Review Article

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Antimicrobial Peptides and Cancer: Potential Use of Antimicrobial-Like Peptides in Chemotherapy

Mizejewski GJ*

Division of Translational Medicine, Wadsworth Center, New York State Department of Health, Albany, NY, USA

***Correspondence:** Gerald J Mizejewski, Division of Translational Medicine, Molecular Diagnostics Laboratory, Wadsworth Center, New York State Department of Health, PO Box 509, Empire State Plaza, Albany, NY 12201-0509, USA, Telephone: 518 486-5900; Email: gerald.mizejewski@health.ny.gov

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Abstract

Antimicrobial peptides (AMPs) constitute host defense peptides found among insects, fish, amphibians, and mammals including man. The targets of AMPs are gram-negative and gram-positive bacteria, fungi, enveloped viruses, and transformed/cancerous cells. The AMPs are broad spectrum antibiotics which display the propensity to serve as therapeutic agents not only in infectious disease, but also in human cancer. AMPs demonstrate unique properties which include cell membrane penetration, destabilization of biological membranes, ability to form and/or interact with membrane channels, and the capability to modulate host immune responses. The three types of AMPs consists a) naturally-occurring; b) artificially synthesized; and c) cleaved peptide fragments from blood and extracellular matrix proteins. The present treatise presents one such example of an AMP-like peptide derived from a naturally-occurring human protein as a potential candidate for future cancer therapy. The biological activities of human AMP-like peptides as cancer therapeutic agents are reviewed and reported in multiple *in vitro* and *in vivo* cancer assays. The possibility of using such human protein-derived peptides as primary and adjunct cancer therapeutic agents is addressed.

Keywords: Alpha-fetoprotein, Peptides, Antimicrobial, Penetration, Chemotherapy, Phospholipids, Cell membranes, Chemokines

Introduction

Antimicrobial peptides (AMPs) serve as potent, broad spectrum antibiotics found in many eukaryotic animal species [1]. They comprise entities of the host defense system present in multiple classes of life forms and constitute a portion of the innate immune response in organisms [2]. AMPs are known to destroy gram-negative and gram-positive bacteria, fungi, enveloped viruses, and transformed/cancerous cells. The AMPs demonstrate the potential to function as novel cancer therapeutic agents due to their ability to lyse and destabilize biomembranes, interact and/or form transmembrane channels, and engage in modulating host immunity against foreign invasive agents [3]. AMPs are known to act in concert

with chemokine activities, histamine release, and angiogenesis regulation [4]. The objective of the present review is to provide evidence that AMPs could serve as ideal candidates for cancer chemotherapy.

Recent advances in the use of AMPs as anticancer agents offer an alternative treatment option for patients undergoing chemotherapy. The AMPs provide a novel means to lesson development of drug resistance, increase tumor cell killing effectiveness, and lower expensive drug costs [5]. The increased therapeutic use of small cationic AMPs have resulted from recent introductions of new peptide drug discoveries employing bioinformatics for improved ribosomal and amino acid synthesis methods

[6]. Bioinformatics technologies have been employed to modify, synthesize, and recombine existing AMPs for transition into anticancer drugs. Such transition AMP peptides have been shown to display high cell penetrating activity, low toxicity, reduced number of side effects, and increased target specificity [7]. Furthermore, AMPs have now been reported to affect both solid tumors (pancreatic adenocarcinomas) as well as soluble cultured leukemic HL-60 cells [8,9]. Finally, increased anticancer effectiveness of AMPs has been demonstrated by the use of acetylation and N-myristoylation-modification of the AMP structure in order to increase cell membrane penetration and intracellular targeting to mitochondrial membranes (Cytochrome-C; Caspases) of human breast cancer cells including MCF-7, MDA-MB-231, and MX-1 cultured cells [10].

The amino acid composition and structure of AMPs distinguish them from the pore-forming/cell-penetrating peptides (CPPs), which comprise short length peptides of 5-10 amino acids [11]. In contrast, the AMPs usually consist of 10-50 amino acids AA and contain two or more cationic AAs, a large proportion of hydrophobic AAs, and are rich in anionic AAs (For peptide comparisons, [53]). The AMPs contain many dipolar ions (Zwitterions) and constitute amphipathic peptides with secondary structures of; 1) alpha-helices, 2) Beta strands; 3) Beta-hairpin loops; and 4) one or more disulfide bonds [12]. The amphipathicity of these peptides allow them to partition and/or permeabilize into the cell membrane bilayer, while forming and/or interacting with transmembrane channels through electrostatic attraction [13]. The plasma bilayer membrane represents the prime target of AMPs. Following cell internalization, such peptides can interfere with a) DNA, RNA, and protein synthesis; b) protein folding; c) signal transduction, d) cell membrane synthesis, and e) certain enzymes [14]. Due to the peptide-induced cell membrane penetration, permeabilization, and subsequent bilayer disruption, most AMPs eventually cause membrane leakage leading to cell death.

The composition of the bacterial cell membrane lacks cholesterol, but is rich in phospholipids such as phosphatidylglycerol, phosphatidylserine, phosphoglycerol, gangliosides, phosphatidylcholine, and sphingomyelin [15]. The phospholipid head groups within the outermost bilayer leaflets are negatively charged, and these are exposed to the outer surface of bacterial cell membranes [16]. The main driving force between the positive charge of the AMPs and the negatively charged bacterial/cancer cell membranes is

electrostatic attraction [17]. Interestingly, transformed cancer cells have a similar negatively-charged outer cell surface bilayer membrane. Thus, the positive charge of the AMP membranes is attracted to the negative-charged bacterial/cancer cell surface membrane [16]. While AMPs display structures with positive-charged and hydrophobic interfaces, there also occurs hydrophobic interaction between the AMPs and the zwitterionic phospholipids of bacterial and cancer cell membranes.

Properties of cell penetrating microbial peptides

The AMPs induce pores and/or channels in the cell membrane for cell entry; however, they also can recruit chemokines and their receptors (i.e. CCR6) to assist in their function [4]. In fact, the AMPs themselves have been referred to a “mini-chemokine ligands”. In comparison, AMPs consist of 15-40 amino-acids, while chemokines contain up to 90 AAs. The AMP-like chemokines are immunostimulatory and are biologically active at low micromolar (1-50 μ M) concentrations. While forming pores and/or channels, the actual translocation of AMPs through the cell membrane requires only 1 to 5 minutes; whereas, receptor-mediated endocytosis process can require 20 to 45 minutes to complete [18]. The AMPs are not attracted to cells displaying a positive charge as discussed above, thus, AMPs do not attack non-cancer mammalian cells. In contrast, cells displaying a lipid bilayer inversion (sphingomyelin to Phosphatidylserine), as observed in cancer cells, is a prime target for AMP membrane insertion. Furthermore, the ion channels which form and/or interact with AMPs are voltage-dependent being selective for cations and/or anions [19].

The AMPs can further be divided into 2 additional forms; 1) the linear peptides, and 2) the cyclic peptides [18]. The linear AMPs consist of α -helix structures, and special amino acid enriched residues (gly, Pro, Arg, His, Trp). The second group is the cyclic peptides which are determined by their cysteine content, (i.e., a single cysteine residue or multiple cysteines) in which 2 or more can form disulfide bridges. The AMPs are thought to employ two mechanisms of cell penetration which are described below; 1) the carpet saturation and 2) the barrel-stave models [19, 20]. The carpet saturation mode is a concentration-dependent mechanism that requires multiple linear monomer peptide insertions whose accumulations result in cell membrane breakdown. The barrel-stave method involves cyclic peptides which form enlarged openings (pores) lined by multiple copies of the peptides.

Types of antimicrobial peptides

There are three types of AMPs that can be isolated or produced, namely, a) naturally-occurring AMPs; b) laboratory synthesized AMPs; and c) AMPs cleaved as fragments derived from naturally-occurring proteins. Naturally- occurring AMPs are produced by many known species including bacteria, fungi, hydra, insects (mastroparin), shellfish, fish, frogs (magainin), and mammals (defensins) [21]. Artificially synthesized AMPs have been produced to treat various infections such as pneumonia (Bacitracin), Hepatitis-C (Boceprevir), and HIV (Enfuvirtide). AMPs have also been produced from cleaved segments (fragments) of naturally-occurring proteins [20]. An example of an AMP cleaved from a natural protein is the cationic peptide C18G, obtained from the C-terminal domain of human platelet Factor-IV. It is further relevant to this discussion that some naturally-occurring insect AMPs (Cecropins A and B) display anticancer properties and are referred to as “anticancer peptides” and reported as such [20, 21]. In like manner, a microbial-like peptide derived from a segment of naturally-occurring human alpha-fetoprotein (AFP) has been demonstrated to possess anticancer (antigrowth) properties [22]. This is of interest since intact AFP is normally known to be a growth enhancing protein. The AFP-derived peptide segment and its sub-fragments have been collectively termed the “Growth Inhibitory Peptide” (GIP) and its properties, characteristics, and activities are described below (Table 1).

Mechanisms of action of AMPs

The AMPs are able to attach and insert into membrane bilayers due to their AA composition, amphipathicity, size, and cationic charge [18]. As mentioned above, they form pores and/or channels in the bilayer membranes by one of three proposed mechanisms, namely, 1) barrel-stave; 2) carpet, and 3) toroidal-pore. In the barrel-stave model, the AMPs form a barrel shape within the cell membrane in which hydrophobic residues are juxtaposed to lipid chains, and by hydrophobic residues that line the wall of the central pore [20]. In the carpet model, membrane thinning occurs between the negative charged phospholipids and the cationic, amphiphilic AMPs [20,21]. In the toroidal model, lipids bend in such a way that the AMPs are positioned nearest to the phospholipid head groups which shape the pore diameter.

The cell surface charge of the plasma membrane is the main target of the AMPs which act by inducing a change

in cell membrane polarization following peptide insertion [21,22]. Such peptide action results in two events, namely; (1) an increase in cell membrane permeability, and (2) reduction of the transmembrane electrical potential. The peptides perturb cell membrane molecular activity which then affects the fluidity of the membrane bilayer. In a demonstrable laboratory procedure, peptides are incubated in trifluoroethanol (TFE) which simulates a lipid bilayer environment and mimics a lipid bilayer structure in which a peptide can traverse the cell membrane bilayer; the TFE together with the “staged” lipid bilayers induces an increase in the alpha-helix content of the peptide which can be measured by circular dichroism [22]. The alpha-helix configuration induces the peptide to corkscrew itself into the cell membrane. However, it is the lipid inversion (flip) between the phosphoglycerol and phosphatidylserine head groups that cause the actual change in the cancer cell surface charge [23]. Linear AMPs form a pore by a 2-step mechanism: 1) the peptide monomer inserts into the membrane as a “pin prick”; 2) the peptide then undergoes self-aggregation (oligomerization) which widens the pore and/or channel. Increasing the salt concentration (osmotic effect) reduces the binding of peptide to the cell surface and the subsequent leakage induced by the peptide [24]. The shorter truncated linear peptides display less pore-forming activity than longer (linear) ones; at least 20 amino acids being optimal for the pore-forming process. Thus, amphipathic AMPs bore a hole, reduce the membrane electrical potential, and cause thinning of the lipid bilayer (carpet model). More specifically, the peptides dissipate the electrical potential across the cell membrane, allowing ion leakage which lowers the proton gradient, thus reducing the membrane potential [25]. The AMPs have an advantage in bypassing the multi-drug resistance pathways which is observed in some cancer chemotherapies.

The Antimicrobial-like “Growth Inhibitory Peptide” (GIP)

The GIP Fragment of AFP, discussed above, is a 34 amino acid (AA) peptide which was identified, designed, and synthesized as a segment from AAs # 464 to 498 of the Human AFP polypeptide chain [26, 27]. The containment of a class of growth regulatory and modulating peptides residing within a polypeptide chain as seen in many circulating blood and extra-cellular matrix proteins appears to be a commonplace occurrence in nature [28]. Once discovered and the AA sequence identified, peptide segments from large proteins can be isolated using directed proteolytic cleavage of the

Table 1: Antimicrobial and Antimicrobial-like Peptides are Listed and Compared According to Their Biochemical and Biophysical Characteristics,Traits,and Properties. Note the similarity of properties.

Characteristics,Traits,Properties	Antimicrobial Peptides (AMP)	AFP-Derived Growth Inhibitory Peptide (GIP 38)	Reference Citations
1)Cell membrane penetration effects	Forms transmembrane Pores and/or channels, reduces the cell membrane potential	Stabilizes cell membrane and interacts potentially with transmembrane channels	[2,3,9,16]
2) Cell method of internalization	Transmembrane channel passage,channel receptor endocytosis	Interacts with membrane channels,non-receptor endocytosis mechanism	[9,15,20]
3) Cell-specific targeting	Microbial cell membrane,plasma membrane of vertebrate (mammals),transformed cancer cells.	Plasma bilayer cell membrane; transformed cancer cells,and bacterial membrane	[4,8,10]
4) Cargo delivery vehicles	Mostly small cargo delivery capability,binds metals,fuses with peptides and proteins	Transmembrane passage of small ligands,binds metals,protein/peptide fusion	[9,12,13]
5) Cell toxicity	Cytostatic and/or cytolytic	Cytostatic only	[4,7,14,15]
6) Amino acid (AA) composition	Largely amphipathic,- contains some positive and negative charged AAs and hydrophobic AAs	Amphipathic form containing positive,negatively charged,and hydrophobic AAs	[10,17,18]
7) Number of AAs in length	12-50 AA	8-34 AAs	[6,7,16]
8) Peptide secondary structure forms	Displays some alpha-helix,Beta sheets,and Beta Hairpin loops	Displays alpha-helix,Beta sheets, β -hairpin loops and disordered structure	[6,7,16,17]
9) Effect on Host Immunity,innate immunity	Promotes and enhances the innate immune response of host organism,initiates Immunomodulation	Enhances the immune response to lectins and serves as MHC antigens for HLA-1 type receptors	[15,16,24]
10) Angiogenic responsiveness	Modulates angiogenesis	Blocks/inhibits angiogenesis	[3,4,21,28]
11) Cytokine chemokine interaction	Induces cytokine/chemokine production pro-inflammatory	Synergistic with cytokine/chemokine receptor activities	[1-3,26,28]
12) Examples of peptides in nature and/or synthesized,and fragments from natural proteins	a) Amphibian-H5, b) human dermcidin c) human defensins, d) Cecropins from insects, e) Magainin and bombisinins from amphibians f) Indolicidin from (Cows) g) prophenin from pigs h) Horseshac crabs	a) (C18G) C-terminal domain of Human Platelet Factor IV b) Hybrid Ceciopin A & B, c) GIP-34 d) GIP-12 e) GIP-14 f) GIP-8	[1,2,9,18,20, 24-31]
AFP = Alpha-fetoprotein,AA = amino acids			

native protein or can be artificially synthesized. The 34 AA GIP has been synthesized as a free peptide segment and subsequently isolated, purified, and biochemically characterized; its biological activities were then determined [29-33]. Although active as a 34 AA segment, the three sub-fragments of GIP-34 have been found to possess individually distinct biologic activities. The three contiguous bioactive GIP sub-fragments consist of; a) a 12 AA amino-terminal segment (GIP-12); b) a middle piece of 14 AAs (GIP-14); and c) a carboxy terminal segment of 8 AAs (GIP-8) called AFPep [34-36]. However, it is the intact GIP-34 fragment and the GIP-8 segment that show the greatest growth inhibitory properties as observed in cancer cells [32-35]. While the GIP-8 appears to inhibit growth largely in estrogen (E) dependent cancers, the GIP-34 was found to inhibit growth in both E-dependent and non-E dependent (basal) cancer growth. The midpiece (GIP-14) can interact and bind with transcription factors, dyes, and cell cycle-associated proteins [33-35]. The mechanism of action of the GIP-34 and GIP-8 fragments have been determined and reported; it is both the GIP-34 and the GIP-8 peptides that are associated with cell membrane penetration, disruption, and subsequent cell growth inhibition [34-36].

GIP-34 is a synthetic amphipathic peptide with an isoelectric point of pH 4.7; it displays a secondary structure consisting of 45% beta sheets and turns, 45% random coil (disordered), and 10% alpha-helix [30, 31]. In contrast, the GIP-8 peptide (both linear and cyclic) is largely of a disordered structure [36]. The GIP-34 and GIP-8 both contain a carboxyterminal type-1 reverse beta-turn structure which serves to enhance cell surface membrane attraction and attachment [37]. Both peptides are cell membrane disrupters capable of bypassing receptor mediated endocytosis and undergoing rapid cell internalization. Following transmembrane passage, the two peptides each become diffusely scattered throughout the cytoplasm within 1.0 hour and by 2.0 hours are localized in the perinuclear region of the cell, an area contiguous with the endoplasmic reticulum [28,30]. The GIP-34 segment is bioactive both as a linear as well as a cyclized peptide [38,39]. The linear GIP-34 peptide was reported to display both dimer and trimer peptide oligomers, while cyclic GIP-34 behaved as a dimer [40].

Bioactivities of the AFP-derived AMP-like peptides

AFP-derived GIPs display other biological activities such as estrogen-associated cytoskeletal interaction

and prevention of birth defects in animal models [41-45]. Plasma membrane electrical activities in both GIP-34 and GIP-8 fragments have been demonstrated by means of electrophysiological procedures using a) Sharps microelectrodes, and b) patch-clamp whole cell recordings in MCF-7 cell cultures [36,41]. *In vivo* recordings indicated that GIP-34 at 10^{-6} M and lower concentration could serve as cell membrane pore forming/channel-interacting peptide coincident with a decreased cell membrane resistance. At higher peptide concentrations (10^{-5} M and greater) GIP-34 was capable of membrane penetration while stabilizing the cell membrane potential at -30 to -45 mVolts [42]. GIP-34 could also act as a channel-interacting (or blocking) peptide coincident with increased cell membrane resistance [42,43]. Both channel-interactive GIP-34 and GIP-8 peptides were capable of interfacing with a cluster of signaling proteins and enzymes (termed a signalplex) located on the inner leaflet side of the cell membrane bilayer [44,45]. Thus, AFP-derived peptides were found to gain entrance into cells via a) cell membrane bilayer penetration and/or b) channel interacting (blocking) modes. A subsequent global RNA microarray analysis of GIP administered to MCF-7 breast cancer (BC) cell cultures confirmed that GIP-34 was capable of down-regulating the RNA of multiple outward flux cation channel proteins resulting in cation channel membrane polarization (See Ref.[34] for ion channel types affected).

The cytoplasmic ripple effect of membrane ion channel polarization via GIP has been reported to affect and halt S-phase cell cycle progression together with growth inhibition in MCF-7 human breast cancer cells [45,46]. The above-mentioned RNA microarray data revealed that treatment of the MCF-7 cells with GIP-34 for 3 days resulted in RNA-downregulation of multiple cell cycle proteins such as Cyclin-E, SKP2, and associated transition checkpoint proteins which prevented Cyclin-E/Cdc2 induced G1- to -S-phase progression [37,41]. GIP-fragments concomitantly blocked ubiquitin-induced degradation of cyclin inhibitors such as p27 KIP and p21 CIP resulting in cell cycle arrest and subsequent mitotic cell growth inhibition. While the labile linear version of GIP-34 forms trimers which are growth inhibitory at 10^{-4} to 10^{-6} molar concentration, the cyclic form (which behaved like a dimer) was inhibitory at 10^{-7} to 10^{-10} molar concentrations [36]. Moreover, both forms were cytostatic without any cytotoxic side effects in xenograft mouse models *in vivo*.

Other biological activities of the AFP-derived peptides

involved cancer cell adhesion studies concerning GIP-34 attachment to a variety of extra-cellular matrix (ECM) proteins linked to metastasis [44,46]. The ECM proteins serve as basement membrane and cell anchor constituents; such proteins include fibronectin, vitronectin, collagen, thrombospondin, fibrinogen, and laminin. The 34-mer peptide was capable of interfering with the attachment of cancer cells to ECM proteins thus preventing cell anchoring. Furthermore, GIP-34 was able to block 90 to 95% of all stages of the platelet aggregation reaction [36,45]. This is noteworthy since intravascular platelet aggregation provides an attachment platform for cancer cell circulating through the bloodstream; this event serves to aid metastatic cell migration through the blood circulation. GIP-34 was also demonstrated to inhibit 95% of new blood vessel formation (angiogenesis) as shown in both chick embryo and Chorioallantoic membrane-based cancer cell assays [36]. Inhibition of tumor angiogenesis denies the formation of new blood vessels that transport oxygen and nutrients to newly developing tumor cell foci; thus, inhibition of angiogenesis prevents further cancer cell growth and subsequent metastasis.

The disruption of cancer cell signaling activities has been shown to disturb, impair, and disable the ability of tumor cells to transduce downstream signals to spread, adhere, and metastasize. GIP-34 has been described as a cell membrane perturbation agent, capable of disrupting tumor cell adhesion, membrane pseudopodial extensions, platelet cell shape, aggregation, and cell agglutination activities [36]. Thus, AMP-like peptides exemplified by GIP-34 might have the potential to serve as chemotherapy agents alone or as adjunct chemotherapeutic agents.

Antimicrobial-like peptides and the immune response

Antimicrobial peptides are quite effective as immune response enhancing agents. Naturally-occurring AMPs are known to attack multiple infectious agents and cancer cells. Thus, AMPs can modulate host immunity against foreign invading agents and modulate the immune response through the cytokine system especially chemokines and their cognate receptors [2, 15]. It follows that AMP-like peptides (i. e. GIP-34) could qualify as immunomodulatory agents as a part of their functional activity repertoire. Concerning the immune response, GIP-34 has been reported to produce an enhancement of the cellular immune response when tested in Concanavalin-A stimulated blast transformation assays employing T- and B- lymphocytes *in vitro* [45]. Using

tritiated thymidine incorporation, GIP-34 was reported to enhance the lectin-induced blast transformation process and did not suppress or block the cell-mediated immune response. In another report, both the middle and terminal AA fragments of GIP have been reported shown to serve as antigenic epitopes for the activation of dendritic (antigen-presenting) cells and T-lymphocytes [46,47]. The entire amino acid sequence of the AFP polypeptide (each being 9-10 residues in length) was individually screened by lymphocyte chemokine assays in which major histocompatibility complex antigenic sites of the HLA-A epitopes were involved; two of the GIP-34 epitotypes were identified in this screen [47]. The GIP epitopes reacted with HLA-A/GIP primed tumor cells in cytotoxicity and interferon/cytokine production and secretion assays. Therefore, it appears that GIP-derived peptide epitopes might be capable of enhancing the immune response in immune compromised patients.

AMP-like Peptide suppression of cancer growth

In pre-clinical studies of *in vitro* and *in vivo* models, AFP-derived peptide (GIP) fragments have been demonstrated to cytostatically suppress the growth of multiple tumor cell types [48]. GIP-34 was shown to suppress the *in vitro* growth of T-cell lymphomas, prostate, lung, hepatomas, and ductal and glandular human breast and ovarian carcinomas. The GIP-34 segment was found to induce more cancer growth suppression than did tamoxifen alone in comparison cell cultures assays [31]. It was shown in another report that I-¹²⁵ labeled GIP-34 could localize and distribute into rodent mammary tumors *in vivo* by 24 hours post IV-injection [31]. In a further report, fluorescent-labeled GIP-34 nanobeads were found to bind the surface of MCF-7 cultured cells and were internalized into the tumor cell cytoplasm. Finally, a follow-up study revealed that neither GIP-34 nor GIP-8 bound to the tumor cell surface receptor described by Canadian investigators, rather, GIP-34 was found to enter MCF-7 cells by cell membrane penetration in various assays [36,45]. Because AMP-like peptides can penetrate the plasma membrane, a receptor-mediated endocytosis process was not reported for GIP transmembrane passage [45]. However, a recent computer-program modeling system suggested that other AA segments on the full length AFP polypeptide have the potential to bind to a variety of proteins including "Scavenger receptors" [49].

Additional GIP biological activities

The AMP-like GIPs may also have the potential to serve in multiple biological capacities which could apply to therapies of human diseases. Such potential clinical applications have been addressed in summaries of published reports based on preclinical studies of animal models and *in vitro* human cell culture studies. Such reports addressed GIP serving as an agent for: 1) cell chemosensitization; 2) cell radiosensitization; 3) binding to allosteric sites on proteins; 4) postsurgical cancer therapy; 5) disabling the tumor-to-micro-environment communication network; 6) disrupting metastatic cancer cell adherence to platelet islet clusters in the bloodstream; 7) enhancement of the adaptive T-cell immune response; and 8) boosting AFP epitope antigen presentation by dendritic cells. All of the preceding studies have been documented by data in previously published pre-clinical studies [35,44,45].

Concluding Statements

The preceding discourse has presented the case that antimicrobial-like peptides could potentially be utilized as adjunct or single agents in the course of human cancer chemotherapy. It has also been previously established that the AFP molecule and its derived peptides could serve as carrier transport vehicles of chemo-drugs for delivery into cancer cells [31,50]. The biomedical literature is replete with reports that intact full-length AFP itself can bind chemodrugs and toxins in both a covalent and/or non-covalent manner [34, 44, 50]. Regarding AFP-derived peptides (GIPs), an international multi-center collaborative task force reported that GIP fragments conjugated to either fluorescein or doxorubicin (DOX) underwent tumor cell uptake and drug delivery.

This study demonstrated that a DOX-GIP-8 conjugate could produce cytotoxic cancer cell destruction better than DOX alone [35]. These studies strongly support the potential future utility of GIP fragments as cell pore/channel interacting cancer therapeutic agents.

Less frequent occurrence in published literature is the co-administration of intact native AFP together with a co-mixture of chemotherapeutic drugs; such is also the case for AFP-derived peptides like GIP. However, repeated injection of full-length native AFP into adult patients with disease (i.e. cancer) might be wrought with dangers as previously reported [51]. Full-length native AFP is bristling with active and occult binding sites, some of which are hidden in molecular crevices and can be exposed by conformational changes in the AFP molecule. In light of

this Stage-1 and Stage-2 clinical trials using human AFP to clinically treat autoimmune diseases failed to progress to Stage-3 testing in human clinical trials [52]. However, similar human clinical trials with AFP-derived peptides have yet to be attempted. In contrast to the use of full-length native Human AFP in the clinic, the use of function-site-specific AMP-like peptides (i.e. GIP) offer a safer, more conservative approach for possible adjunct therapy for cancer and other diseases such as autoimmune disorders. Full-length recombinant human AFP should cautiously be viewed as a dangerous molecular "loose cannon" protein replete with unpredictable bio-reactive sites that might be directed toward unwanted target cell growth enhancement. Continuous injections of full-length AFP into human patients might possibly convert dormant or senescent target cells into benign/malignant growths, or worse, micro-clusters of newly developing transformed cancer cells. Native full-length AFP is known to display three Epidermal Growth Factor (EGF) 30-40 AA repeats, one repeat on each of the three domains of the AFP polypeptide chain [49]. Such EGF repeats might contribute to the growth enhancing properties reported for intact native AFP. Administering full-length recombinant human AFP to patients with potentially undetected growing malignant tumor foci would be akin to adding gasoline to a fire. A word to the wise should be sufficient for the use of full-length recombinant or purified AFP to treat human diseases and/or disorders.

Overview

In summation, AMPs as anticancer drugs should now be viewed as future chemotherapy candidates with a low propensity to drug resistance, high drug delivery efficiency, low side effects, and increased targeting capabilities. For further studies on the AMP-like peptides (i.e. GIP), (the reader is directed to References [53-55]).

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Conflicts of interest

The author declares that there are no known conflicts of interest in the preparation of this manuscript.

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