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Review

Alpha-fetoprotein and derived peptides as biomarkers and biologic response modifiers: Summaries, updates, and prospectives

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.

ABSTRACT

Alpha-fetoprotein (AFP) is a tumor-associated fetal protein that promotes growth and well-being in the fetus during pregnancy and further serves as a biomarker for birth defects and various cancers. Although AFP regulates growth in both fetal and cancer cells, it can also inhibit cell growth in environments undergoing shock, stress, and inflammation. The present review addresses AFP and its derived peptides, not only as disease/ disorder biomarkers, but as biologic response modifiers in multiple bodily functions and interactions. In order to present these events, the present treatise was divided into four discussion sections, namely, 1) AFP structure and function; 2) AFP as a human disease/disorder biomarker; 3) AFP-derived peptides in their role in cancer growth and metastasis; and 4) AFP peptides as potential biotherapeutic agents. Each section discusses and summarizes activities of AFP peptides in events such as cell migration, adhesion, cell-to-cell interaction, transduction signaling, angiogenesis, carcinogenesis, metastasis, and growth regulation.

KEYWORDS: alpha-fetoprotein, DNA repair, biomarker, cell cycle, metastasis, growth, cancer, peptides, therapy.

*Email id: gerald.mizejewski@health.ny.gov

1. Introduction

1.1. Alpha-fetoprotein structure and function

1.1.1. Historical

Mammalian alpha-fetoprotein (AFP) is classified as a member of the albuminoid gene superfamily consisting of albumin, AFP, vitamin D (Gc) protein, and alpha-albumin. Molecular variants of AFP have long been reported in the biomedical literature. Earlier studies identified isoelectric pH isoforms and lectin-binding variants of AFP, which differed in their physiochemical properties, but not in amino acid composition. Genetic variants of AFP, differing in mRNA kilobase length, have been extensively described in rodent models during fetal/perinatal stages, carcinogenesis, and organ regeneration [1, 2]. Following the advent of monoclonal antibodies in the 1970-1980s multiple antigenic epitopes on native AFP were detected and categorized, culminating in the identification of six to seven major AFP epitopes. During this period, various AFP-binding proteins and receptors were reported to inhibit certain AFP biological activities [3, 4]. Concomitantly, human and rodent AFPs were cloned, and the amino acid sequences of the translated proteins were divulged. Once the amino acid composition of the AFP molecule was known, enzymatic fragments were identified, and synthetic peptide segments synthesized [5]. Following discovery of the AFP molten globule form, the existence of transitory, intermediate forms of AFP were acknowledged

and their physiologic significance was realized [5]. In past reviews the various isoforms and variants of AFP have been described in light of their potential biological relevance [2, 4].

1.1.2. Alpha-fetoprotein, isoforms, epitopes, and conformational variants

The above discussion and cited reviews have attempted to familiarize the reader concerning the classification, nomenclature, and status of the various isoforms, epitopes, peptic fragments and conformational variants exhibited by AFP. Such forms have been incorporated into the design of immunoassays, purification strategies, physiochemical studies, genetic variants, and bioassays for AFP. As previously stated, [2], AFP has also been employed as a biomarker carrier vehicle for anticancer drugs, and the biomodulation of both ontogenic and oncogenic growth. The cloning of the AFP gene and its translated protein product has further enabled the design and use of short-specific peptide segments obtained from recombinant and synthetic peptide technologies as probes for biological activities, subcellular trafficking, and therapeutic targets [2, 4]. With the documentation of AFP and its derived peptides as regulators of growth, studies of carrier vectors for genetic manipulation and histocompatibility modulation have been pursued. It can only be expected that more imaginative and creative uses of AFP and its derived fragments will be employed as the future dictates. The past euphoria regarding the success of the Human Genome Project at the DNA level has given way to a recognition of the enormity and importance of the present ongoing challenge, i.e., Proteomics. The structure and function of the AFP, as described here and in previous reviews serve as examples of this future proteomic challenge.

1.2. Alpha-fetoprotein as a biologic response modifier

In prior reviews, the structure of α-fetoprotein (AFP) has been addressed in consideration of AFP membership and position in the albuminoid supergene family and its relation to other gene family members [2-4]. Ontogenetic AFP gene expression has been described in view of AFP cell culture mRNA variants derived from various tissues at different times during development and carcinogenesis. The multiple molecular forms of AFP have also been discussed in relation to reports of various AFP binding proteins and cell surface receptors. Such reviews proceeded to also present AFP as a potential model of a modular/ cassette-bearing protein based on sequence comparison with cleaved fragments of prohormones and biological response modifiers. Such cleaved peptidic fragments could potentially serve as peptide messengers and enactors of vascular, neuroendocrine, and digestive biological activities [2-4].

Once it was discovered that AFP binds to serine proteases, AFP-associated cytoskeletal, extracellular matrix, and cellular adhesion interactions were identified [2, 3]. AFP employed as a carrier/transport protein based on structural relationships was further elucidated by analysis of the various ligands bound to AFP and its hormonal interactions [5, 6, 8]. Since AFP binds to heavy metals, the question was posed that AFP could function as an antioxidant. An analysis of the AFP relationship to transcription factors, tumor suppressors, and homeodomain proteins followed, and such studies were interfaced with the concept of programmed cell death in light of second domain amino acid sequence matches with the AFP molecule [3, 5]. Emphasis was then placed upon the AFP comparison to homeodomain protein sequence stretches since AFP is a fetal, phase-specific protein found throughout embryogenesis, histogenesis, and organogenesis [2]. In keeping with histogenesis, the association of AFP with eye lens protein development was subsequently uncovered. Finally, reviews of AFP sequence analysis presented in light of the members of the immunoglobulin superfamily, autoimmune disorders, and various disease states began to be revealed. These discussions summarized regions of presumptive matched protein/peptide identities on each of AFP's three domains [4, 5].

It can be predicted that some of the biological roles of AFP remain unknown, since this has been an ongoing work in progress. The *in vitro* carrier/ transport ligand-binding function has proven the easiest to demonstrate. Furthermore, a growth regulatory role for AFP can no longer be denied and is presently an accepted concept. The immunoregulatory roles of AFP are undergoing

continued clarification in studies employing recombinant AFP and its domains, subdomains and/or fragments [9-11]. In the author's opinion, AFP is hardly a fetal substitute for albumin since albumin is present in nearly all vertebrate classes together with alpha-albumin, while AFP is present only in birds and mammals [3, 4]. Only two short identical amino acid segments are common to all AFPs and ALBs. One is in domain 2 (16 AA long) and the other is in domain 3 (17 AA long). The domain 1 site resides in a region of platelet and apoptosis related proteins, while the domain 3 site is localized in an area with high homology with AFP binding sites for binding to fatty acids, estrogens, and protein factors (58% over 31 AA). Clearly, both regions were found to display albumin-associated functions.

The present discussion was further intended to focus on AFP as a biologic response modifier in order to expand the reader's perspective regarding the functional roles of AFP. By employing a repository of biomedical AFP publications from the literature, attempts to reconcile computer sequence comparisons with AFP domain, subdomain, and motifs/cassettes with already published proteins continue to be researched [3-5]. Although computer findings did not always have a published counterpart, inroads have been made to provide both a rationale and justification for further studies of AFP physiology reflecting its domain and subdomain structure and peptide fragments. More intense investigation of the functional roles of AFP should prove mutually beneficial to the biomedical community as well to the biotechnology industry.

1.3. Protein binding and receptor interactions with alpha-fetoprotein

Human alpha-fetoprotein (HAFP), a tumor-associated fetal protein, is well-known clinically as a biomarker for both fetal defects and malignant tumors. However, less well-known are the proteins that interact and/or bind to HAFP. Such protein-to-protein interactions include a multiplicity of entities which include; a) cell surface receptors; b) intracytoplasmic binding proteins; and c) protein intermolecular complexing agents which are both serum circulating and non-secreted or cell-bound [12]. Some AFP receptors are located on tumor cells, others have been detected on monocytes, macrophages, inflammatory, and lymphoid-associated cells. The intracytoplasmic AFP binding partners include nuclear receptors, transcription-related factors, cell cycle checkpoint and DNA repair proteins, caspases, and apoptotic-associated proteins. Finally, the AFP intermolecular complexing groups of proteins can be found in the blood circulation as well as in the intracytoplasmic compartments. The plethora of proteins interacting/ binding to AFP further attest to the wide varieties of biological activities (i.e. growth promotion) in which AFP engages.

In recent years, it is now evident that HAFP displays activities with an array of cell surface receptors, cytoplasmic binding proteins, and circulating inter-molecular complexes. A previously reported concept of a universal tumor receptor for HAFP must be denied in view of the multiple documented reports of receptors and binding proteins on AFP as reported in the biomedical literature [10, 12, 13]. One such class of cell surface scavenger receptors was found not only on tumor cells but on monocytes, macrophages, cells of the leukemic stem cells, and the lymphoid cell lineage [14]. A second class of AFP receptors has been identified as mucin- associated cell surface proteins [15]. Because HAFP is an established growth promoting protein, it would be logical that a fetal protein could be active as growth factor at the cell surface, as well as in cytoplasmic compartments. However, as stated above AFP is uniquely equipped to promote growth but can also inhibit growth under certain conditions of microenvironmental stress due to oxidative, pH, osmotic, metabolic, signal transducing, and excessive ligand concentrations, both in fetal disorders and in malignant tumors [2, 6-8]. Such a growth inhibitory function would permit AFP to temporarily halt gestational growth until fetal damage is repaired and normal growth could be resumed during pregnancy. Thus, AFP appears to serve as a multi-tasking molecular Swiss Army Knife during embryo/fetal development and in cancer.

1.4. Alpha-fetoprotein update and cytoplasmic trafficking in cancer immune-associated cells: Relevance to adaptive immunity

The cellular and uptake trans-passage of alpha-fetoprotein (AFP) through the cytoplasm has long

been known in the literature *via* a progressive fashion. Moreover, recent novel immunohistochemical studies have provided valuable new insights into the intra-cytoplasmic fate of endocytosed AFP in multiple cells including both cancer and immune-responsive cells [10-13]. Employing biochemical inhibitors and antibodies to various endosomal cytoskeletal-associated proteins and enzymes, investigators have tracked in detail the organelle pathway following AFP cell uptake to its final cytoplasmic destination [12]. Such recent contributions highlight and contrast two different cytoplasmic trans-passage routes for AFP [10]. One pathway involves endocytosis followed by endosomal vesicular transport of AFP free of ligands, while the other route consists of ligand-bound (fatty acids/steroids) AFP as a component of a nutrient in-and-out cell shuttle system. Finally, recent reports have distinguished between antigen-presenting dendritic cells employing the scavenger mannose receptor (CD206), and hepatoma cells utilizing the CD36, LOX-1, and SRB1 scavenger receptors [5].

It can be deduced from the above discussion that the cell uptake and trafficking of AFP through the cytoplasm can follow either of two diverse and circuitous pathways [10, 11]. One such pathway involves the endosomal cytoskeletal-transport system; the other is a drop-cargo shuttle system utilizing circulating carrier proteins for cell depositions. In the former pathway, AFP is taken up by a receptor-mediated endocytotic process after which the AFP-receptor complex fuses with early endosome vesicles. The fused AFP receptor-complex contained in the endosome traverses a cytoskeletal-guided trafficking (transport) network involving a host of enzymes and scaffolding proteins. These protein entities include tyrosine kinases, GTPase, dynamin, actin filaments, adaptin, kinesin, and dynein motor microfilaments [2, 9]. The final destination of the AFP-receptor complex pathway leads to a cytoplasmic perinuclear compartment wherein AP-2 and Auxillin serve to release the AFP-to-receptor complex from its encompassing endosome (now referred to as a late endosome) [10]. The freed separated receptor is re-cycled back to the cell membrane and the free AFP is stored within the perinuclear compartment. At this point, AFP is available to be processed within the MHC-Class II histocompatibility system which leads to proteolysis and subsequent antigen presentation to T-Lymphocytes present at the dendritic cell surface.

An alternate pathway following AFP cell uptake involves a carrier protein-cargo drop-off system mediated through organelle structures such as the trans-reticular Golgi network, lysosomes, and endoplasmic reticulum (KDEL) transport systems [10]. In these cytoplasmic organelle networks, serum carrier proteins such as AFP can transport and drop off cargos such as fatty acids, steroids, and drugs into cells for nutrition and growth during development and/or in malignant states. In summary, the route of AFP delivery into cells can impact either immune-stimulatory or growth enhancement/therapeutic effects depending on the cell type and ligand-bound status of the carrier AFP fetal protein [14].

1.5. Nonsecreted cytoplasmic alpha-fetoprotein: role in intracellular signaling and regulation

The notion of a non-secreted cytoplasmic-bound form of alpha-fetoprotein is not a new concept in AFP biological activities. Cytoplasmic AFP (CyAFP) has been a long-known but neglected protein in search of a function other than a histochemical and serum biomarker. In a prior report, CyAFP was presented as an “old” protein with a newly described intracellular function [16, 17]. In 1976, CyAFP was shown to be a synthetic product of hepatoma cells utilizing 14C-Leucine incorporation and demonstrated by auto-radiographic procedures. The synthesis of CyAFP without secretion from cells was demonstrated to occur in both malignant and non-malignant cells encompassing hepatomas, ascites fluid cells, immature rodent uterine, MCF-7 breast cancers, and cell extracts from human breast cancer patients [16]. In a following study using computer protein matching and alignments in AFP versus members of the nuclear receptor superfamily, a consecutive series of leucine zipper (heptad) repeats in AFP was found, suggesting a means for protein-to-protein binding interactions [17]. The potential for heptad heterodimerization between protein-binding partners provided the rationale for proposing that CyAFP could possess the capability

to form molecular hetero-complexes with cytoplasmic-based nuclear receptor transcription factors. Recent investigations have since provided experimental evidence that CyAFP is indeed capable of colocalizing, interacting, and binding to transcription-associated nuclear factors. Such proteins can modulate intracellular signaling leading to regulation of transcription factors in the initiation of growth in human cancer cells [18]. Although circulating serum AFP is known as a growth-enhancing factor during development, cytoplasmic AFP likewise has a functional role in oncogenesis, growth, and metastasis of adult liver pre-cancer and cancer cells.

The colocalization or complexing of CyAFP with karyophilic transcription factors in the cell cytoplasm proposes that a molecular mechanism is in place to enable protein-to-protein interactions to occur which could block nuclear receptor entry into the nucleus. Although a concept of heterodimerization *via* heptad repeats provides a plausible mechanism for the observed colocalization of CyAFP with the RAR, the dimerization concept has still not been fully elucidated. Even though fluorescence resonance energy transfer analysis (FRET) indicated that CyAFP forms a complex with the retinoic acid receptor (RAR), the precise mechanism or basis for heterodimerization interaction is still under investigation. The demonstration by computer matching and alignment analysis has provided a rationale to explain the binding together of CyAFP and RAR (60% homology matching) in a heterodimerization fashion [17]. The proposed mechanism for CyAFP blocking the nuclear import of RAR is that the complexing together of CyAFP with RAR could cover up the nuclear localization signal on the RAR molecule, thus preventing its transnuclear passage. However, further experimental verification is underway to determine the precise mechanism.

It is evident from the published reports that CyAFP plays a definitive role in intracellular signaling and regulation. It was demonstrated that CyAFP is capable of blocking the cytoplasm-to-nuclear transpassage of RAR across the nuclear membrane. By impeding the entrance of nuclear receptor through the nuclear pores, CyAFP could indirectly influence and modulate physiological events occurring in the nucleoplasm such as nuclear receptor binding to HREs and its subsequent gene expression. Further examples of such CyAFP-regulated interaction with transcription proteins include the expression and activities of CyAFP with caspase-3, Fn14, GADD153, nuclear receptors, CXCR4, apoptotic associated proteins, PTEN, PI3K, AKT, and mTOR [16]. Thus, it would be conceivable that a cytoplasmic-residing protein (i.e., CyAFP) could affect and influence intranuclear activities from a cytoplasmic location outside the nucleus.

1.6. Alpha-fetoprotein contains third domain interaction sites of DNA repair-associated proteins

Although much has been published on the domain structures of human alpha-fetoprotein (AFP), the AFP third domain (AFP-3D) has emerged as an important AFP receptor binding fragment regarding the binding, docking, and interaction sites for hydrophobic ligands, multiple receptors, ion channels, and cell cycle proteins. In keeping with previous reports, studies have demonstrated beyond a reasonable doubt that certain amino acid (AA) sequences on AFP-3D can provide a docking interface for protein-to-protein interactions (complexing) with DNA repair proteins [19] By means of a computer software program designed to study such '*in silico*' interactions, certain AA sequences on AFP-3D were identified which could plausibly interact with a group of DNA damage-sensing and repair (DDSR) proteins. The DDSR proteins identified included: A) BRCA1 and BRCA2, B) FANCI and FANCD2, C) nibrin, D) ATM and ATR, and 5) DNA-PK kinase. Following the computer mapping of the AFP-3D with DDSR protein interaction sites, the computer program-derived AFP-AA identification sequences were further examined for similarities and comparisons to previously reported ligand, receptor, channel and other protein interaction sites on AFP-3D [19]. Literature searches revealed that the association of AFP with the DDSR proteins showed computer correlations not only with clinical serum AFP levels, but also with CyAFP which can interact with transcription factors, cell death (apoptosis) proteins, nuclear receptors, and enzymes (caspases) [20, 21]. The DDSR proteins

that interacted with AFP were further shown to be involved with cell cycle checkpoint proteins, cyclins and their dependent kinases, and ubiquitin ligases. Finally, both clinical and experimental reports on the AFP-3D association with DDSR proteins were found to be consistent with other published '*in silico*' computer findings.

Evidence has now been reported that several mutated proteins of the DNA-damage/repair pathways are associated with cancer susceptibility, tumorigenesis, and enhancement of tumor progression, most notably in breast and ovarian cancer, and in other cancers as well [20]. The BRCA and FA-related protein mutations leading to anemia cause such patients to develop tumors later in life. A significant connection of AFP to FA-mutated DNA repair proteins is reflected in the elevations of serum AFP in anemic patients. There are further correlations which exist with breast cancer and its associated BRCA1/BRCA2 mutated proteins. Sarcione *et al*. has reported that a circulating bound form of AFP, as opposed to circulating free AFP, exists in some female breast cancer patients [20, 21]. As discussed above, it has been reported that CyAFP is present in normal developing cells, as well as cancer cells. The non-secreted cytoplasmic CyAFP form has been shown to participate in kinase regulation, transcription, apoptosis, and nuclear gene expression [16, 18]. A proposed mechanism of CyAFP interaction within cells involves the heterodimerization of CyAFP with cytoplasmic proteins such as caspases, transcription factors, and nuclear receptors [18]. Furthermore, the reported observations of interaction of AFP-3D with cell cycle proteins, together with the DNA-repair proteins' association with cell cycle checkpoints, allows one to speculate that AFP could mask, interfere, enhance, or interpose (involved) itself into the DNA-repair process of the cell cycle checkpoint regulation pathway. Previously reported RNA microarray analyses with AFP peptides are consistent with this supposition [9]. The above studies would query whether CyAFP (by means of the third domain) is an important regulatory factor in the overall program of DNA repair during cell cycle progression (see Table 1).

2. Role of alpha-fetoprotein as a biomarker of human disease

2.1. Does alpha-fetoprotein as a biomarker also contribute to the mortality and morbidity in human hepatocellular carcinoma patients?

The fifth most common cancer worldwide is hepatocellular carcinoma (HCC), while being the third leading cause of global cancer-related deaths. Although HCC incidence is less frequent in North America, it is a more common malignancy in Asia and Africa associated with a high rate of mortality and morbidity due to ineffective therapies against liver cancer growth, invasion, and metastasis [23]. It is well established that serum alpha-fetoprotein (AFP) is the "gold standard" biomarker for liver cancer; however, less known are the biological activities of AFP involved in liver cancer carcinogenesis, growth, proliferation, and metastasis. Clinicians are well aware that increasing AFP serum levels parallel disease progression and demise of HCC patients, but many doctors are less knowledgeable in the lethal growth-promoting properties of AFP as an autocrine stimulator of hepatoma cell proliferation. The present discussion addresses the mortality and morbidity concerning AFP in the genesis, growth, progression, and spread of HCC and emphasizes the perilous consequences of AFP-supported growth in human liver cancer even after liver resection and transplantation. Thus, AFP is not just a biomarker for HCC but also an ardent growth promoter of liver cancer and tumor progression.

It is now evident that serum and cytoplasmic AFP are not just bystander agents but are active participants in all phases of liver transformation, growth, proliferation, and metastasis of the HCC malignant state. Hence, AFP is intrinsically involved in the overall process of cancer progression from carcinogenesis to metastasis. Moreover, AFP induces malignant behaviors by: 1) stimulating expression of oncogenes and transcription factors; 2) inhibition of the apoptosis process; 3) promoting growth of cancer cells; 4) enhancing drug resistance, and 5) increasing cancer cell invasion and metastasis. Earlier reports have demonstrated that AFP is a lethal compound involved in HCC cell apoptosis, shielding and

Table 1. Alpha-fetoprotein (AFP) structure and function.

Alpha-fetoprotein topic	Biological roles, components, activities	References (#) cited
1). Alpha-fetoprotein structural forms, variants, derivatives, gene family	- Carbohydrate and pH isoforms; - 6-7 immunologic epitopes (antigens); - conformational variants - mRNA expressed variants - peptic fragments, 3 domains - member of albuminoid gene family	1-5
2). Alpha-fetoprotein as a biologic response modifier	- enhances fetal, cancer growth - serves as an anti-oxidant - transports metals, drugs, steroids - acts both as an acute/chronic inflammatory agent - binds to cells and extracellular matrix	4-11
3). Alpha-fetoprotein binding and receptor interactions	- binds to certain cell surface receptors - enhances protein-to-protein interactions - complexes with serum proteins - binds to intracytoplasmic proteins - attaches to monocytes, macrophages, lymphocytes	8-15
4). Alpha-fetoprotein uptake and cytoplasmic trafficking	- undergoes receptor endocytosis - cytoskeletal interactions and binding - forms an endosome vesicle - reacts with kinesin, dynein, dynamin - cargo transport, perinuclear localization	10-16
5). Alpha-fetoprotein as a non-secreted cytoplasmic protein	- binds to intracytoplasmic proteins - partakes in intracellular signaling - undergoes heptad heterodimerization - binds transcription and growth factors such as caspase-9, FN14, RAR Receptor, GADD153	14-18
6). Alpha-fetoprotein interacts with DNA repair proteins	- interacts with DNA damage-sensing and repair proteins, and transcription factors - inhibits apoptosis (cell death) - interacts with BRCA1, BRCA2, FANC1 proteins	16-21

escaping from lymphocytes, cell cycle progression, and signal transduction pathways [24]. In many of the reports cited earlier, knockout of the AFP gene inhibits and represses HCC tumor growth, while the transfection of the AFP gene into non-AFP hepatomas serve to initiate HCC tumor genesis, growth, and progression. It is apparent that the removal and/or absence of AFP from the liver tumor environment eliminates much of the tumor lethality and reduces HCC growth, while the presence of AFP adds to the lethality of such tumors [25]. Although AFP is known as the "gold standard" biomarker for HCC, as stated above, it must be emphasized that AFP itself is a highly effective growth factor and not all clinicians may be aware of its immense potential for enhancing HCC growth and patient decline. Indeed, it has recently been reported that AFP contains three epidermal growth factor sequence motifs, one on each domain of the AFP polypeptide, which serves to enhance cancer growth [14].

Although AFP contributes to successful fetal growth during pregnancy, full-length AFP should now be recognized to play a lethal role in the growth, development, and progression of HCC. In conclusion, it is of utmost importance that "recombinant" AFP should not be employed as a therapeutic agent to treat human cancer disease

and other autoimmune disorders. Since AFP is a "loose cannon" bristling with protein-to-protein interaction sites, it would be instead more prudent to use specific AFP-derived domain fragments and/or peptide segments for any type of therapy in humans if it were ever approved (see later, Ref. #71). Hypothetically, the ultimate positive therapies for HCC should include 1) downregulation of the AFP gene, 2) use of microRNA and small interfering RNAs against AFP, and/or 3) AFP peptide therapies and/or peptide vaccination against HCC (see Table 1).

2.2. Alpha-fetoprotein (AFP) as a biomarker in gastric cancer

Gastric (stomach) cancer (GC) is the fourth leading cause of cancer deaths worldwide. Alpha-fetoprotein (AFP)-secreting gastric cancer (AFP (+) GC) represents an aggressive, less common subtype of stomach cancer exhibiting poor prognosis, low patient survival times, high progression rates, and liver metastases. No standard treatment regimen is presently in practice although multimodal therapies have been employed; while some drug resistance has been encountered in chemotherapy, AFP (+) GC is known to be more lethal than AFP non-secreting tumors with a patient medial survival time of only 14 months. The reason for the increased mortality and morbidity of AFP-secreting GC has not been completely understood although multiple factors have been forwarded. Such factors could include later stage diagnosis, unresectable metastases, rapid tumor growth, high mitotic rates, and elevated cellular expression of growth-promoting proteins [26]. However, in recent years, a better understanding of the physiological (biological) activities of AFP as a growth regulatory cell-signaling factor has emerged. Such studies have now established that AFP itself, acting through a cell surface receptor, is a potent tumor growth promoter as demonstrated in the present discussion [20] (see below).

AFP (+) GC is an aggressive, poorly differentiated tumor of the stomach often accompanied by unresectable metastatic lesions and elevated AFP serum levels. This AFP-secreting stomach adenocarcinoma differs clinically from the conventional type of gastric cancer by having a poorer prognosis and lower survival time of patients which is attributed to liver metastasis and lymphovascular invasion among other traits. Moreover, AFP (+) stomach cancer further exhibits increased mitosis and cell migration, high proliferative rates, and rapid tumor progression [23] similar to liver cancer. The greater mortality and morbidity rates of the AFP-secreting stomach cancers may be largely due to the presence of AFP as a growth promoter [21]. Circulating serum AFP is an autocrine-enhancing growth factor with high binding affinity to AFP receptors residing on the cell surface of multiple tumor types, including gastric tumors [20, 28]. In fact, I-125-radiolabeled AFP peptides were found to specifically localize in the stomach at 20 times the blood levels (tissue-to-blood ratios) in biodistribution studies employing mice [28].

Reports in the biomedical literature have further demonstrated several AFP growth-enhancing activities resulting from conditions such as inhibition of apoptosis, presence of solute carrier transporter proteins of chemo drugs, mucin interaction, and mRNA down-regulation of cell cycle kinases and checkpoint proteins [24]. Cytoplasmic bound AFP (CyAFP) can regulate cancer cell growth, proliferation, progression, and metastasis. The future clinical goal of treating of AFP (+) secreting tumors should someday entail the elimination or down-regulated expression of the AFP molecule [25]. This endeavor could be accomplished by recently published procedures which employed inhibitory mRNA for AFP; silencing of AFP synthesis (siRNA) or gene modulation and editing procedures intended to eliminate AFP production [24]. The knowledge of such biochemical procedures which could possibly contribute to rapid demise of AFP (+) secreting gastric and other tumors should prompt further investigation into such reported procedures. The present discussion is intended to serve as an impetus for investigators to pursue potential new and novel treatment options for AFP-secreting gastric cancers. Armed with such information, clinicians would also be better informed to consider new and novel treatment options for GC patients displaying a non-threating clinical status.

2.3. Alpha-fetoprotein as a diagnostic biomarker for head and neck germ cell tumors

Germ cell tumors (GCT) represent an assorted group of benign and malignant neoplasms derived from primordial germ cells [29]. Although most GCTs reside in the retro-peritoneal cavity, such tumors can also exist in the midline axis of the body including the mediastinum including head and neck regions. The cervical areas encompass the oral cavity, neck region, eye orbits, nasopharynx, and oropharynx [30]. Both benign and/or malignant teratomas (yolk sac) can develop in tissues such as mandible, gingiva, upper lip, epignathus, floor of the month, base of the skull, scalp, lingual, and craniofacial areas. Alpha-fetoprotein (AFP) is a well-known and highly utilized biomarker for teratomas of the head and neck regions [29]. AFP levels have long been employed as a "first response" biomarker for the cervical GCTs because AFP is a known marker of aggressive disease. Both serum AFP and CyAFP play a notable role in the diagnosis, follow-up, and monitoring of post-surgical resurgence and/or reduction of GCT mass. Thus, serum levels of AFP have long served as an indicator of tumor activity, chemotherapy response, presence of malignancy, and cancer cell transformation. In this discussion, it is demonstrated that circulating AFP, in the blood of certain GCT bearing patients, is a superior biomarker to hCG, CEA, and lactate dehydrogenase, while CyAFP, together with established histopathological markers, greatly aid in confirming GCT diagnosis [29].

Germ cell tumors (i.e., teratomas) are an assorted group of benign and malignant neoplasms derived from primordial germ cells. Most GCTs of the fetus/neonate are benign, except yolk sac tumors; such teratomas can be expressed as either mature or immature [31]. The immature tumors display both the higher serum AFP levels and the maximal tumor spacial dimensions than the mature teratomas. Many GCTs contain calcifications and strands of partially differentiated segments of hair, bone, muscle, and other bodily tissues. Tumor masses can further be detected by antenatal and perinatal sonography assisted by physical exam (palpation). AFP determinations in the prenatal and perinatal periods have proven invaluable in the measurement of both maternal serum and amniotic fluid AFP levels involving GCTs [30, 31]. The most recently accepted chemotherapy regimen for GCT patients includes cisplatin, bleomycin, and etoposide [7]. In the past, GCTs have been misdiagnosed as lymphadenopathies and lymphangiomas (2); however, AFP is not elevated in most lymphatic tumors.

Measurements of AFP serum levels and CyAFP presence have proven extremely helpful in the diagnosis of germ cell tumors, since AFP is a biomarker of aggressive disease. However, AFP levels should not be considered absolute discriminatory factors of germ cell tumor type or maturity. Serial monitoring of SAFP levels should be performed in order to analyze the kinetics of serum AFP levels over extended time periods which could reflect neoplastic growth or the lack thereof. Hence, AFP measurements can serve as indicators of patient response following surgical procedures, radiation, and chemotherapies. Decreasing AFP levels could indicate a neoplastic mass reduction and possibly reduced metastases, whereas, increased AFP levels mark a neoplastic relapse. It has been revealed in further discussions, that serum AFP determinations for GCTs can be superior to other serum biomarker in most instances. Moreover, determination of CyAFP presence in combination with glypican, placental alkaline phosphatase, and alpha-I-antitrypsin provides a very useful combination for histopathological examination of the tumor [32].

2.4. Alpha-fetoprotein as a biomarker of immunodeficiency diseases: Relevance to ataxia telangiectasia and related immune disorders

Human alpha-fetoprotein (AFP) involvement as a biomarker in immunodeficiency disorders (IDD) has received less attention in the biomedical literature due to the recent focus on AFP epitope analysis, vaccine development, and regulation of T- and B-cell immune responses. Thus, the literature is currently devoid of reviews or summaries which encompass the use of AFP as a biomarker in the various types of IDDs. In order to update and increase present knowledge regarding an AFP-IDD relationship, the present discussion addresses the presence of AFP serum levels in patients with IDDs such as acquired immunodeficiency syndrome (AIDS), severe

combined immunodeficiency (SCID), adenosine deaminase deficiency (ADD), and in pregnant women infected with the human immunodeficiency virus (HIV) [33]. Prior treatises have applied a proteomic approach in order to study possible protein-to-protein interactions of AFP with chemokines and other protein effectors of IDDs. Additional evidence has been obtained from AFP domain structure analysis, especially ligand binding in the AFP third domain, in search of possible protein-to-protein interaction partners contributing to IDD states. Finally, attempts have been made to elucidate the relationship of immunodeficiencies in ataxia telangiectasia patients to AFP involvement both at the molecular and clinical levels.

Elevated levels of the AFP biomarker in IDDs suggest an association between AFP synthesis/ secretion and deficiencies in the immune system. Although previous studies have included AFP serum levels during pregnancy in women with HIV, IDD patients also display elevated AFP levels in infancy, childhood, adolescence, and in adult immune disorders. The question of why AFP levels are elevated in AT patients is not presently known. AFP in such patients is known to be of hepatic origin [34] in which AFP levels gradually increase with age [35]. Although the AFP serum level increase runs parallel to the age-related worsening of the AT disorder, a link between the degree of neurological disease and AFP levels has not been established. Other than a few clinical oxidative stress and cirrhotic cases, little or no evidence of chronic liver disease/ damage has been reported in autopsied AT patients [35]. It is of interest that such studies indicated that p53 and related family member proteins act as regulators of AFP gene expression during fetal liver development [36]. Thus, the AFP gene in the neonatal AT liver may be under aberrant transcriptional control in conjunction with possible defects in DNA regulatory proteins required for hepatic cell maturation. That is, the AFP gene may not be completely turned off by methylation and/or other regulatory control factors. Liver stem cells, which do not secrete AFP, surround the hepatic bile ducts and give rise to the non-parenchymal oval cells capable of secreting AFP [32]. The oval cells further serve as progenitor cells of the liver parenchymal (non-AFP secreting) cell population. It may be that an abnormal (high) density population of oval cells exists in the childhood/adult AT liver which continues to secrete AFP into the bloodstream. This event could be confirmed by histochemical staining and should be pursued. The continued state of immature cells in the liver, verifiable by immunohistology, might further explain the continued elevated levels of AFP in AT patients. Thus, the liver of AT patients might persist as an organ composed of an aberrant, unbalanced mixture or immature and mature cell types composed of stem, oval, and parenchymal cells.

2.5. Alpha-fetoprotein (AFP) and inflammation: Is AFP an acute and/or chronic phase reactant biomarker?

Even though alpha-fetoprotein has long been implicated with inflammation during pregnancy and adult liver dysfunction, the biomedical literature lacks sufficient knowledge of such a relationship. Clarification of the role of alpha-fetoprotein in the inflammatory response has been explored in the present discussion regarding AFPs participation as a positive and/or negative phase inflammatory reactant [38]. Inflammation follows a complex succession of vascular changes involving alternations in blood and lymphatic vessels both at local intracellular sites and at the organ (liver) level. The inflammatory response may result in either an acute phase or develop into a chronic phase following injury or insult from foreign bodies, microbes, toxins, carcinogens, or autoimmune self-antigens. The site of inflammation attracts pleomorphic cell infiltrates which secrete various chemical mediators such as cytokines, chemokines, interferons, histamines, and a host of others. It has been demonstrated that alpha-fetoprotein can serve both as an acute and a chronic phase reactant depending on its stage of ontogeny [38]. It was found that alpha-fetoprotein functions as a positive acute phase reactant in the embryo, fetus, and placenta during pregnancy. In contrast, alpha-fetoprotein proceeds to function as a negative acute phase protein in the postnatal and adult period of life, especially following liver dysfunction and toxic insult. In chronic viral-induced inflammation of the liver, especially viral hepatitis, alpha-fetoprotein appears to serve as a positive phase inflammatory reactant [39].

Previously, the relationship between AFP and inflammation has not been fully clarified and elucidated in the literature. It is apparent from reported discourses that AFP could indeed be an inflammatory reactant serving at different times, both as a positive and a negative acute response agent. The positive or negative response appears to be dependent on the ontogenetic stage at which AFP is being expressed and secreted. During acute inflammation in pregnancy, AFP levels are increased in conditions such as pre-term birth, intra-uterine growth retardation, prematurity, fetal-maternal bleed, premature membrane rupture: also included are placental inflammatory events such as chronic villitis, ischemia, intervillis thrombosis, and chorioamnionitis [40]. From such reports, it is apparent that AFP can serve as a birth delivery inflammatory biomarker at term pregnancy. Such term pregnancy lesions can result from placental insufficiency and poor weight gain in late pregnancies. AFP was also found to be elevated in acute experimental liver injury such as chronic virus-induced inflammation of the liver due to chemical and dietary hepatotoxins. AFP can also serve as a negative phase reactant in the postnatal period as exemplified in experimental turpentine injections, clinically as in neonatal death, and in adult liver dysfunction. Thus, the dual role of AFP as in indicator or biomarker of inflammation should be further explored in the future.

2.6. Alpha-fetoprotein as a biomarker and autoantigen in hepatocellular carcinoma and juvenile batten disease

Failure of immune tolerance leads to production of autoantibodies to self-antigens. The repertoire of autoantibodies detected in cancer patients can indicate the presence of autoimmune disease. Alpha-fetoprotein (AFP) autoantibodies have been found in patients with hepatocellular carcinoma (HCC) and in juvenile Batten disease (BD), a neurodegenerative condition involving autoimmunity. Variant conformational forms of AFP together with exposed occult antigenic determinant sites on the AFP polypeptide resemble the features of a disordered, denatured protein which can impair central immune tolerance. These aberrant structural protein forms can lead to the persistence of autoantibody production by immune-sensitized B-lymphocytes. Thus, it is not surprising that AFP, a self-antigen, can induce autoimmune responses in the human body. This section discusses the molecular and antigenic properties of AFP which make it a disordered protein, and its ability to induce autoantibody production to AFP cryptic intrinsic epitopes in both HHC and BD patients [41].

It is now apparent that AFP among other self-proteins can serve as targets of an autoimmune response resulting from hidden epitopes exposed on unfolded proteins in the brain of BD patients. Similarly, autoantibodies to AFP-hidden epitopes were also detected in hepatocarcinoma patients. Cryptic epitopes can become exposed to the immune system secondary to certain events, such as trauma and necrosis, which may act by inducing conformational change modifications of the protein and/or in the course of processing proteins [42]. Human and rodent AFPs are immunogenic in xenogeneic animals, while they do not induce immune responses in their species of origin [43]. In summary, the unveiling of hidden epitope(s) on AFP may result in the induction of autoantibodies, indicating the activation of B and T lymphocyte clones which were not eliminated during development of immune tolerance [45, 44]. Since denatured intermediate forms derived from tertiary-folded AFP resemble those of a disordered protein, it is logical to predict that AFP could indeed induce immune responses as observed in various human diseases. Lastly, AFP has now been proposed as a biomarker for fetal, infant, and juvenile anemia [46] (see Table 2).

3. Alpha-fetoprotein-derived peptides in cancer growth and metastasis

3.1. Functional activities of the alpha-fetoprotein-derived growth inhibitory peptides

Alpha-fetoprotein (AFP), known largely as a growth-promoting agent, is known to possess growth-inhibitory short amino acid sequences identified as occult epitomic segments in the third domain. The present discussion addresses the multiple biological activities of this AFP-derived peptide segment termed the “Growth Inhibitory Peptide” (GIP), which constitutes a 34-amino acid

Table 2. Alpha-fetoprotein (AFP) as a biomarker of human disease.

Alpha-fetoprotein topic	Biological roles, activities	References cited
1). Alpha-fetoprotein as a biomarker for liver cancer	- a biomarker for hepatocellular carcinoma (HCC) - enhances HCC growth, proliferation, metastasis - serves as an autocrine growth stimulator - increases both mortality and morbidity	14, 22-25
2). Alpha-fetoprotein as a biomarker for gastric (stomach) cancer	- a biomarker for poorly differentiated tumors - biomarker for unresectable tumors - increases mitosis and cell migration - marker of tumor high proliferation rates - inhibits tumor cell death (Apoptosis)	23-28
3). Alpha-fetoprotein as a biomarker for head and neck germ cell tumors	- AFP serum levels greatly increase - tumors located in cervical areas, oral cavity, neck region, eye orbits, nasopharynx. - teratomas located in mandible upper lips, scalp, base of skull, gingiva	26-31
4). Alpha-fetoprotein as a biomarker for immunodeficiency diseases	- a biomarker for ataxia and related disorders, severe combined immunodeficiency, adenosine deaminase deficiency, AIDS, SCID, and HIV. - chemokine, virus load, HIV during pregnancy	32-36
5). Alpha-fetoprotein as a biomarker for acute and chronic inflammation	- increased vascular alterations - results from foreign bodies, microbes, yeast, fungi, toxin, carcinogens, infections - involved with chemokines, interferon, histamines, and cytokines	37-40
6). Alpha-fetoprotein as a biomarker for autoimmune disease in HCC and juvenile Batton's disease	- self-antigens in liver cancer and in neurodegenerative Batten disease (BD) - autoantibody production from liver (HCC) and from brain areas in BD. - involvement of B-and T-lymphocytes/ autoantibodies - denatured, disordered proteins form antigens	41-46

fragment taken directly from the 3rd domain full-length 609 amino acid AFP molecule. The GIP segment has been chemically synthesized, purified, characterized, and subjected to a variety of bioassays [47]. The GIP has a proven record of growth suppression in both fetal and tumor cells, but not normal adult cells. Even though the GIP mechanism of action of cell entry has been elucidated, GIP is also known to participate in a variety of biological activities such as endocytosis, angiogenesis, and cytoskeleton-induced/cell shape changes [48]. In this section, a survey of the functional roles of the GIP is presented which encompasses multiple organizational levels of GIP involvement which includes: 1) total Organism, 2) organ, 3) tissue, 4) cell, 5) plasma membrane, 6) cytoplasm, and 7) nucleus levels. At the cell membrane interface, the actions of GIP are discussed concerning cell aggregation, agglutination, adhesion, and migration in light of GIP serving as a possible decoy ligand and/or soluble receptor. Regarding cytosolic activities, GIP has been reported to inhibit various cytoplasmic enzyme activities, modulate apoptotic events, and regulate cytoplasmic signal transduction (MAP kinase) cascades [49, 50]. Concerning the nuclear compartment, GIP is capable of complexing with the estrogen receptor to block nuclear entry, thus indirectly affecting estradiol-induced estrogen receptor transcription.

It can readily be observed that GIP activity is not limited to phylum, class, genus, or species. GIP was found to be active in cells of organisms ranging from crustaceans to mammals, encompassing frogs, birds, mice, rats, and humans. The GIP is not limited to cell or hormone specificity; it activates growth suppression in multiple models, such as tadpole metamorphosis, insulin-injected chick embryos, and E2-induced toxicity in fetal mice [51, 52]. GIP has also been involved in suppressing several cytoplasmic enzyme activities. It is at the cell membrane that GIP demonstrates its most diverse functionality, by interfering with a variety of cell surface events. GIP affects basic physiological functions of many cell types throughout the animal kingdom; these include cell surface disruption and cell cycle inhibition [9]. Data based on immunohistochemical findings have further suggested that GIP might serve as a decoy peptide ligand for the family of G-coupled receptors [53, 55]. Although a present working hypothesis has favored a decoy peptide receptor-mediated endocytosis, further research has elucidated other modes of action of GIP (see below).

3.2. Growth inhibitory peptide as a biotherapeutic agent for tumor growth, adhesion, and metastasis

The present survey further discusses the biological activities of the alpha-fetoprotein-derived peptide termed the GIP, a synthetic 34-amino acid segment produced from the full length 609 amino acid AFP molecule [54, 55]. GIP has been shown to be growth-suppressive in both fetal and tumor cells but not in adult terminally differentiated cells [52, 53]. Even though the mechanism of action of growth suppression of this peptide has recently been elucidated, GIP is known to be highly interactive at the plasma membrane surface in cellular events involving cell penetration, cell contact inhibition and cytoskeletal-induced cell shape changes [9, 48]. GIP was shown to be growth-suppressive in 38 of 60 cell-cultured human tumor types (NCI Screen) and to suppress the spread of tumor cell metastases *in vivo* in human and in mouse mammary cancers. The AFP-derived peptide and its subfragments were further shown to inhibit tumor cell adhesion to extracellular matrix (ECM) proteins and to block platelet aggregation; thus, it was found that GIP could inhibit cell spreading/migration and metastatic infiltration into host tissues such as lung and pancreas [47]. It was further reported that the cyclic versus linear configuration of GIP determined its biological and anti-cancer efficacy [49]. Genbank amino acid sequence identities with a variety of integrin alpha/beta chain proteins provided further evidence of GIP's linkage to inhibition of tumor cell adhesion and platelet aggregation [38, 55]. The combined properties of tumor growth suppression, prevention of tumor cell-to-ECM adhesion, and inhibition of platelet aggregation suggest that such interactions of GIP present promising targets as anti-metastatic agents. It was concluded that GIP represents a growth inhibitory motif possessing properties that allow it to initiate and disrupt cell surface events such as adhesion, migration, metastasis, and aggregation of tumor cells followed by cell cycle inhibition of growth.

It is evident from the above discussion that the AFP-derived GIP is a major participant in tumor cell surface and cell cycle interactions. Moreover, GIP is a capable suppressor of tumor proliferation in both rodent and human cancer models. It has also been demonstrated that GIP's overall biological activity is dependent on its oligomeric state, specifically its trimer (linear) as compared to monomer (cyclic) configuration [38, 49]. In studies using a cell adhesion assay, it was demonstrated that GIP inhibited tumor cell adhesion against multiple ECM proteins, many of which serve as basement membrane and platelet clotting constituents. GIP-coated coverslips were further employed to demonstrate that tumor cell attachment, migration, and spreading were negatively modulated by AFP-derived peptides. In assays using activated platelet suspensions, GIP was shown to be capable of blocking all phases of the platelet aggregation process against a variety of agonists. Prior to translocation to the cell cytoplasm, the events of GIP's action appear to occur primarily as cell surface activities being representative of cell adhesion, migration, aggregation, cell-to-cell contact, agglutination, cytoskeletal-mediated cell shape and form, and endocytosis [50]. These conserved actions indicate that GIP appears to be a cell membrane disruptive agent that can block or disable integrin-associated

and platelet-dependent physiological actions on tumor cells growth, progression, and metastasis.

3.3. Alpha-fetoprotein growth inhibitory peptides as potential leads for cancer therapeutics

α-Fetoprotein (AFP) known largely as a growth-promoting agent possesses a growth inhibitory motif identified as an occult epitopic segment of the protein molecule. The purified 34-mer GIP exhibits complex aggregation behaviors; initially, trimeric oligomers are formed that demonstrate growth inhibitory activity as shown in rodent uterine bioassays [49]. These rodent growth assays served to confirm GIP as an anti-growth and later as an anti-cancer agent in studies that followed. In solution, the trimers convert slowly to dimers containing intrapeptide disulfide bonds; such dimer forms are inactive in the antigrowth assays. Cysteine-to-alanine analogues of the GIP retain the antigrowth properties, while similar cysteine-to-glycine and cysteine-to-serine analogues demonstrate little, if any, growth regulatory activity [53]. Chemical modifications of the cysteine residues also have little influence on the antigrowth activity of the GIP. Fragments of the 34-mer possess variable biological activities of their own, with an octamer form near the carboxyl terminus displaying estrogen-dependent antigrowth activity similar to that of the 34-mer. It was further observed that the GIP could bind both Zn^{2+} and Co^{2+}; the Co^{2+} peptide complex was shown to have a distorted tetrahedral symmetry, involving coordination of two cysteine and two histidine residues [49]. The Zn^{2+} GIP complex has antigrowth activity and does not form the intrapeptide disulfide bond characteristic of the free GIP in aqueous solution. GIP was tested *in vitro* for anticancer activity and was found to suppress the growth of 38 of 60 human cancer NCI cell lines, representing nine different cancer types [24]. *In vivo* studies of the GIP, certain analogues, and its fragments revealed anticancer activities in both isograft and xenograft animal tumor transplants. Further, the 2C → 2A replacement analogue was active against a breast tumor *in vivo* and *in vitro* and a prostate cancer *in vitro* [53]. Thus, it was proposed that the GIP, its analogues, and its subfragment peptides can potentially serve as lead compounds for future cancer therapeutics [49].

The AFP-derived 34-mer peptide and its three fragments have been demonstrated to suppress both ontogenic and oncogenic growth in cell culture and various animal models. Such peptides could serve as prime candidates for templates or lead compounds toward the development of anticancer drugs because their pre-clinical efficacy has already been demonstrated. Because the peptides act on various proliferating tissues and a multitude of cancer types, the peptides could potentially provide site-directed targeted drug delivery to a variety of different types of cancer cells [28]. Small organic mimics (such as polyphenols) could be modeled to GIP and its fragments to provide second-generation drugs with oral and/or nasal mist modes of delivery. Finally, the AFP-derived peptides could find utility in the identification of molecular targets for drugs intended as biomodulation cancer therapies and to provide “proof of concept” in identifying targets for future peptide mimics [49].

3.4. Alpha-fetoprotein growth-inhibitory peptides as chemotherapeutic agents for tumor growth and metastasis

This discussion further describes the biological activities of an AFP-derived peptide described above as the growth-inhibitory peptide (GIP). GIP has been shown to be growth-suppressive in both fetal and tumor cells and in immature mouse uteri. Prior to elucidating GIP as a cell membrane disrupter and as a cell cycle inhibitor, GIP was shown to engage in cell surface events such as cell aggregation, hemagglutination, and cytoskeletal-induced cell reactions [48]. GIP was shown to be growth-suppressive in many human tumor cell types and to suppress the spread of tumor cell migration, spreading, and metastases in human and mouse mammary cancers [50]. It was further found that the different oligomeric states (cyclic vs. linear configuration) of the GIP determined its biological and anticancer efficacy (see above). Thus, the combined properties of tumor growth suppression, membrane perterbation and reduction of tumor metastases represent promising areas for GIP serving as a chemotherapeutic agent.

It is evident from prior studies that the AFP-derived GIP could serve as an adjunct therapeutic agent in both tumor cell growth and metastases. GIP might

also serve as a decoy ligand to receptors and could blunt adenyl cyclase downstream signal transduction [40, 50]. Further observations have suggested that the growth cell cycle inhibitory mechanism of action of GIP is common to many different types of animal cells [51, 52]. The other shared events of GIPs action appear to occur primarily as cell membrane surface activities as discussed above. Such conserved actions suggest that GIP is a cell membrane-reactive agent that can disrupt or disable cell membrane activities which can ultimately translate to inhibition of tumor growth, progression and metastasis.

3.5. Targeted delivery of anti-cancer growth inhibitory peptides derived from human α-fetoprotein: review of an international multi-center collaborative study

As discussed above, GIP is a 34-amino acid peptide composed of three biologically active subfragments. GIP-34 and its three constituent segments have been extensively studied for a multitude of biological activities. The GIP-34 and GIP-8 subfragment have been characterized as anticancer therapeutic peptides [50]. A multicenter study was initiated to elucidate the means by which these peptide drugs could be targeted to tumor cells [28]. The first study established which cancer types were specifically targeted by the GIP peptides including both *in vitro* and *in vivo* investigations. It was demonstrated that radiolabeled peptide (125-I GIP) can be specifically localized *in vivo* to rodent breast tumors at 24 hrs post-injection. These radionuclide studies provided evidence that a cell surface AFP receptor may be a participant; this was suggested in a further study using fluorescent-labeled GIP-nanobeads which were shown to localize at the plasma membrane of MCF-7 breast cancer cells. Finally, it was then demonstrated that GIP conjugated to doxorubicin (DOX) drugs underwent tumor cell uptake. In subsequent studies DOX-GIP conjugates induced cytotoxic cell destruction indicating the utility of GIP subfragments to serve as cancer therapeutic transport agents. In previous reports, two candidate cell surface receptor families were proposed which correlated with multiple published reports for the two AFP receptor families namely, the 1) Scavenger Receptor Family; and 2) the mucin receptor family [14, 15].

It is now apparent the AFP molecule itself can bind to two or more cell surface receptors, while the GIP fragment itself has no such receptors. Earlier publications based on computer modeling systems suggested plausible binding of AFP to G-coupled seven-transmembrane receptors, such as the GPR30 estrogen-binding membrane receptor [61]. However, the binding of GIP-34 and GIP-8 to GPR-30 could not be confirmed [57]. Further, it is long known that the GIP sequence is not fully exposed on native, compactly folded circulating HAFP; only an AFP conformational change can expose the GIP-34 segment following exposure to stress and shock extracellular environments. The search for GIP cell entry has been fully elucidated in that cell membrane penetration and a channel alteration mechanism is involved following conversion to an unfolded or molten globule form of the entire AFP molecule [9]. Similar peptide forms are found in gene families of proteins related to pattern recognition binding.

3.6. Mechanism of cancer growth suppression of alpha-fetoprotein-derived growth inhibitory peptides (GIP): Comparison of GIP-34 versus GIP-8 (AFPep)

As discussed above, GIP is a 34-amino acid segment of the full-length human AFP molecule that inhibits tumor growth and metastasis. Both the GIP-34 segment and its carboxy-terminal 8-mer segment, (termed GIP-8), were found to be effective as anti-cancer therapeutic peptides against multiple human cancer types [58]. Following the uptake of GIP-34 and GIP-8 into the cell cytoplasm, (see above), each follows slightly different signal transduction cascades en route to inhibit pathways concerning tumor cell growth and proliferation. The parallel mechanisms of action of GIP-34 versus GIP-8 are demonstrated to involve the interference of signaling transduction cascades that ultimately result in: (1) cell cycle S-phase/G2-phase arrest; (2) prevention of cyclin inhibitor (p27, p21) degradation; (3) protection of p53 from inactivation by phosphorylation; and (4) blockage of K^+ ion channels opened by growth enhancers such as estradiol and epidermal growth factor (EGF) [8]. The overall mechanisms of action of both peptides have been described in light of their differing modes of cell attachment and uptake.

These studies have been solidly confirmed and fortified by RNA microarray analysis following electrophysiologic measurements of cell membrane conductance and resistance. As a chemotherapeutic adjunct, the GIPs could potentially serve in alleviating the negative side effects of: (A) tamoxifen resistance, (B) uterine hyperplasia/cancer, and (C) blood clotting, (D) Herceptin antibody resistance, (E) cardiac (arrest) arrhythmias; and (F) doxorubicin's bystander cell toxicity [8].

GIP-34 has the advantage of being both a cell penetrating peptide (CPP) and a channel blocker (microbial peptide) depending on peptide concentrations as demonstrated by electrophysiologic studies. The CPPs are known to gain entrance into cancer cells by disrupting or disturbing the bilipid cell surface and cork-screwing itself into the plasma membranes of cells displaying an overall net negative cell surface charge as seen in many call cancer types [59, 60]. Hence, cells destined for apoptosis including cancer cells are known to undergo a cell membrane-lipid inversion by switching (substituting) sphingomyelin and/or phosphatidylcholine for phosphatidylserine, thus shifting a negative charge to the cancer cell apical outer surface [61]. The negative-charged cell surface not only flags cells for targeted apoptosis, but also designates the cell as a candidate for cell penetration, and transmembrane passage. Thus, CPPs do not attach or bind to positively charged normal cells, but rather to cells displaying (flagged by) a higher net negative charge on their surface, as found in cancer cells [28]. This phenomenon could provide a basis of specificity for targeting cancer cells, but not bystander non-cancer cells. The ion channels affected by CPPs are largely voltage-dependent and are selective for cations such as Ca^{++}, K^{+}, and Na^{+} ions. GIP-34 has been confirmed to affect voltage-gated K^{+}-channels as shown in RNA microarray data and in electrophysiology studies [9]. In contrast, short amino acid sequence peptides like GIP-8 do not show CPP activities, but instead exhibit channel blocker activity that eventually results in down-regulation of ion channels. Due to the cell membrane disruptive mode of action, it may be possible that drug resistance to chemotherapeutic agents could be by-passed by use of both peptides [62]. Finally, both peptides might serve as allosteric drugs in the that peptides can dock and target amino acid sequence stretches at a site other than that of the major ligand binding pocket; these data have been demonstrated by computer modeling of peptide (GIP) to protein docking sites [14, 15] (see Table 3).

4. Anti-microbial, cell penetrating, and alpha-fetoprotein-derived peptides as therapeutic agents

4.1. Cell-penetrating versus antimicrobial peptides: Comparison of potential use as cancer therapeutics

The use of peptides in cancer therapies has gained recent attention in the biomedical community due to their short half-lives and selective targeting advantages. At the forefront of potential peptide candidates, the cell-penetrating peptides (CPP) and, antimicrobials peptides (AMP) await implementation and inclusion into the armamentarium of future cancer therapeutics. Although similar in some properties, the use of each of these two peptide types for cancer therapies differ due to their intrinsic amino acid composition, hydrophobic properties, cell membrane targeting capabilities, secondary structure manifestation, mode of cell membrane encounter, permeabilization, cytoplasmic destination, and functional capabilities. While CPPS are involved with cell pore penetration and cargo (drugs, chemicals, etc) delivery, AMPs are characterized by cell membrane disruption/destabilization of bilayer cell membranes, channel and/or pore formation, and immune response enhancement [63, 64]. Past reports have compared and contrasted the properties, characteristics, traits, and function of each peptide type followed by discussion of the advantages and disadvantages of their possible adaptation for clinical use [65]. Finally, the clinical fate of these peptides have been described as well as the methods required to evaluate their suitability for use in future cancer therapy.

Future improved developments of CPPS and AMPs might include procedures such as: 1) insertion of organelle signal localization amino acid (AA)

Table 3. Alpha-fetoprotein (AFP)-derived peptides in cancer.

Alpha-fetoprotein topic	Biological roles, activities	References cited
1). Alpha-fetoprotein-derived peptides (GIP) (Growth Inhibitory Peptides) Functional Activities	- development of an AFP-derived peptide termed "Growth Inhibitory Peptide" (GIP) - Bioassay development, physical properties, - studies of cell entry and uptake, - participates in cross-talk and signal transduction within cytoplasm	47-55
2). Alpha-fetoprotein-derived GIP as a biotherapeutic agent for cancer	- GIP shown to inhibit cancer growth, progression, adhesion, contact inhibition, cell migration, aggregation, and metastasis - interacts with cell membrane as disrupter - serves as a cell membrane contact agent - importance of peptide oligomeric states	9, 48, 49, 53-55
3). Alpha-fetoprotein-derived GIP as a lead for cancer therapeutics	- production of peptide analogs for cancer cell attachment, spreading, and migration - displays complex aggregation behaviors similar to trimers and dimers - GIP binds to zinc and cobalt metals - suppresses growth of nine different cancers	24, 28, 49-53
4). Alpha-fetoprotein-derived GIP as a chemotherapeutic agent for cancer suppression	- GIP suppresses growth of 38 of 60 cancers *in vitro* cell lines and *in vivo* mouse mammary tumors - reacts with cytoskeleton for cell shape changes - suppresses human breast cancer growth in multiple cell culture lines	28, 48, 50-52
5). Alpha-fetoprotein-derived GIP in targeted cancer cell delivery studies	- studies of GIP targeted to various tumors - use of GIP subfragments of GIP-34, GIP-8 - radiolabeled GIP organ biodistribution studies - cell surface membrane localization studies	14, 15, 28, 50, 52-57
6). Alpha-fetoprotein-derived GIP; Mechanism of cancer cell growth inhibition	- GIP interferes with cell cycle progression of the G1 to S-phase and G2 phase to mitosis - prevents cell cycle inhibitor degradation, - induces cell cycle arrest and prevents p53 inactivation by phosphorylation - blocks K-channel activity	8, 9, 28, 58, 60-62

sequences during peptide structure synthesis, and 2) enhancement of cell targeting specificity using new and novel membrane electrostatic alterations [66, 67] The use of additional transporter partner peptides with differing AA sequences and uptake kinetics could aid in achieving optimal low micromolar intracellular concentrations of peptides required to influence various cell membrane and cytoplasmic interactions. On a comparative functional basis, use of CCPs and AMPs could readily compete with rival vector transport methodologies such as electroporation, magnetofection and lipofection, and dendrimer, and nanoparticle formation [67]. Greater understanding of the functional potential roles for CPP and AMP will require implementation of techniques employing solid state NMR for membrane disruption, X-ray diffraction, fluorescent dye tracking, circular dichroism, and polarization interferometry for study of ion channel formation

[68]. In summation, CPPs and AMPs have yet to approach “prime time” usage in the rapidly advancing field of cancer therapeutics [69].

4.2. Antimicrobial peptides and cancer: Potential use of antimicrobial-like peptides in chemotherapy

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. 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 [70]. The three types of AMPs include a) naturally-occurring; b) artificially synthesized; and c) cleaved peptide fragments from blood and extracellular matrix proteins. The present discussion presents one such example (GIP-34) of an AMP-like peptide derived from the naturally-occurring human AFP protein as a potential candidate for future cancer therapy. The biological activities of human AMP-like peptides as cancer therapeutic agents have been reviewed and reported in multiple *in vitro* and *in vivo* cancer assays [70].

This discussion presents 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 [28, 69]. The biomedical literature is replete with reports that intact full-length AFP itself can bind chemo-drugs and toxins in both a covalent and/or non-covalent manner [53, 15, 69]. Regarding AFP-derived peptides (GIP), a prior international multi-center collaborative task force reported that such GIP fragments conjugated to either fluorescent or doxorubicin (DOX) underwent tumor cell uptake and delivery of their carrier drugs. (see Ref. 50). This study demonstrated that a DOX-GIP-8 conjugate could produce cytotoxic cancer cell destruction better than DOX alone [28]. Such 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) could be wrought with dangers as previously described [70] (see above). 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 these events, Stage-1 and Stage-2 clinical trials have been conducted using recombinant human AFP to clinically treat autoimmune diseases; these trials failed to progress to Stage-3 testing in human clinical trials [72]. In contrast to the use of full-length native human AFP in the clinic, the utility of functional-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. 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 [14]. Such EGF repeats could readily contribute to enhancing growth of small cancer foci within the intended patient. Such small cancer foci may not be detectable on magnetic resonance imaging (MRI) and computer assisted tomography (CAT) scan devices.

4.3. Breast cancer, metastasis, and the microenvironment: disabling the tumor cell-to-stroma communication network

Breast cancer (BC) is the leading cause of cancer-related deaths in women worldwide. However, the majority of cancer mortalities can be attributed to cancer cell metastasis to distal organs/tissues rather than the primary tumor mass itself. The microenvironment surrounding the main tumor mass, as well as its final migratory destination,

plays a crucial role in the survival, growth, proliferation, and progression of BC [73]. Intercellular stromal cells and components of the microenvironment surrounding a tumor comprise a nurturing cubicle that establishes a communication network of cross-talk and signaling between the tumor cells and the extracellular matrix (ECM) which includes interstitial cells. This network connection enables the tumor cells to engage in metastatic-associated activities such as cell adhesion, invasiveness, mobility, migration, cell shape cytoskeletal changes, cell-to-cell contact, and basement membrane degradation [74]. An untapped therapeutic approach that might disable the communication network between cancer and stromal cells could aid in providing such an unmet need in treating metastatic disease. The intravenous administration of naturally occurring protein-derived peptides to patients might have the potential to occupy, saturate, and block receptors and metastatic binding proteins at the interstitial/ECM communication interface with tumors [75].

It has been proposed that both naturally occurring protein-derived and synthetic peptides, ranging from 8-50 AA or larger, might be candidates capable of disabling tumor-to-microenvironment communications. Such network connections are essential for supplying vascular and nutrient supplies from the ECM to the tumor; such critical locations are required for tumor survival and subsequent metastasis. The microenvironmental components surrounding tumors are required for cell nourishment and for successful cell detachment from the primary mass while taking advantage of the migration and adhesion (protein/peptide) factors already present in the extracellular and stromal cell areas.

This discussion highlights the many natural protein-derived peptides in the human body whose activities are already involved in cell adhesion, mobility, contact, angiogenesis, blood clotting, and tumorigenesis. In certain instances, a protein-derived peptide could display an opposite action from its protein of origin such as angiogenic inhibition versus angiogenic enhancement of new blood vessel growth [74]. In another example of protein-derived peptides, an amino acid segment of the protein is not cleaved from the preproprotein, but rather exposed following a conformational change of the tertiary-folded polypeptide (i.e. GIP). As a final example, metabolic stresses of excessive ligand concentrations can temporarily convert the growth enhancing AFP molecule into a transitory growth inhibitory protein. This transitory form of AFP then refolds back to its tertiary structure and full length refolded AFP once again displays the property of growth enhancement.

4.4. Breast cancer, chemokines, and metastasis: A search for decoy ligands of the CXCR4 receptor

Breast cancer (BC) is the leading type of malignant tumors in young to middle-aged women worldwide. Moreover, the survival rate in BC-patients is only 20% when associated with metastatic disease. The high mortality rate observed in BC women with metastatic disease has precipitated a major challenge revealing an unmet need to develop new therapeutic strategies in treating metastatic cancer [75]. One such approach has involved utilization of chemokines and their receptors as therapeutic targets for cancer cell metastasis. It has been previously established that a definitive correlation exists between overexpressed CXCR4 chemokine malignant cell receptors and cancer cell growth, invasion, and migration [76]. It is also widely accepted that the CXCR4 receptor, complexed to its CXCL12 ligand, plays a major role in establishing migratory pathway gradients for cancer cells migrating to distal tissues/organ sites. It would follow that chemokine decoy ligands, such as peptide antagonists and inhibitors, might serve to induce receptor blockage and impede subsequent intracellular signaling. Such peptide ligands, both synthetic and natural, are known to contribute to reducing cancer cell growth, invasion, adherence, and migration. The present discussion highlights several existing synthetic CXCR4 receptor-ligand peptide antagonists and discusses a possible strategy to develop naturally occurring human protein-derived candidates.

There is a critical need for therapeutic strategies capable of disrupting and dispersing circulatory cancer cells intended for metastatic spread to targeted host organs. The chemokine receptor

CXCR4 has been highlighted as a key player in the gradient channeling of disseminated tumor cells onto their metastatic pathway toward distant organs. Furthermore, the CXCL12/CXCR4 axis has been identified as a major factor in the promotion of BC cell growth, progression, angiogenesis, invasion, adherence and migration [77]. The CXCL12/CXCR4 complex is gradually being accepted as a prime therapeutic target to impede BC metastasis to distal organs [78]. Attempts to design and develop peptide decoy ligands as antagonist (inhibitors) of CXCR4 function have produced a host of emerging synthetic peptide candidates replacing a previous "promise unfulfilled" group that had failed to achieve clinical therapeutic usage. This cause was partially due to their propensity to interfere with CXCR4 intrabody global signaling and blockage of non-malignant cell functions. Although synthetic "designer peptides" and natural plant extracts have been found to be capable of inhibiting certain functions of CXCR4 receptors, few if any were able to block global receptor expression/signaling without interfering with normal cell homing and migration activities. Naturally occurring protein-derived peptides (i.e. GIP) have been proposed as potential candidate decoy ligands for CXCR4 that might fulfill functions not always addressed by synthetic peptide antagonist ligands. AFP peptides could be proposed as being capable of serving as decoy ligands for use in receptor blockade and neutralization by providing additional antagonist/ inhibitor activities to the existing armamentarium of synthetic peptides originally designed to impede metastasis [75].

4.5. Cancer cell cycle inhibitors (CCIs): Potential therapeutic strategies for CCI cell targeting and drug delivery

Cancer is a genetic instability disorder caused by the accumulation of successive gene mutations. Breast cancer (BC) and its metastases are the most common cause of cancer in young and older women. It is well known that abnormalities in the positive and negative modulators of the cell (growth) cycle occur frequently in many cancers including breast carcinomas (BC) [80]. Recent advancements in clinical studies have been reported regarding BC patient's survival times using a class of aromatic heterocyclic drugs termed cell cycle inhibitors (CCIs). These third generation FDA-approved CCI synthetic drugs are cyclin-dependent kinase 4/6 inhibitors which act by inhibiting progression of the G1-to-S phase transition of the cell cycle. However, deleterious side effects of these drugs can affect five main areas of patient well-being namely, 1) bone marrow depletion; 2) gastrointestinal disease; 3) increased risk for infections; 4) cardiac wave interval delays and 5) CCI drug resistance [79]. In view of reports conceiving BC patient survival times, seeking new and novel therapeutic options for CCIs has gained new importance. In order to achieve improved patient care and survival, the potential use of antimicrobial-like peptides (i.e., GIP) as CCIs is presently addressed regarding cancer cell targeting, plasma membrane penetration, and intracellular drug delivery [80].

The cell growth cycle plays a crucial role in DNA syntheses and the cell preparation and progression for mitotic cell division, replications, growth, and proliferation. The cyclin-dependent kinases, such CDK4/6 and CDK2, are the pivotal drivers of cell growth in combination with cyclin-D and Cyclin-E, respectively [81]. These Cyclin/CDK complexes normally serve to enhance cell cycle progression from the G1-to-S phase transition into the cell cycle [79]. However, the naturally occurring CCIs, such as p21 CIP and p27 KIP, are susceptible to mutational alterations leading to defective function of the retinoblastoma and p53 gene products observed in breast cancers [80]. It was the presence of dysfunctional natural CCIs in cancers that led to the pharmacological development of synthetic heterocyclic CCIs (Palbociclib, ribociclib, abemaciclib), especially the CDK-specific third generation of such drugs [82]. As noted above, these synthetic CCIs have demonstrated notable BC growth inhibition and improved survival times in patients. Unfortunately, the synthetic CCIs produce a significant array of deleterious side effects as described above.

In view of the above clinical disadvantages in using CCIs, the present discussion attempts to offer potential new and novel therapeutic options for the clinical chemo-drug use in BC patients. One potential therapeutic approach would involve the utilization of antimicrobial-like peptides (AMPs)

and/or peptide mimetics to prevent the adverse side effects, drawbacks, and disadvantages attributed to synthetic CCI drugs [66, 70]. The AMPs have been clinically employed to boost host immunity, bypass antibiotic drug resistance, reduce inflammation to microbial infections, and are now being poised for use as anticancer agents [66, 80]. The AMP-like peptides demonstrate few if any side effects in preclinical human cell culture and animal studies; moreover, these show excellent cell targeting and drug delivery properties.

In summation, one could propose that an ideal therapeutic strategy for BC patients might be to combine the advantages of both the heterocyclic CCI drugs and the AMPL peptides. Hypothetically, this could entail either one or both of two possibilities, namely, a) separate injections (administrations) of a CCI in combination with an AMPL peptide into the same patient; and/or b) injection of a patient with a heterocyclic CCI drug conjugated to an AMPL peptide. Such procedures might represent the “best of both worlds” for cancer chemotherapeutic strategies.

4.6. Disintegrin-like peptides derived from naturally occurring proteins: A proposed adjunct treatment for cancer therapy

Disintegrins constitutes a group of small proteins or peptides (45-85 amino acids) that function as natural antagonists of integrin receptor-dependent cell activities [3]. The integrins themselves comprise a superfamily of hetero-dimeric (alpha and beta chains) transmembrane cell surface receptors whose functions encompass cell adhesion, growth, migration, and angiogenesis. In contrast, the disintegrins are comprised of groups of two types of molecules, namely, a) short proteins or peptides comprising insect and animal venoms; and b) intrinsic sub-domain sequence fragments or short motifs present on large mammalian metalloprotease enzymes. Certain disintegrins bind specifically to tri-amino acid sequences (RGD, LGD etc.) located on integrins beta-1 and beta-3 chains of the hetero-complex integrins [83]. Binding at such sites can inhibit or block cell migration, angiogenesis, metastasis and platelet aggregation [84]. Recently, small disintegrin-like peptides derived from naturally occurring proteins have likewise been reported to inhibit cancer growth and adhesion functions associated with integrin-dependent cell activities. The present discussion describes examples of such disintegrin-like peptides and provides support for their proposed use in adjunct cancer therapy.

It is plausible that interference with integrin signaling by DIs or DILs could provide a rational basis for the development of adjunct treatment modalities for cancer growth, progression, metastases, and angiogenesis. Anti-integrin antibodies, disintegrins and DI-models have been used in preclinical anti-cancer therapy studies. Integrin interruption of the adhesive interaction of tumor-to-tumor cells and platelets has been shown to arrest cancer cell growth progression and metastasis [14]. Disintegrin-like agents that block or interfere with the initial attachment of integrins to ECM components, might possibly blunt signal transduction events potentially inhibiting cell proliferation, migration/invasion, angiogenesis, and platelet aggregation [74-75, 86-87]. These agents could serve to constitute a formidable armamentarium of non-toxic anti-cancer agents.

Recombinant and chimeric forms of DTs and ADAM subdomain fragments have been synthesized for use in studies in integrin inhibition of tumor growth, proliferation, adhesion, migration and angiogenesis of cancers such as liver, breast, lungs and melanomas, [84, 85, 90]. In addition, the DI-like GIP has been reported to induce apoptosis in both radio and-chemo-sensitized cultured lymphocytes [87]. It has been further reported that the ADAM-22 disintegrin subdomain is an active participant in the development of breast cancer resistance during endocrine hormone therapy in women [59, 60]. In lieu of this latter report, GIP administered to cultured MCF-7 human breast cancer cells for 7 days was shown to down-regulate the expression of ADAM-22 by 30-fold as determined by global RNA microassay analysis [9]. These data demonstrated that GIP treatment in MCF-7-cultured cells clearly down-regulated the expression of ADAM-22; in effect, this event might be capable of blocking the development of hormone resistance in breast cancer cells [88-89] (see Table 4).

Table 4. Alpha-fetoprotein-derived peptides as cell-penetrating and antimicrobial peptides.

Alpha-fetoprotein topic	Biological roles, activities	References cited
1). Alpha-fetoprotein-derived GIP: Cell-penetrating and anti-microbial peptides' usage	- Growth inhibitory peptide (GIP) can serve both as a cell-penetrating peptide (CPP) and as an antimicrobial peptide (AMP). - includes phospholipid inversion, negatively charged cell membranes, cation membrane channels, flagged for cell death by leucocytes.	63-69
2). Alpha-fetoprotein derived GIP as an anti-microbial peptide (AMP) for cancer therapy.	- produces pores by channel formation, - serves as adjunct chemotherapeutic agent - acts as a host defense peptide - AMPs react against bacteria, fungi, enveloped viruses, and transformed cancer cells	3, 15, 28, 69, 70-72
3). Alpha-fetoprotein-derived GIP as an inhibitor agent against breast cancer growth and metastases	- GIP can disable the tumor cell-to-stroma cell communication network - GIP can inhibit stromal cell-to-extracellular matrix (ECM) cross-talk and signal transduction -GIP can occupy and block cell surface receptors	73-75
4). Alpha-fetoprotein-derived GIP as a decoy ligand of the CXCL12/CXCR4 chemokine complex against metastasis.	- CXCR4 chemokine receptor can be targeted for cancer cell metastases therapy - the chemokine ligand/receptor complex directs and forms gradient pathway migration for metastatic breast cancer cells - GIP blocks metastatic migration	74-78
5). Alpha-fetoprotein-derived GIP as a cell cycle inhibitor to arrest growth of cancer cells.	- GIP was shown to inhibit cancer growth by blocking CDK4/6 and CDK2 kinases including Cyclin-D and E activities. - this inhibition results in cell cycle arrest - GIP, similar to commercial drugs, results in cancer cell inhibition and good patient survival	9, 79-82
6). Alpha-fetoprotein-derived GIP as a disintegrin-like peptide for use in cancer therapy.	- disintegrins are peptides (venoms) that block platelet aggregation and clotting - disintegrins are present in snakes and insects - can inhibit cancer cell adhesion, cell-to-cell contact, and arrest metastasis spread	9, 14, 59, 60, 83-87

5. Future directions and prospects

Both biological and biochemical research endeavors, based on the physiological activities of GIP, have been extensively pursued. Future directions of present research efforts should focus on 1) mechanism of action of AFP/GIP growth arrest, and 2) AFP/GIP therapeutic applications to cancer; these activities encompass the development of AFP-derived peptide drug delivery systems and *in vivo* storage/stability studies [9]. Investigational attention is also being directed to the nature of the peptide-to-cell surface interaction and on cell-free systems which constitute the cytoplasmic milieu. The search for additional molecular targets of the GIP molecule and its fragments could be pursued *via* Genbank identity/similarity matching, computational identity searches (BLAST programs), and computer modeling of protein/ peptide interactions [90, 91]. The separation, synthesis, and recombinant production of AFP subdomains and fragments could provide a novel source of unique or modified pharmaceutically derived AFP fragments for testing in such diverse

fields as endocrinology, hematology, immunology, and neurology. Finally, the pursuit of GIP mimetics should be followed by seeking; 1) polyphenol substitutes that could supplant the expensive use of peptide synthesis methods, and 2) the synthesis of short "active site" molecule-derived lactone cyclic esters and/or comparable Lactam family-linked chemical agents.

In the future, it might be feasible to administer short peptides to metastatic patients by injections, infusion, and osmotic pumps, or *via* sublingual routes to patients in early and/or late metastatic disease. Such peptides are also capable of down-regulating the expression of metastasis-associated proteins as previously described for GIP [9]. Peptides are short half-life molecules with good targeting properties, and adequate target binding (loading/off-loading) affinities. Natural or synthesized peptides could potentially serve as treatment adducts in combination with next generation therapeutic cancer drugs. Peptide binding, occupation, down-regulation, and saturation of ECM proteins in the interstitium could possibly serve to disable and block primary tumor growth and/or metastatic nesting sites from their ECM communication networks [91-94].

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CONFLICT OF INTEREST STATEMENT

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