other using tight junctions.[62]

Human proteins are again a source of pro-apoptotic peptides. Lan-7, derived from the sandostatin analog TT-232 by substitution of the disulfide bridge with a lanthionine monosulfide bridge, was able to induce the apoptosis of human ovarian carcinoma 2008 cells.[40]

Regarding apoptotic peptides from vegetable origin, two peptides (11- and 15-mer) released by trypsin action from the *Abrus precatorius* agglutinin, a well-known protein with antitumor activity, exhibited an antitumor effect in both B16 melanoma and Ehrlich's ascites tumor models, basically by reducing the size of the lesions (both weight and volume) through a direct killing mechanism (apoptosis is suggested by the authors, although no further evidence on this is provided), and by inducing activation of immune cells.[45]

Apart from being a source of amino acids, carbohydrates, calcium and phosphorus, beta-casein can be partially hydrolyzed with the help of different endopeptidases to produce beta-casein phosphopeptides (beta-CPP). In its dimeric form, a preparation of beta-CPP has been shown to induce apoptosis in several tumor cell lines.[20]

Disorganization of tubulin structure

Some antitumor peptides display their effect after binding to tubulin, the major component of the cellular microtubules. These peptides interfere with chromosome segregation by avoiding the formation of the mitotic spindle, and, therefore, efficiently inhibiting the proliferation.[63] The family of cytotoxic peptides denominated Dolastatins is produced by *Dollabella auricularia*, a mollusk species from the Indian Ocean, and Western and North-Western Pacific Ocean. Dolastins family includes linear and cyclic peptides, as well as synthetic peptides such as D-amino acids.[4] All the members of this family have a pronounced antiproliferative bioactivity against several cancer cell lines, and the mechanism of action seems to rely on mitosis inhibition in the target cell by binding to overlapping domains on the surface of β-tubulin, and, therefore, halting the mitosis process.[64]

Cytotoxicity

Some antitumor peptides are directly toxic for cancer cells, and thus considered cytotoxics. In scientific literature, we can find several examples of cytotoxic peptides isolated from different sources. Peptides isolated from soybean meals, characterized by their resistance to simulated gastrointestinal conditions, displayed high cytotoxic bioactivity against tumor cell lines isolated from the colon, liver and lung.[65] One of the venom proteins from the scorpion *Buthus martensii*, AGAP-SYPU, showed strong antitumor

(cytotoxic) activity against both Ehrlich ascites tumor and S-180 fibrosarcoma models *in vivo*.[18] Finally, a recently discovered family of cytotoxic peptides is the cationic peptides, the so-called "KLAK," which is arranged tridimensionally in amphipathic α-helices and disrupted bilayer membranes.[32] Given that cationic peptides are not efficiently internalized within the cells due to their net charge, they are usually conjugated with cell-penetrating motifs, or to receptor ligands.[32]

Unknown mechanisms

Whereas the effect of several antitumor peptides has been experimentally shown, but the precise molecular mechanism of action remains uncharacterized. This is the case of the mollamides, a family of cyclodepsipeptides isolated from *Didemnum molle*, an Ascidian species inhabiting the Indo-Pacific Oceans.[66] Trunkamides are another family of antitumor peptides structurally analogous to mollamides; the major molecular change is that they contain a thiazoline ring in their cyclic conformation.[4] Interestingly enough, Trunkamide A has already undergone preclinical trials with promising antitumor effects.[66]

Clinical trials

In spite of the amount of data pointing to a potential usefulness of antitumor peptides, and while many of their mechanisms of action are known and described, not so many peptides have undergone the further research needed to provide proof of their clinical usefulness. In fact, most of the clinical trials implying peptides and cancer involved vaccination of the patients with tumor-associated antigens (TAAs). For instance, a cancer vaccine consisting of 20 mixed prostate-derived peptides has concluded its Phase I trial to further study its effectiveness in castration-resistant prostate cancer.[67] In other studies, TAAs were used to condition specific immune cells subsets in order to develop a specific response against a given tumor. For instance, dendritic cells (CD1c) isolated from blood and conditioned with a set of restriction peptides derived from prostate-specific antigen and other prostate cancer related antigens, were safely loaded back into the blood stream, making this strategy suitable for Phase II trials on prostate cancer.[68] Therefore, vaccination or immune cell conditioning with specific TAAs has been the strategy of choice for many of the clinical trials registered in the databases[69] and, to date elpamotide treatment, which is a peptide derived from vascular endothelial growth factor receptor 2, displayed moderate effects in patients with advanced biliary tract cancer, with a response rate of 18.5%. This peptide has thus successfully concluded this Phase II and Phase III trials with promising results in terms of survival.[70,71]

Another safe and suitable peptide for Phase II trials is LY2510924. This is a peptide that blocks the C-X-C motif receptor 4 (CXCR4), expressed during tumor progression.[72] Interestingly, some antitumor peptides have been used in clinical trials due to their ability to act as carriers for other drugs. This was the case of the aforementioned cilengitide, which is a cyclic peptide fused to the RGD motif, and which is currently in clinical phase II for several tumors and in clinical phase III for glioblastoma.[9] Another RGD-fused peptide, (18)F-FPPRGD2 PET/CT, is a radiopharmaceutical peptide that has been shown to be safe for breast cancer visualization by positron emission tomography in a Phase I trial.[73] In this regard another tumor-homing sequence, NGR, was fused to the human tumor necrosis factor (hTNF). This fused peptide was successfully delivered into colon cancer cells, and this strategy has shown promising results for the treatment of treatment-resistant colorectal cancer and in relapsed ovarian cancer patients in Phase I trials.[74,75]

Finally, other clinical strategies involved using radiolabeled peptides which are specific for tumor receptors. Peptide P2045, a somatostatin analog of 11 aa, is able to bind to somatostatin receptors, which are expressed in lung cancer cells. The labeling of P2045 with rhenium 188 has been used in Phase I to deliver 2.1 MeV beta radiotherapy, but no information as to whether this strategy has entered Phase II is available.[76] The same is true for ABT-510, a modified peptide of 9 amino acids that is an analog of thrombospondin-1, a powerful antiangiogenic molecule. Although the peptide was well tolerated during the Phase I trial, no additional information is available on its clinical usefulness.[77]

Conclusions

Currently we have an extensive knowledge on the sequence and molecular mechanism of action of several antitumor peptides, but very few have been applied to the clinical practice. Although there is huge potential for the application of antitumoral peptides in the fight against cancer, only the mechanism of action and sequence of few of them are known. Peptide sources (plants, animals, foods, wastes, etc) remain unexplored so far, and currently there is not a resource allowing calculation of the probability of a given peptide sequence to be potentially antitumoral. In the near future, *in silico* prediction of those peptides coupled to algorithms able to predict their potential bioactivity, and in combination with in *vitro*/*in vivo* validation of their bioactivity, will provide the scientific community with an extensive database of novel bacteria-encrypted antitumor peptides. Finally, and at the light of the clinical trials involving antitumoral peptides, new linking methods and molecules are required in order

to improve their performance and to extend its shelf-life, notably through gastrointestinal/bloodstream passage.

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The authors declare that there are no conflicts of interest.

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