Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: July 31, 2023

Citation: Mizejewski, GJ, 2023. An Alpha-fetoprotein derived Peptide Suppresses Growth in Breast Cancer and Other Malignancies: A Review and Prospectus. Medical Research Archives, [online] 11(7.2). https://doi.org/10.18103/mra.v11i7.2.4147

DOI: https://doi.org/10.18103/mra.v11i7.2.4147

ISSN: 2375-1924

REVIEW ARTICLE

An Alpha-fetoprotein derived Peptide Suppresses Growth in Breast Cancer and Other Malignancies: A Review and Prospectus

Dr. Gerald J. Mizejewski

Wadsworth Center, New York State Department of Health
Biggs Laboratory, Empire State Plaza, Albany, NY 12237, USA

Gerald.mizejewski@health.ny.gov

Abstract

Growth Inhibitory Peptide (GIP) is an alpha-fetoprotein (AFP) derived peptide found during human pregnancy, which gradually disappears following childbirth in both the woman and the newborn. Following a stress-induced conformational change in the AFP molecule, GIP is exposed on the protein surface from a concealed occult site on the unfolded full-length AFP. The exposed 34-amino acid GIP peptide then targets, blocks, and suppresses malignant growth in the mammalian body. In the present report, GIP has been demonstrated to inhibit cell growth in vitro in nine different types of cancers including breast, prostate, and ovarian among others. GIP can further assist in preventing blood clotting, arresting growth via the cytoplasmic growth cycle, suppressing tumor blood vessel angiogenesis, and inhibiting circulating cancer cell metastasis. In further studies, GIP has been reported to suppress cancer growth in 38 of 60 different cancer cell culture lines. The growth suppressed human breast cancer cell lines included MCF-7, T-47D, Bt-547, MDA-MB-231, MDA-MB-435, in addition to mouse mammary tumor implants and xenografts. Thus, GIP was found to suppress and inhibit cancer growth in both in vitro and in vivo preclinical studies.

Keywords: Alpha-fetoprotein, oncofetal protein, growth regulation, cell cycle, breast cancer, prostate cancer, malignancy, metastasis

I.Introduction

Human Alpha-fetoprotein (HAFP) is a tumor-associated oncofetal protein present during ontogenic and oncofetal growth phases. The fetal protein is synthesized during development in fetal liver, yolk sac, gastro-intestinal tissues, and in adult liver cancers.[1] HAFP has a molecular mass of 69 kDa and is largely alpha-helical, composed of three domains of 200 amino acids (AA) each.[2] HAFP is a single-polypeptide chain containing 3-5% carbohydrate; it exhibits a triplicate domain structure configured by intramolecular loops dictated by disulfide bridging, resulting in a helical V- or U-shaped structure first demonstrated by Luft and his associates.[3] The GIP peptide itself was first discovered in the author's (GJM) laboratory in 1996. See Table -1 legend, Ref-GIP

In the clinical laboratory, HAFP polypeptide has been employed as a tumor and gestational age dependent fetal defect marker with dual utility as a screening agent for neural tube defects and aneuploidies;[4,5] it has also been utilized as a serum tumor marker in adults for liver, yolk sac, and germ cell cancers.[6] In recent years, AFP has been determined to be a growth factor for both fetal and tumor cells, such as breast cancer, and it enhances growth in both cell types.[7,8] In contradistinction to its growth promoting features, the HAFP molecule can undergo a conformational change that transiently converts the growth promoting fetal protein into a molten globule form which exhibits growth inhibition.[9]

Table #1: The Growth-suppressive (cytostatic) screening results* of Human AFP derived Growth Inhibitory Peptide (GIP) for multiple types of human tumor cells cultures*. Cells were exposed to the peptide for six days, fixed, and stained with sulforhodamine-ß. None of the cells' lines were dependent on estrogen for growth.

Human Tissue of Origin	Cell Line Designation	Tumor Tissue Type	Conc. Range (Molar)	% Growth Inhibition	Growth Response Degree
Colon	KM-12	AC	10^{-5}-10^{-7}	75	Suppression
	HCC-299	AC	10^{-5}-10^{-7}	80	Suppression
	Colo-205	AC	$10^{-5'}$	10	Slight Suppression
	HCT-116	AC	10^{-5}-10^{-7}	75	Suppression
Ovary	OVCAR-3	AC	10^{-5}-10^{-7}	80	Suppression
	SK-OV-3	AC	10^{-5}-10^{-7}	60	Suppression
	IGROV1	AC	10^{-5}-10^{-7}	75	Suppression
	OVCAR-4	AC	10^{-5}-10^{-7}	85	Suppression
Breast	MCF-7	AC	10^{-5}-10^{-7}	80	Suppression
	MDA-MB-231	AC	10^{-7} only	80	Suppression
	MDA-MB-435	AC	10^{-5}-10^{-7}	70	Suppression
	BT-549	AC	10^{-6}-10^{-7}	25-40	Moderate Suppression
	T-47D	AC	10^{-5}	25	Slight Suppression
Prostate	PC-3	AC	10^{-6}-10^{-7}	80	Suppression
	DU-145	AC	10^{-5}-10^{-7}	90	Suppression
Non-Small Cell Lung	HOP-62	CA	10^{-5}-10^{-7}	75	Suppression
	NCI-H226	CA	10^{-5}	5-10	Slight Suppression
	NCI-H460	CA	10^{-5}-10^{-7}	80	Suppression
Melanoma	UACC-62	Epithelial	10^{-4}-10^{-7}	80	Suppression
	SK-MeL-28	Squamous	10^{-4}-10^{-7}	35	Mild Suppression
	SK-MeL-5	Squamous	10^{-5}	10	Slight Suppression
	SK-MeL-2	Squamous	10^{-5}-10^{-7}	50-75	Moderate Suppression
	UACC-257	Squamous	10^{-5}-10^{-7}	75-80	Suppression
Central Nervous System	SF-295	CA	10^{-5}-10^{-7}	80	Suppression
	Sf-539	CA	10^{-5}	15-20	Slight Suppression
	U-251	CA	10^{-6}-10^{-7}	45	Moderate Suppression
	SNB-75	CA	10^{-6}-10^{-7}	50	Moderate Suppression
Kidney	TK-10	Renal CA	10^{-4}-10^{-7}	85	Suppression
	RXF-393	Renal CA	10^{-6}-10^{-7}	45-50	Moderate Suppression
	A498	Renal CA	10^{-4}-10^{-7}	75	Suppression
	ACHN	Renal CA	10^{-7}-10^{-7}	80	Suppression
	CAK-1	Renal CA	10^{-5}-10^{-7}	50-75	Moderate Suppression
White Blood Cell	K-562	Leukemia	10^{-7}	45	Moderate Suppression
	Molt-4	Leukemia	NA	10-15	Slight Suppression
	SR	Leukemia	10^{-7}	25	Slight Suppression
	RPMI-8236	Leukemia	10^{-6}-10^{-7}	15-25	Slight Suppression
	CCRF-CEM	Leukemia	10^{-5}	5-10	Slight Suppression

AC= Adenocarcinoma, CA= Carcinoma

*National Cancer Institute Therapeutics Screening Program, Bethesda, MD, used with permission.

Data derived and extracted from Ref.[15,16,20].

Ref. GIP: Mizejewski., Dias, JA, Hauer CR, Henrikson KP, and Gierthy J. Alpha-fetoprotein derived synthetic peptides: assay of an estrogen-modifying regulatory segment. Mol Cell. Endocrinol 1996 118: (1-2):15-23

Table 2: Breast Tumors Suppressed by Growth Inhibitory Peptide (GIP)

Cell Line Designation	Organism of Origin	Cancer Tissue Type	Receptors Detected	Tumor Cell Morphology	Oncogenes Detected	In Vitro/ In Vivo Model	Growth Response Peptide	% Growth Inhib.	Optimal Conc. (M)	Treat. (Days)
MCF-7	Human	Glandular adenocarcinoma	Estrogen	Epithelial	Wnt 7h	In Vitro (CC)	Suppression	80	10^{-7}	6
T-47D	Human	Ductal carcinoma	Prolactin Progesterone Estrogen Androgen	Epithelial	Wnt 7h	In Vitro (CC)	Suppression	25	10^{-5}	6
BT-549	Human	Ductal papillary	Estrogen	Epithelial	NR	In Vitro (CC)	Suppression	55	10^{-6}	6
MDA-MB-231	Human	Glandular adenocarcinoma	EGF TGFα	Epithelial	Wnt 7h Wnt 3	In Vitro (CC)	Suppression	90	10^{-7}	6
MDA-MB-435	Human	Glandular adenocarcinoma	EGF TGFα	Epithelial	Wnt 7h Wnt 3	In Vitro	Suppression	70	10^{-7}	6
GI-101 TAMOX-Resis	Human	Ductal carcinoma	EGF Estrogen ER	Epithelial	Neu	In Vitro	Suppression	75	.5µg/day	65
						In Vitro	Suppression	88	10^{-12}	6
EMT-6	Murine	Mammary derived sarcoma	EGF TGFβ	Spindle	NR	In Vivo	No effect	00	None	28
6WI-1 Ref #1	Murine	Adenoacanthoma	NR	Squamous (ascites-adapted)	NR	In Vivo	Suppression	47	1µg/day	12
MCF-7 (foci assay)	Human	Glandular adenocarcinoma	Estrogen	Epithelial foci (over growth)	Wnt 7h	In Vitro (CC)	Suppression	65	10^{-10}	14

Data derived and extracted from Refs.[15,16,20,21].
NR= not reported
CC= cell culture
TAMOX-Resis= tamoxifen- resistant
Ref. 33= Breast Cancer Res. & Treat Vol. 63:41-52 2000

EGF = Epidermal Growth Factor
TGFα= Transforming Growth Factor alpha
Wnt = Wingless Drosophilia Homalog
Acanthoma= Adenoid squamous cell carcinoma
Murine= Mouse

II. Physical Features of GIP:

Some of the most potent growth inhibitors known to date include peptide fragments derived from abundant plasma or extracellular matrix (ECM) full-length proteins that themselves do not inhibit growth until cleaved from the mother protein. The containment of a class of growth modulatory peptide segments included within the structure of several circulating body proteins (their intrinsic peptides) appears to be a recurring theme in the field of signal transduction and growth regulation. A recent published example was an occult (cryptic) binding site for tenascin within the fibronectin molecule.[10] The encrypted tenascin binding site was only detectable on cleaved proteolytic fibronectin fragments and when fibronectin itself was in an extended linear configuration.[11]

Human AFP has been reported as a serum protein capable of assuming a molten globular configuration (form) following exposure to various stress/shock environments and excessive on-and-off loading of ligands.[12,13] This loading effect often occurs following activation of a "hot spot" on the protein; the hot spots are characterized as buried sites within the molecule which are tightly packed.[14] It is the third domain of HAFP that contains an occult "hot spot" named, growth inhibitory peptide (GIP) amino acid segment that lies encrypted in the protein's native, compactly folded tertiary form.[15] This site has been reported to become accessible via a conformational change involving a rotational molecular hinge.[16] The encrypted growth inhibitory peptide (GIP) epitope on HAFP is not detectable using present day commercial polyclonal or monoclonal antibodies produced against full-length AFP. However, polyclonal antibodies have been produced against the free GIP peptide.[9] This hidden site has been shown to be exposed by partial unfolding of the native full-length protein following exposure of the fetus to stress/shock cell/tissue environments.[17,18] The 34-amino-acid sequence of this occult peptide segment, produced as a synthetic peptide fragment, has been purified, characterized, and assayed for biological activity.[17,18,19] A major trait of the 34-mer synthetic peptide was found to be a growth suppressive property with both neonatal and tumor cells; hence the name, Growth-Inhibitory Peptide (GIP). However, GIP has multiple other functions as described below.

III. The Multiple Functions of Growth Inhibitory Peptides

A summary of additional functions engaged by the 34-mer GIP are varied and multiple in number. The GIP segment is involved in the following developmental activities,[20-22]:

1. Inhibition of immature rodent uterine growth;
2. Protection from insulin and estrogen toxicity in pregnancy;
3. Inhibition of blood vessel angiogenesis surrounding tumors;
4. Prevention of toxic hyperestrinism in pregnant mice;

In studies of human cancer growth,[15,16,20,21,23] GIP was capable of:

A. Inhibition of estrogen-dependent and independent breast cancer growth (in vivo and in vitro);

B. Suppression of growth of tamoxifen-resistant breast cancer cells;
C. Blockage of cell-to-cell contact inhibition in breast cancer cells;
D. Inhibition of cancer growth in 38 of 60 different cell cultured lines including breast, prostate, ovarian, central nervous system cancers, melanoma, kidney, lung, and colon;
E. Growth suppression in multiple human breast cancer cell lines including MCF-7, T-47D, Bt-547, and in Sarcoma 6WI-1 isografts in the mouse 6WI-1, and in vivo hollow fiber cancer assays in the NCI;
F. Inhibition of platelet aggregation in vitro studies;[15,16,19]

The remarkable aspect of all in vivo assays was the total lack of any GIP-induced harmful and/or toxic side effects. Finally, as a cell surface membrane disrupter, GIP has been demonstrated to inhibit/and suppress cancer cell spreading, migration, cell-to-cell contact, cell-to-extracellular matrix, and cancer metastasis in various animal models.[15,16]

A) The mechanism of action of the growth inhibition and cell membrane disruption of GIP-34 has been uncovered and is now well understood. Overall, the growth suppression of GIP-34 involves interference with the cell growth cycle, multiple cell signaling transduction cascades, and protein-to-protein cross-talk interactions. Blockage of the internal cancer cell growth cycle results in the following:[20-27]

1. Cell cycle "G1-to-S-phase" arrest;
2. Prevention of p27 and p21 cyclin inhibition by ubiquitin degradation;
3. Protection of p53 from inactivation by phosphorylation;
4. Blockage of K+ ion channels and transient receptor potential channels (TRP) formed by estrogen and epidermal growth factors;[20-24]

B) Additionally, acting as a chemotherapeutic adjunct agent, GIP is capable of alleviating the side effects of:[20,21]

1. Tamoxifen resistance;
2. Uterine hyperplasia;
3. Preventing blood clotting;
4. Herceptin antibody resistance;
5. Radio-resistance and chemo-resistance of drugs;
6. Cardiac arrhythmias;
7. Doxorubicin bystander cell toxicity.

C) Finally, GIP-34 could further serve as a cancer preventative and therapeutic agent by:[7,8,25,31,32]

1. Acting as a decoy ligand for the CXCR4 chemokines receptor to inhibit cancer metastasis;
2. Mimicking disintergrins by inhibiting cancer cell growth, migration, angiogenesis, and cell spreading;
3. Blocking circulating tumor cells from initiating the metastatic process;
4. Disabling cell-to-stromal cell communication by inhibition of cytoskeletal factor activities required for cell migration and cell shape alterations;
5. Serving as antimicrobial peptides and cell-penetrating peptides for cell entry, drug delivery, and pore/channel formation.

IV. Uses and Additional Functions of Growth Inhibitory Peptide (GIP) including Physical Features

GIP is a peptide fragment derived from a naturally occurring protein called alpha-fetoprotein as discussed above. The peptide exhibits long shelf-life, and the lyophilized powder can be stored in a dry state at room temperature, and in the dark for long periods of time. GIP can be given by oral administration and could be developed into a pill-form (capsule) for future human medication and cancer prevention. The peptide is well-tolerated in animal studies, is mechanistically novel, and can be used in combination with/ or conjugated to chemotherapeutic drugs such as tamoxifen and doxorubicin.[20,21] A major advantage of using the GIP peptide is that no toxic side-effects have ever been observed or reported in over 1,000 animals utilized in pre-clinical trials, even at extremely high doses. Some of these effects of the GIP peptide can be explained by its cytostatic rather than its cytotoxic activity. 15 The evidence for the lack of toxicity in animals was determined by observation and measurement of body weights, cage activity, fur texture, individual organ weights, histological analysis, behavioral activities, longevity, and animal death records. The peptide can further complement the use of tamoxifen by alleviating the uterine hyperplasic side effects when administered in peptide-tamoxifen combinations.[20] Tamoxifen binds to the human estrogen receptor (ER), but does not activate it, while GIP is able to bind to the receptor and inhibit the serine-118 phosphorylation of the ER. In comparison, GIP is similar to tamoxifen, in that, it is capable of binding to the ER.[27-33]

The GIP peptide has the advantage of being both a cell penetrating peptide (CPP) and a channel blocker depending on the peptide concentration as demonstrated by electrophysiologic studies employing measurement of amperage and voltage potentials.[30,31] The CPPs are known to gain entrance into cancer cells by disrupting or disturbing the bilipid cell surface membrane and corkscrewing themselves into the plasma membranes of cells which display an overall net negative cell surface charge as usually displayed by cancer cells.[30,31] Hence, cells destined for apoptosis including cancer cells, are known to undergo a cell surface membrane lipid inversion (lipid flip) by switching sphingomyelin with phosphatidylcholine or phosphatidylserine, thus providing a negative charge to the apical surface of the cancer cell.[3] The negative-charged cell membrane surface not only flags cells intended for targeted apoptosis by white blood cells, but also designates the cell as a candidate for cell penetration and transmembrane passage. Thus, CPPs like GIP, do not attach or bind to positively charged normal cells, but rather to cells displaying a net negative charge on their cell surface, as observed in all cancer cells. This procedure could provide the basis for target specificity of the peptide in searching out cancer cells, and not bystander non-malignant cells. The ion channels affected by CPPs (GIP) are largely voltage-dependent and are selective for

cations such as Ca++, K+, and Na+ ions. GIP has been confirmed to affect voltage-gated K+ channels as shown in microarray data analysis and in electrophysiology studies.[26-28] In contrast, a short amino acid sequence peptide of GIP (GIP-8), does not show CPP activities, but instead exhibit channel blocker activity that eventually results in down-regulation of ion channel passage.[21]

V. Advantages in the use of GIP as a Biomedical Treatment Aid:

Other advantages in the use of GIP-34 stems from its potential use as radiosensitizing and chemosensitizing agents as demonstrated in previous publications.[20,21] One such report involved irradiated thymocytes described by Mizejewski et al.[15, 16] These results showed that gamma X-ray exposure of mouse thymocytes incubated overnight in the presence of 10-8 M to 10-10 M GIP enhanced apoptosis in irradiated thymocytes. Such results suggested that GIP could be utilized as a tumor cell radio-sensitizing agent prior to or during chemotherapy. In additional studies, GIP was employed as a chemo-sensitizing agent when used prior to treatment in combination with tamoxifen or doxorubicin.[20,21] In both instances, the anti-cancer effect of GIP combined or conjugated to such a chemotherapy drug was enhanced in cell cultures of T47D breast cancer and glioblastoma cells; furthermore, it may be plausible that the drug resistance reported in chemo-agents could be bypassed by the use of GIP peptides. Additionally, the GIP peptide might serve as an allosteric drug in that the peptide can dock to intrinsic target docking sequences at a site other than that of the major ligand binding pocket of the protein; as was demonstrated by computer modeling of the GIP-to-protein docking studies.[23,25,36,37]

Another unexpected advantage of using a GIP fragment of AFP was found to be enhancement of the immune response(blast transformation) to lectins such as Con-A and to serve as AFP antigenic epitopes for T-cell sensitization of cell-mediated immune responses. In the induction of a T-cell mediated immune responses, two juxtaposed sequences on GIP-34 were demonstrated to serve as epitopes for antigen presentation to dendritic cells and T-cells as a means to induce production of cytotoxic lymphocytes directed against AFP bound to hepatoma cells.[38] Thereby, GIP should be capable of serving as a growth suppressive agent against hepatoma cells in culture; a published report has confirmed this prediction.[37,38-40] In other reports, the GIP segment was found to be effective as an anti-angiogenic factor during chick development and in mouse cancer cell cultures.[16] Furthermore, GIP may prove to be efficacious as a breast cancer therapeutic agent if used in conjugation with tamoxifen therapy due to both their anti-uterotrophic (hyperplasia) properties; additionally, GIP could be used as an inhibitor of platelet aggregation to aid in preventing blood clots observed in human patients undergoing tamoxifen treatment and various vaccinations.[15,16] Hence, GIP could also be effective as an anti-metastatic agent due to its ability to inhibit cell spreading, platelet aggregation, and cellular adhesion to ECM proteins.[15]

VI. Development of a Nucleotide Sequence of GIP: Potential for a "Trojan Horse" Cancer Therapy

Chinese Investigators have attempted to apply a genetic engineering process in vitro by constructing a nucleotide (DNA) sequence of GIP intended for cancer gene therapy.[40] These scientists produced a translated nucleotide sequence of GIP, developed expression vectors, special primer sequence designs, transfection plasmids, and amplification of the gene for GIP. RNA was originally extracted from the full-length AFP secreted from hepatoma cells in culture; then by employing reverse transcription, cDNA was obtained. Cultured cancer cells of human liver, lung, prostate, and breast were infused with the GIP- gene construct. The gene product was administered to the cancer cells on days 2, 4, 6 and cells harvested on day 7. Isolated cells were subjected to flow cytometry followed by MTT sulforhodamine analysis for cytotoxic activity; the results showed significant growth suppression in all cancer cell types tested. This study demonstrated the "proof-of-principal" that gene therapy could be applied to cultured cancer cells by infusion of the GIP nucleotide sequence into cells via a "trojan horse" concept. Thus, growth suppression (and possibly lethality) of cancer cells could be induced by introduction of the GIP gene sequence into such cells to initiate growth inhibition from within the cancer cell.

VII. Use of GIP in the Diagnosis and Treatment of Human Patients in each of the Four Stages of Cancer Development

The use of GIP in cancer treatment must, by the very nature of GIP, be used against either

1) Very early small tumor growth (foci);
2) Post-surgical ablation of medium-to-small tumor growths or;
3) Small metastatic cell foci.

The GIP peptide would be no match for large (golf ball/ping-pong ball sized) tumors. Such a cancer would be too much of a tumor load for GIP treatment to have any effect. GIP is cytostatic (not cytotoxic) and arrests further growth but does not destroy tumor tissue already present. Small tumor cell foci, when growth suppressed, have been observed to eventually die by a process termed "programmed cell death" (apoptosis). If GIP could prevent further growth of small tumor foci, it could find a niche in the various cancer preventative treatment modalities already in existence. Alternately, GIP could be employed to prevent cancer foci from even growing in the first place. Dr. Barbara Richardson showed that HAFP in pregnancy already does this, i.e., initiates cancer chemoprevention in pregnant women.[41] Human pregnancy AFP protects women from postmenopausal breast cancer 20 years after a full-term delivery.

Diagnostic Approaches:

GIP could have two venues for cancer diagnosis, namely,

1) The transformed AFP (tAFP) ELISA kit for blood analysis used for pre- and post-surgical monitoring,
2) Tumor localization of small tumor masses (foci) and metastatic clumps of tumor cells.

Primary tumor cell localization and metastatic sites could be revealed via GIP labeled with radioactivity, fluorescence, or heavy metals (Zn^{2+}, Fe^{2+}, Co^{2+}, etc.). Heavy metals would allow PET scanning (Positive Emission Topography) and/or nuclear magnetic resonance (NMR). GIP might be very useful in locating and imaging small in situ tumor masses in Stage -I and II, in nearby lymph nodes (Stage III), and in distant metastatic sites (Stage IV). GIP labeled with Biotin/avidin could also be used for staining of pathology slide specimens obtained from surgery.

Therapeutic Approaches

For therapeutic uses, GIP could find application in several formats, namely, GIP-conjugated drugs, bio modulated growth arrest, and tumor cell-induced platelet aggregation inhibition of metastasis.

A) GIP-conjugated Chemo-drugs

The GIP molecule could be conjugated to a whole host of chemotherapeutic drugs that would allow drug delivery to tumor cell for destruction (cell killing). In this capacity, GIP could be converted into a cytotoxic drug by permanent conjugation of the toxic drug (i.e. doxorubicin) to the GIP molecule and sending it into a cell (like a smart bomb). Also, GIP could be labeled with radioactive nucleides (i.e. I^{131}) to deliver lethal doses of radioactivity into cancer cells. Fatty acids could be bound to GIP for delivery into cells; microwave beams directly pinpointed to the tumor site have been shown to cause cell destruction by total cell membrane lysis. Such cytotoxic modalities could be applied at each cancer stage (I through IV) provided the cell/tissue mass was small. It would be especially effective following post-surgical ablation of the primary tumor.

B) Biomodulated Growth Arrest:

GIP injections, osmotic pumps, or pellet depositions could also be applied at any cancer stage (I through IV) with a small tumor body burden. GIP as a biological response modifier, could be particularly effective as a radio-sensitizing agent. GIP would first be applied for a defined period of time, then the tumor mass could be irradiated in a pinpoint fashion. This could result in cancer cell death as previously observed in isolated cell preparations. GIP administered as pellets and/or from osmotic pumps should also be effective as an adjunct agent for chemotherapeutic drug treatment. GIP, given prior or together with cytotoxic drugs (cisplatin, doxorubicin) could arrest growth in small cancer foci, synchronize the cells, and thus prime the cell for cytotoxic exposure at the G1 phase prior to cell division. Thus, GIP could physiologically arrest the cancer cell growth making them more vulnerable to chemotherapy.

C) Tumor-Induced Platelet Aggregation (TIPA):

TIPA was first described by Gasic in the early 1970s. Tumor cells migrating in blood vessels are routinely observed to congregate with circulating platelets with the total complex attaching to the blood vessel wall. The attachment allows metastasizing tumor cells to gain anchor points in their vascular migratory journeys. The blood of cancer

patients is already in a hyper-coagulable state and blood clotting easily occurs attracting more platelets during the clumping process. Once anchored to the blood vessel wall, the tumor cells detach and migrate through the walls of the blood vasculature gaining access into cells of the surrounding tissues. GIP can prevent this invasion by interfering/inhibiting the formation of platelet aggregation sites so that tumor cells have nowhere to attach to blood vessel linings. In doing so, GIP could prevent, inhibit, or dampen the formation of metastatic migratory routes that allow tumor cell passage into distant tissues. Such a process a metastatic migration could inhibit cancer stage III and stage IV.

VIII. Conclusions:

It must be clearly stated that the in vitro and in vivo studies of the GIP peptide were performed mostly in cell culture and in small animal preclinical studies using multiple cell cultures and hundreds of mice, rats, and other rodents. It was also determined that GIP would not be effective against large tumor masses as found in many human adults. Since GIP is a cytostatic and not a cytotoxic anti-cancer agent, the AFP-derived peptide would find better use as a cancer preventative and post-surgical agent rather than a cancer chemotherapeutic drug. As described above in Section IIIA, GIP inhibits cell growth by blocking cell cycle growth progression causing arrest at the checkpoint of the G1-to-S-phase of the cell growth cycle. Thus, as a result of GIP treatment, the cancer cell cycle is prevented from progression to the mitosis phase, the growth of the cancer cells is halted, and cancer cell proliferation is prevented. In summation, the GIP peptide could also be effective as a one-a-day capsule cancer preventative agent directed against small cancer foci or clusters that arise daily in the human body.

DISCLOSURES:

Funding:

None; no U.S. federal grants were used in the preparation of this paper.

Conflicts of interest:

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

Acknowledgement:

The author extends his thanks and gratitude to Ms. Sarah Andres for her commitment and time expenditure in the skilled typing and processing of the manuscript, references, and tables of this report.

CORRESPONDING AUTHOR:

G.J. Mizejewski, Ph.D

Division of Translational Medicine, Molecular Diagnostic Laboratory

Wadsworth Center, New York State Department of Health

Biggs Laboratory, Empire State Plaza

Albany, NY 12237

Telephone: 518-486-5900

Email: Gerald.mizejewski@health.ny.gov

References

1. Mizejewski, G.J. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp. Biol. Med. 2001. 226(5):377-408.

2. Mizejewski, G.J. Alpha-fetoprotein as a biologic response modifier: relevance to domain and subdomain structure. Proc. Soc. Exp. Biol. Med. 1997. 215(4):333-362.

3. Luft, A.J., Lorscheider, F.L., Structural analysis of human and bovine AFP by election microscopy, image processing, and circular dichoism. Biochem. 1983 22:5978-5981.

4. Mizejewski, G.J. Biological roles of aloha-fetoprotein during pregnancy and perinatal development. Exp. Biol. Med. 2004. 229(6): 439-463.

5. Mizejewski, G.J. Physiology of alpha-fetoprotein as a biomarker for perinatal distress: relevance to adverse pregnancy outcome. Exp. Biol. Med. 2007. 232(8):993-1004.

6. Mizejewski, G.J, Head and neck germ cell tumors: effectiveness of alpha-fetoprotein as a diagnostic biomarker, BAOJ Cancer Research & Therapy. 2017. 4:52-58

7. Mizejewski, G.J, Breast Cancer, metastasis, and the microenvironment: disabling the tumor cell-to-stroma communication network, Journal of Cancer Metastasis and Treatment, 2019 Doi: 10.20517/2394-4722.2018.70.

8. Mizejewski, G.J. Breast Cancer, chemokines, and metastasis: a search for decoy ligands of the CXCR4 receptor. Journal Neoplasms. 2018 1:1-5.

9. Mizejewski, G.J. Should "transformed alpha-fetoprotein" be considered a potential biomarker for adverse term pregnancy risk: an opinion letter. Gynecology and Women's Health Care. 2020 2:1-6.

10. Ingham KC, Brew SA, Erickson HP: Localization of a cryptic binding site for tenascin of fibronectin. J Biol Chem 2004. 279: 28132-28135

11. Podolnikova NP, Yakubenko VP, Volkov GL, Plow EF, Ugarova TP: Identification of a novel binding site for platelet integrins alpha IIb beta 3 (GPIIbIIIa) and alpha 5 beta 1 in the gamma C-domain of fibrinogen. J Biol chem. 2003. 278: 32251-32258

12. Uversky NV, Kirkitadze MD, Narizhneva NV, Potekhin SA, Tomashevski AY. Structural properties of AFP from human cord serum: The protein molecule at low pH possess all the properties of the molten globule FEBS Lett. 1995. 364: 165-176.

13. Uversky NV. Narizhneva NV, Ivanova TV, Tomashevski AY. Rigidity og human AFP tertiary, structure is under ligand control. Biochemistry. 1997. 36:13638-13645

14. Dudich IV, Semenkova LN, Dudich EI. Reversible conformational changes in the teritary structure of the human AFP molecule induced by ligand-protein and protein-protein interactions. Tumor Biol 1990. 19:34

15. Muehlemann M, Miller KD, Dauphinee M, Mizejewski GJ. Review of Growth Inhibitory Peptide as a biotherapeutic agent for tumore growth, adhesion, and metastasis. Cancer Metastasis rev. 2005. 24:441-467

16. Mizejewski GJ, Butterstein G. Survey of functional activities of alpha-fetoprotein derived growth inhibitory peptides: Review and Prospects. Curr Protein Pept Sci. 2006. 7:73-100.

17. Bennassayag C, Rigoud V, Hassid J, Nunez EA. Does high polyunsaturated free fatty acid level at the feta-maternal interface alter steroid hormone message during pregnancy? Prostaglandins, *Luekoirienes and Essential Fatty Acids.* 1999. 60(5-6).393-399.

18. Vallete G, Martin ME, Benassayog C, Nunez EA (1989). Conformational changes in rodent and human alpha-fetoprotein: Influnece of fatty acids at biophysics Acto (BBA)- Protein structure and molecule EnzymoLogy. 1997(3): 302-312.

19. Mizejewski GJ, MacColl R. Alpha-fetoprotein growth inhibitory peptides: Potential leads for cancer therapeutics. Mol Cancer Ther. 2003. 2: 1243-1255.

20. Mizejewski GJ, Mirowski M, Garnuszek P, Maurin M, Cohen B.D, Poiesz BJ, Posypanova, GA, Makarov, VA, Severin ES, Severin SE. Targeted delivery of anti-cancer growth inhibitory peptides derived from human alpha-fetoprotein: review of an International Muilt-Center Collaborative Study. J. Drug Target. 2010. 18(8):575-588.

21. Mizejewski GJ, (2011) Mechanism of cancer growth suppression of alpha-fetoprotein derived growth inhibitory peptides (GIP): Comparison of GIP-34 versus GIP-8 (AFPPep). Updates and Prospects. Cancers. 2011. 3(2):2709-2733.

22. Mizejewski GJ, Smith G, Butterstein G. Review and proposed action of alpha-fetoprotein growth inhibitory peptides as estrogen and cytoskeleton-associated factors. Cel Biol. Int. 2004. 28(12):913-933.

23. Mizejewski GJ. The alpha-fetoprotein (AFP) third domain: a search of AFP interaction sites of cell cycle proteins. Tumor Biol. 2016 37(9):12697-711. Epub 2016/10/27.

24. Mizejewski GJ. Breast Cancer and cell cycle inhibitors (CCIs): potential therapeutic strategies for CCI cell targeting and drug delivery. Current Advances in Oncology Res. & Therapy (Issue-1) 2019: 1-8.

25. Mizejewski GJ. The third domain ligand binding fragment of alpha-fetoprotein: detection of Metastasis-associated molecular targets. Cancer Therapy & Oncology. 2017 6:1-8.

26. Mizejewski GJ. Breast cancer and transient receptor potential (TRCP) cation channels: Is there a role for non-selective TRP channels as therapeutic cancer targets: a commentary. Intl. Journal of Cancer Res. And Development. 2017. 2:4-6.

27. Mizejewski GJ. The third domain fragments of alpha-fetoprotein (AFP): mapping AFP interactions with selective and non-selective cation channels. Curr. Topics Peptide Protein Res. (in press) 2016.

28. Mizejewski GJ. Cancer, circulating tumor cells, and metastasis: could protein-derived peptide fragments impede brain metastasis? Journal of Cancer Metastasis and Treatment, 2018. 10:205-225.

29. Mizejewski GJ. Disintergrin-like peptides derived from naturally occurring proteins: a proposed adjunct treatment for cancer therapy. Intl J Res Mol. Mech. 2020. 5(2): 2381-3318.

30. Mizejewski GJ. Antimicrobial peptides and cancer: potential use of antimicrobial-like peptides in chemotherapy. J. Cancer Biol. Therap. 2019. 5:233-242.

31. Mizejewski GJ. Cell-penetrating versus antimicrobial peptides: comparison of potential use as cancer therapeutics Journal of Oncology Research Forecast. 2019 2:1013-1015.

32. Mizejewski GJ, Muehlemann M, Dauphiee M. Update of alpha fetoprotein growth inhibitory peptides as biotherapeutic agents for tumor growth and metstasis. Chemotherapy 2006. 52(2): 83-90.

33. Vakharia D. Mizejewski GJ. Human alpha-fetoprotein peptides bind estrogen receptor and estradiol and suppress breast cancer. Breast Cancer Res. Treat. 2000. 63(1):41-52.

34. Mikhail Bogdanov, Jun Xie, Phil Heacock, William Dowham. To flip or not to flip: lipid-protein charge interactions are a determinant of final membrane protein topology. J Cell Biol. 2008 Sep 8; 182(5):925-35.

35. Mizejewski GJ. Review of the adenocarcinoma cell surface receptor for human alpha-fetoprotein; propsea identification of a widespread mucin as a the tumor cell receptor. Tumour Viol. 2013. 34(3):1317-1336.

36. Mizejewski GJ. The alpha-fetoprotein third domain receptor binding fragment: in search of scavenger and associated receptor targets. J. Drug Target. 2015. 23(6):538-551.

37. Mizejewski GJ. Alpha-fetoprotein (AFP)-derived peptides as epitopes for hepatoma immunotherapy: a commentary. Cancer Immunol. Immunother. 2019. 58(2):159-170.

38. Butterfield LH, Ribas A, Dissette VB, Lee Y, Yang JQ, et al. A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. Clin. Cancer Res. 2006. 1: 2817-2825

39. Butterfield LH, Koh A, Meng W, Vollmer CM, Ribas A, et l. (1999) Generation of human T-cell responses to an HLA-A2. 1-restricted peptide epitope derived from alpha-fetoprotein. Cancer Res 1999. 59: 3134-3142.

40. Zhang C, Jiang W, Li H, Hou W, Li G: "Enhanced Hepatoma-cell GIP-36, Peking University Chinese Patent filing, number CN201410166756.3A/B, 2014, patent pending.

41. Richardson BE, Hulka BS, David Peck JL, Huges CL, van den Berg BJ, Christianson RE, Calvin JA. Levels of maternal serum alpha-fetoprotein (AFP) in pregnant women and subsequent breast cancer risk. Am L Epidemiol 148:719-727.1998

42. Gasic GJ, Gasic TB, Galanti N, Johnson N, Murphy S. Platelet-tumor-cell interactions in mice. The role of platelets in the spread of malignant disease. Int J Cancer. 1993. 11:704-718.