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Research Article

Unveiling the Relationships of Calcium Ions, Transient Receptor Potential Channels and Fetal Peptides with Calcium Induced Cell Death. A Review and Commentary

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ABSTRACT

Extracellular concentrations of vascular calcium ions are high, while intracellular levels are three logs lower in concentration. However, high calcium levels in the cell cytoplasm are toxic to cells invoking programmed cell death (apoptosis) and/or autophagy. The bioregulation of calcium influx into cells (both normal and malignant) is tightly regulated by a family of cell membrane non-selective cation channels termed "Transient Receptor Potential" (TRP) channels. Such channels allow the passage of calcium, magnesium and manganese into cells. At the cell bilayer membrane, the TRP channels are embedded in a cluster of cell signaling proteins, (including protein kinases), known as a "signal plex." This molecular signaling complex is essential to maintain, support and stabilize the TRP channels within the bilayers of the cell membrane. The TRP channels within the signal plex cluster can interact with an alpha-fetoprotein-derived peptide (GIP) which is capable of affecting both the TRP cell membrane voltage current and the electrical resistance. These electrophysiological effects render the TRP channel susceptible to a GIP-induced voltage effect which locks the calcium channel in an open position for a defined time period. Hence, an unrestricted and non-regulated opening of the TRP channel can result in Ca++ cytoplasmic toxicity and ultimately, cell death via apoptosis.

Keywords: Alpha-Fetoprotein, Bone marrow, Breast cancer, Calcium channels, Cations, Cancer, Cell cycle, Cell death toxicity, Chromosome, DNA repair, Gene instability, Prostate cancer, Stem cells.

1. Calcium Presence in the Body

Calcium (Ca^{++}) ions are essential for bodily cell maintenance, regulation and homeostasis in man and other mammals. All cells in the body are bathed in extracellular fluids very rich in Ca^{++} ions (10^{-3} M), while intracellular Ca^{++} ion concentrations are 3 logs lower in concentration[1]. However, normal cell membranes are permeable only to lipid soluble components and are impermeable to calcium ion influx. Thus, Ca^{++} ions are passed into cells via ion channels. Excess Ca^{++} ions are usually pumped out of the cytoplasm itself and into two other cell organelle storage compartments. Thus, excess calcium ions are normally transported first to the mitochondria and secondly into storage in the smooth endoplasmic reticulum (ER). Excess Ca^{++} ions can be pumped out of the ER by Ca^{++} associated ATPase enzymes following their activation[2]. However, in certain cases, alterations due to damage and/or disruption of the cell membrane bilayer can disable the cell surface membrane permeability barrier resulting in increased Ca^{++} ion influx into the cell cytoplasm. Overall, calcium ions are biologically very active, being capable of disrupting the cell's metabolic homeostasis resulting in excess cytoplasmic accumulation of Ca^{++} ions which can induce cytoplasmic toxicity and eventually cell death[3,4].

2. Calcium Presence in Cytoplasmic Organelles

Ca^{++} ions are toxic to cells when present in excessively high cytoplasmic concentrations as stated above[5]. However, biological cells require calcium for the proper functioning of the mitochondria (MT), an organelle that requires Ca^{++} to aid in supplying ATP energy for cell function. Moreover, the influx of high levels of cytoplasmic Ca^{++} ions can cause the MT to deplete energy (ATP) stores to become dysregulated and imbalanced; this condition results in cells literally "suffocating" for lack of energy[6]. Ultimately, calcium ions are normally stored in the endoplasmic reticulum (ER) but can also be negatively affected (overburdened) by high levels of calcium in the cell cytoplasm. The uptake of cytoplasmic Ca^{++} ions into both the MT and the ER can occur by means of cell channels and pore openings, respectively, in both organelles[7]. In the MT, Ca^{++} channel pores (see below) are localized in the outer membrane of the MT which also opens when exposed to high reactive oxygen species (ROS) stimulation. Likewise, calcium channels can also be opened in the ER when activated by nitric oxide levels[8,9].

3. The Transient Receptor Potential (TRP) Calcium Channels

Within the cell surface bilayer membrane, certain calcium ion channels can be activated by chemo- and thermal sensitizing agents such as spices, temperature and reactive oxygen species **(Table-1)**. These Ca^{++} channel types are tightly regulated by a family of non-selective cation channels called "transient receptor potential" (TRP) channels consisting of 6 different families with multiple numbers of family sub-members[10]. These members (and sub-members) include the following: 1) the canonical member (no sub-members) (TRPC); 2) the melastatin member, TRPM (1-8 sub-members); 3) mucolipin member, TRPML (1-3 sub-members); 4) polycystic member, TRPP (1-5 sub-members); 5) vanilloid member, TRPV (1-6 sub-members); and 6) a single ankyrin member, TRPA1. All such receptor subfamily members display six transmembrane domains (TD1-TM6) containing carboxyl (C) and amino-terminal (N) regions located on the intracellular side of the cell membrane with the pore forming loop situated between transmembrane domains (TD5-TD6). The prominent differences between the 6 TRP channel families lie in their C- and N-terminal cytosolic domains; these contain putative protein interactive and regulatory motifs[11] (see below).

Table 1: Listed below are the chemosensitizing agents, spices and environmental sensors or irritants that activate and/or stimulate the cation selective and non-selective channels of vertebrate cells. Such channels interact with a family of proteins referred to as Transient Receptor Potential (TRP)* channels (See Legend).

Name of Channel Stimulation/Sensor Activation Chemosensitizing Agent	Type of Cation TRP Calcium Channel Activated	Functions of Activated TRP Channels	Type of Cancer cells Affected, Suppressed	References Cited
1) Mustard seed or oil spice (Allythiocyanate)	TRP A1, TRP M8, TRP V1, TRP V4	Cell cycle arrest, apoptosis, blocks cell migration and invasion	Lung, liver, digestive tract, pancreas, colorectal cancer	59
2) Ginger Spice	TRP V4, TRP V1	Induction of programmed cell death i.e., apoptosis	Embryonic kidney cells, colorectal cancer	60
3) Wasabi (Japanese Horseradish)	TRP A 1 (Ankyrin)	Triggers cell signals to suppress programmed cell death, lipid peroxidation	Colon cancer, pancreatic, central nervous system, lung and breast	61,62
4) Cayenne Pepper, Hot Chili powder, Capsaicin, Camphor	TRP V1, TRPC6, TRPM2, TRPM7, TRPV6	Upregulates liver apoptosis or programmed cell death, growth factor	Breast carcinoma, Renal cell cancer, cervix cancer, non-cell lung cancer. Hepatoblastoma kidney, colorectal, thyroid cancer, stomach cancer	63,64
5) Menthol (peppermint oil) mint leaves (powder)	TRP M1, TRP M2, TRP M7, TRP M8, BK channels	Increases cell migration, spreading, motility, limits metastasis, tumor growth	Prostate cancer, Brain glioblastoma, skin cancer melanoma, lymphoma	65,66
6) Garlic	TRPA1, TRPM1	Activation of dendritic endothelial cells	Mast cell cancers, T-cell lymphoma, prostate, skin cancer, melanoma	67
7) Cinnamon (Cinnamaldehyde)	TRPA1, TRPM8	Gut mobility, inflammation, pain, O_2 stress	Head and neck, and squamous cell cancers	68
8) Cannabis, tetrahydrocannabinol	TRP A1, TRP V4	Induces apoptosis, programmed cell death	Lung cancer	69

Legend:

*The Transient Receptor Potential (TRP) superfamily can be subdivided into 6 subfamilies: these subfamilies include: 1) TRPC (canonical); 2) TRPV (vanilloid); 3) TRPM (melastatin); 4) TRPA (ankyrin); 5) TRPP (polycystin); 6) TRPML (mucolipin).

Overall, the TRP channels also constitute the: A) the heat and cold sensory channels (not shown) such as TRPV1 (cold) and TRPM8 (heat) sensitive channels. The chemosensory channels (sensitive to capsaicin and menthol) comprise the remainder channels in the TRPV, TRPM and TRPA subfamilies.

The TRPnon-selective cation channel proteins can be described as being grouped as homo- or heterotetramers within the bilayers of the cell plasma membrane. The transmembrane domains are α-helical proteins numbered 1 to 6 of which comprise the aminoterminal domains and (1 to 3) constitute the ligand (cation) sensing domains. The α-helical domain #4 comprises a more specific sensing ligand region, while α-helices #5 and 6 constitute the selectivity core channel through which the cations flow. The TRP channel displays consensus sequences and additional specialized regions both at the aminoterminal and carboxyterminal ends; namely, ankyrins 1,2,3,4, sites, coiled coil-N-terminus sites; a calmodulin (CAM) site and an inositol P3 (IP R) receptor site . As shown below, the third domain peptide fragments of alpha- fetoprotein (AFP) have been mapped to display AFP interactions with non-selective TRP cation channels in silica (See Tables 2 & 4) (16).

Activation of TRP channels can occur by several different chemosensors. Direct activation can result from endogenous sensors agents such as 1) diacylglycerols; 2) phosphoinositides; 3) eicosanoids; 4) anandamides and 5) reactive oxygen species (ROS) by products. Channel-activating chemosensor substances may also include various spices and other environmental agents[12] **(see Table-1)**. In summary, TRP channels are ubiquitously expressed throughout the mammalian body in multiple cells organs and tissues. The TRP channels are non-selective for cations such as Ca^{++}, Mg^{++} and Mn^{++} and are expressed in neurological, cardiovascular, metabolic, blood vascular and pulmonary tissues as well as multiple types of cancers[13]. Overall, TRP channels are involved in important biological functions in both health and disease conditions involving signal transduction mechanisms, sensory and mechanical activation, environmental sensors agents and translational products.

4. The TRP Channels in Signal Transduction Mechanisms (the Signal plex)

The location of the TRP channels within the cell membrane is such that the channel itself can be activated in concert with several other proteins that cluster together with the TRP cation channel at its point of insertion within the cell membrane. The entire cluster of different proteins in conjugation with the TRP channel is collectively called the Signalplex (signal molecular complex)[14]. Thus, the TRP channel proteins exist in macromolecular assemblies comprising nine different multiple signaling protein components[15]. The molecular members of the signalplex proteins comprise an array of potential targeting and scaffolding proteins comprising TRPs and 8 other different proteins; all of which are localized at the cell membrane bilayer and comprising the entire molecular complex[15,16]. The nine total members of the signalplex consist of the following: 1) the TRP ion channels; 2) phospholipase-C (beta); 3) Rhodopsin; 4) Protein Kinase-C; 5) Calmodulin; 6) Myosin; 7) Beta-2-adrenergic receptor; 8) Na^{+}/H^{+} Cotransporter; and 9) the ezrin-radin-moesin complex (44).

Thus, the TRP channel can be activated, modulated or modified by stimulation and participation in conjunction with members of the constituent protein members of the signalplex[17]. It appears that this complex of proteins is essential to maintain, form scaffolds and stabilize the presence of the TRP channel in proximity to the microvilli of the cell surface membrane. The molecular complexes are also in juxtaposition to lipid rafts linked to caveolae proteins near the microvilli. Lipid rafts consist of glycosphingolipid and cholesterol-enriched membrane microdomains associated with the transmembrane channel proteins of the plasma membrane[18,19]. A main member of the signal activation of the TRP channel is the phospholipase-C molecule which activates inositol 1, 4, 5-triphosphate, diacylglycerol (DAG) and polyunsaturated fatty acids (PUFAs). Further activation can be initiated by Ca^{++} release from storage areas in the smooth endoplasmic reticulum and in mitochondria[20,21]. It is also known that certain members of this signalplex are capable of linkage to G-protein coupled receptor signal transduction factors and to the actin cytoskeleton framework of the cell.

5. Proposed Scope of the Present Review

The present review proposes a plausible cell surface targeting of therapeutic AFP-derived fetal peptides (GIP) which can interact with the Transient Receptor Potential (TRP) calcium channels; such studies have used information from both: A) clinical data from patients bearing prostate and breast cancers and B) cultured human LNCaP and MCF-7 cells in culture[22,23]. Hence, the TRP Ca^{++} channels present plausible targets for a novel anti-cancer therapy using peptides derived from fetal proteins such as alpha-fetoprotein[24,25]. Calcium (Ca^{++}) is both an extracellular and intracellular signaling cation that is essential for cell growth, proliferation and survival in both normal (non-malignant) and cancer cells. Furthermore, calcium is a universal second messenger cation employed in cell cycle progression, cell proliferation and programmed cell death (apoptosis)[8,26]. TRPs are barely detectable (baseline levels) in normal healthy cells and tissues, including benign cell growth. In contrast, TRP channels are highly overexpressed in prostate and breast cancer bearing patients and in as well as in multiple other cancer patients[27,28]. Furthermore, TRPs were the first Ca^{++} channel to be clearly identified as targets both in cells and tissues from clinical cancer patients' specimens and in cultured cancer cells. This is because TRPs are highly overexpressed in cancers and especially in human breast MCF-7 and prostate LNCaP cell cultures lines. It is interesting that prostate LNCaP cultured cells display TRPs but were found to display less of the conventional L-type voltage Kv dependent Ca-channels (i.e., the dihydropyridine) type. Thus, in comparison, TRPs are not voltage-dependent channels but rather non-selective cation channels. TRP channel overexpression further correlates with cancer tumor grade progression in patients; thus, TRP levels were found to correlate with the degree of clinical malignancy in human prostate, breast and in other cancer types[29]. TRPs are highly expressed–in advanced prostate and breast cancers, metastatic cancers and androgen-insensitive cancer lesions. As suggested above, TRPs are also useful for pathology staging (biomarker) of human cancers and to predict clinical cancer outcomes. Hence, the scope of the present report is to present a plausible link between calcium ion signaling, TRPs, fetal peptides and calcium-induced cell death.

6. Evidence for Involvement of an Alpha-fetoprotein Derived Peptide with TRP Calcium Channel Signaling

A. Alpha-fetoprotein and Derived Peptides: Alpha-fetoprotein (AFP) is a tumor-associated fetal mammalian glycoprotein present during ontogenic and oncogenic growth[30,31]. Human alpha-fetoprotein (HAFP) is secreted by the yolk sac and fetal liver during pregnancy and can be re-expressed in adult cancers such as teratomas, hepatomas and yolk sac tumors of the ovary[3,4].

Thus, in the clinical laboratory, HAFP has been employed both as a tumor and gestational age-dependent fetal defect marker with utility first, as a screening agent for neural tube defects and aneuploidies and secondly, as a serum tumor marker for adult cancers[32,33]. In recent years, AFP has further been determined to be a growth regulatory protein for both fetal and tumor cells. AFP comprises a 70-kDa single chain polypeptide containing 3-5% carbohydrate and is a member of the albuminoid gene family[32].

B. Published Properties of the Growth Inhibitory Peptide (GIP): The Growth Inhibitory Peptide (GIP) is a synthetic peptide fragment derived from the naturally occurring fetal alpha-fetoprotein (AFP) polypeptide present during mammalian pregnancy[34]. An encrypted GIP peptide segment lies buried within the full-length AFP molecule and AFP can undergo a transformal change exposing the peptide in the presence of fetal stress/shock environments[34,35]; Thus, the GIP segment can be temporarily exposed following a conformational change of the entire AFP fetal protein. The exposed GIP segment on AFP has been found to be used within the fetus to prevent unwanted (dysregulated) fetal growths during pregnancy. Appropriate repair proteins and other signaling transduction pathways are coincidently utilized in re-establishing the monitoring, regulation and homeostasis of growth within the developing fetus.

Overall, Growth Inhibitory Peptide (GIP) can best be described as an AFP derived peptide sequence buried within the internal protein folds of the AFP molecule during human pregnancy. Following pregnancy, serum circulating full-length AFP levels are gradually reduced in both the woman and in the newborn[30]. During pregnancy, when a stress/shock induced conformational change in the fetal AFP molecule takes place, the GIP peptide becomes exposed on the full-length AFP protein surface stemming from within a concealed site buried within the tertiary folded AFP[35]. Within recent years, the exposed 34 amino acid GIP peptide segment has been isolated, purified and biochemically characterized; hence, GIP has been found to target, block and suppress malignant growth in man and other mammals[36-38]. In later published reports, GIP was demonstrated to target cancer cells and to inhibit cell growth of nine different types of cultured cancer cell types which included breast, prostate and ovarian among other cancers[39-41]. GIP can further participate in biological activities such as platelet blood clotting, arresting growth cell cycle, suppressing tumor blood vessel angiogenesis and inhibiting circulating cancer cell metastases **(Table-2)**. In further studies, GIP has been reported to suppress cancer growth in 38 of 60 different cancer cell culture lines provided by the National Cancer Institute (NCI). Finally, the mechanism of action of cancer cell suppression in vitro and in vivo has now been elucidated and published[42].

Table 2: Global RNA Microarray: Expression of 716 transcripts was significantly altered after 8 days of treatment with GIP as compared to treatment with the scrambled peptide. Four hundred thirty were down regulated, while 286 were upregulated.

Microarray Data: Transcripts displaying 1.0 or larger log fold (log base 2.0) decrease for genes associated with cell division and proliferation processes.		
Gene Title	Fold Decrease (-)	Cell Function
I. Cell Cycle Regulation		
1. F-Box/Wd40, Domain- 10 (FBXW10)	-14.9	P27 degradation
2. Checkpoint Suppressor-1 (CHES1)	-9.2	S-phase checkpoint
3. Cyclin-E**	-4.6	Regulates G-S transition
4. SKP2**	-4.3	Mediated p27 degradation
5. Calpain	-32.5	Cell cycle progression
6. CDC20 Cell Division Homolog	-4.3	Activates ubiquitin
II. Ubiquitin-Associated Proteins		
1. SUMO/Sentrin/SMT3 Specific Protease (SENP3)	-2.1	Lysine targeting ubiquitin
2. Ubiquitin Specific Protease-49 (MGC20741)	-2.1	Ubiquitin enzyme
3. Ubiquitin Ligase Protein Complex (KIAA0804)	-2.1	Protein degradation
III. Apoptosis Associated Proteins		
1. p53-regualted apoptosis-inducing protein 1 (P53AIP1)	-9.8	Mediates apoptosis
2. Epithelial Membrane Protein 1 (EMP1)	-5.6	Promote Carcinogenesis
IV. Calcium Associated proteins		
1. Phospholipase C, epsilon 1 (PLCE1)	-8.0	CDC25-associated
2. Solute carrier family 22 (SLC22A16)	-6.1	Cation transporter
3. Dystrophin (DMD)	-6.1	Muscle/ECM connection
4. Cadherin 13(CDH13)	-4.9	Cellular adhesion
V. Channel Associated Proteins		
1. Potassium voltage-gated channel (KCNB2)	-8.0	Shab ion channel
2. Transmembrane Channel like 5 (TMC5)	-5.2	Ion transporter
3. Potassium voltage-gated channel, KQT like (KCNQ3)	-4.0	Cation signaling
4. Calcium Channel, Voltage dependent 2 (CACNA2D4)	-2.0	Calcium signaling
5. Calcium/Calmodulin-dependent Kinase (CAMK2B)	-1.9	Calcium regulation
6. Calcineurin A gamma (PPP3CC)	-1.8	Calcium phosphate 3 protein
7. Calcium Channel, Voltage Dependent (CACNC6)	-1.8	Calcium transport

**= real time PCR

C. Review of Growth Inhibitory Peptide (GIP) as a TRP channel Agonist:

An interactive electrophysiological study employing GIP interaction with TRP Ca^{++} channels in cultured MCF-7 human breast cancer cells, was previously performed and reported[43-45]. It was demonstrated that GIP, administered to human breast cancer cells in cultures, affected the electrical voltage conductance in the cancer cell surface membrane involving TRP channels. Compared to calcium influx into normal cells, the measured inward voltage current across the cancer cell membrane increased with GIP treatment, while the TRP membrane electrical resistance decreased in the Ca^{++} channel **(Table-3)**[46]. Earlier studies using chemosensitizing agents (Table 1) had shown that TRP Ca^{++} ion channels were involved with regulating calcium influx entry into cancer cells[43]. Thus, GIP (at 1.0-10 µMol) was found to impose an increased Ca^{++} influx flow into the MCF-7 cancer cell cytoplasm. GIP was found to interact with TRPs of the M1 and M6 Melastatin subfamilies in computer simulations of peptide-to-protein binding modeling studies, referred to as "molecular docking" **(Table-4)**.

Table 3: Amino Acid sequencing Matching of Alpha-fetoprotein (AFP) derived Growth Inhibitory Peptide (GIP, P149) with Various Cation Channel-Associated and Calcium Interacting/ binding Proteins.

		% Identity/Similarity	% Total
Hum GIP #445	L S E D K L L A C G E G A A D I I I G H L C I R H E M T P V N P G V G N	100/100	100
Fragments P149 (GIP)*	1. GIPa | 2. GIPb | 3. GIPc		
Xen Na/K ATPase (#252)	L S C T R L I A C C Y G N C T G A I X H L C X X T N L S S I	36/23	59
Na Chanel Protein (#55)	Y V Q D Q L Q A C G E G	58/25	83
Hum Calmodulin (#27)	L S E I E L L	71/0	71
Rab Ca Channel-P (#32)	G L L P C A E G	63/25	88
Carp Ca Channel-P (#333)	L C G E G A A G L	33/11	44
Piso ATP-syn A (31165)	A A N L T A G H L L	45/45	90
Hum Calcitonin R (#210)	N S M I I I I H L C	50/30	80
Pig Calcitonin R (#195)	N S I I I I I H L V	50/30	80
Rat Calcitonin R (#195)	N S I I I I I H L V	50/30	70
Hum calreticulin (#3692)	I Q S I I V G H L G	50/20	70
Yeast Calmodulin (#21635)	N R I G Q L C I R	66/11	77
Hum Calcitonin (#1550)	L C I R H S F T P A	60/30	90
Mus K-Chanel P (#18)	L C I R G T L T P R	60/20	80
Bov ATP-channel (P) (#385)	C I Q F E L P P V N	50/30	80
Rat Ca/ATPase (#660)	C I H N Q M Q P V H	60/40	100

*GIP can be divided into three smaller peptide fragments, termed GIPa, GIPb and GIPc (see references).

Table 4: Molecular docking and protein interaction sites on alpha-fetoprotein and Growth Inhibitory Peptide (GIP) were identified. Such sites were localized by means of proprietary computer software (Peptimer Discovery platform). See legend below.* The amino acid segment of human alpha-fetoprotein and Growth Inhibitory Peptide (GIP) that were probed for computer interaction sites employing allosteric modulation designs. The single letter protein amino acid code was used for the Human Alpha-fetoprotein amino acid sequence 445-480 as follows:

NH2- L S E D K L L A C G E G A A D I I T G H L C I R H E M T P V N P G V N P G V G Q-COOH

Acc #	Protein	Function	Computer Hit
1) O14757	Ser/Thr protein kinase CHK2	Regulates S-phase, G2/M checkpoint	Helical region near kinase for ser178
2) AAC37594	BRCA1	Cell cycle checkpoint for DNA damage response	Tumor suppressor region near carboxy terminus
3) NP_005188	CHES1	Cell cycle checkpoint regulator	Glu/Ser kinase substrate site
4) NP_004761	KCNNB2	Volt-gated K-channel outflow	Channel pore
5) NP_113669	SLC22A16	Carnitine, cation transporter	Cation binding region
6) BAA037730	Calpain	Cys protease; Ca^{++} binding; DNA-repair	Ca^{++} binding region
7) NP_009125	Protein kinase CHK2	S-phase checkpoint regulator of the cell cycle	FHA region, a fork head transcription factor phosphatase; *NLS zip code site
8) NP_005188	Checkpoint Suppressor (CHES1)	Suppresses checkpoint mutations of cell cycle proteins	QS kinase substrate sites, fork head region for transcription
9) CAD12456	Titin	Ca^{++} dependent, myosin-binding ECM muscle protein	Ca^{++} binding domain
10) CA140077	CNNM2	Cyclin-M2	Transporter associated region
11) AAH58266	TRP-M1; Transient receptor potential (M-1 family)	A cation channel of the melastatin family; expressed in metanocytes associated with development of melanomas, permeable for Ca^{++} and Mg^{++} ions	TRP consensus site; EWKFAR
12) NP060124	TRP-M6 Transient receptor potential (M-6 family)	A channel permeable to Ca^{++} and Mg^{++} ions. Regulates parathyroid hormone production, energy production and DNA maintenance	TRP consensus site: LPXPFXPSPK

*Note: that GIP displayed direct computer hits on the melastatin family of the TRP calcium channels, TRP-M1 and TRPM6.

The above published studies demonstrated that GIP was found to increase the membrane current flow across the cancer cell channel within the cell cytoplasm. More channel proteins. These targeted pinpointe over, the increased electrical potential across the Ca^{++} channel occurred in a range of -30 to -45 mV; this voltage imposed a clamp which keeps open the Ca^{++} channel which persisted for 90 minutes in a freeze frame fashion[44,45]. Additional studies employing "patch-clamp" electrophysiological procedures confirmed the extended open channel time period induced by GIP. These studies proved to be effective in stabilizing the cell membrane voltage current block while decreasing the channel electrical resistance; this effect was found to occur in TRP channels in both prostate and breast cancer cultured cells. In summary, upon measurement of the electrophysiological voltage conductance and resistance, GIP treatment demonstrated a substantial channel current increase accompanied by a decreased membrane resistance in cancer cells compared to cells employing control scrambled AA peptide sequences. Thus, it is interesting to note that such TRP Ca^{++} channels are also known to be involved in regulation of the cell growth cycle in cancer cells and might account for the reported GIP-induced cell growth arrest found in multiple cancer cell types[23,26].

Thus, in two distinct cultured cancer cell lines, the GIP peptide enhanced the channel flow of Ca^{++} into the cancer cells contributing to the intracellular Ca^{++} ion increase and dysregulation. In subsequent reports of GIP treatment in MCF-7 cells, supporting GIP inhibition data were garnered from several study sets including a) data from a global RNA microarray analysis combined with; b) a computer program analysis of protein-to-peptide pairing study and c) amino acid sequence comparisons. All together, these data set studies suggested that GIP peptides could serve to modulate TRP Ca^{++} channel passage in both cultured MCF-7 breast cancer and LNCaP prostate cancer cells[23,46]. As shown in these other reports, it had been known that calcium ion functioning and transport could affect both cell cycle growth arrest and the induction of apoptosis and autophagy. Thus, GIP is capable of imposing an increased influx of Ca^{++} ions into cancer cells for a defined period of time in a non-regulated/restricted fashion. If this event occurs, the addition of chemosensitizing agents (such as the spices in Table 1) could contribute to the initial opening of Ca^{++} channels as a first step in allowing an increased flow of Ca^{++} into the cancer cell cytoplasm.

7. Calcium-induced Cell Death

Calcium (Ca^{++}) ions are essential for maintenance of bodily cell function but can also be toxic to cells in high concentrations. All cells require physiologic levels of Ca^{++} ions to maintain their homeostasis, but the accumulation of excessively high cell concentrations of Ca^{++} ions into the cytoplasm can result in an imbalance in intracellular organelle bioregulation of energy and homeostasis, resulting in cell toxicity and death. Biomedical researchers have taken advantage of this observed toxic cell effect in the development of therapeutic drugs that could be employed to destroy malignant tumor cells[25]. Such cell compartments include substances, membranes and subcellular organelles involved in the apoptotic process (programmed cell death); these include calcium channels, mitochondria, cytokines, transcription factors and the smooth endoplasmic reticulum. Overall, unregulated and unrestricted Ca^{++} ion channel membrane flow can result in a constant stream of calcium ions into the cell cytoplasm. The resultant unregulated activation of such cell membrane (TRP) Ca^{++} channels can lead to a huge toxic influx of Ca^{++} ions into the cancer cell cytoplasm.

The mode of action of cell toxicity produced by the calcium overload of the cell's mitochondria energy supply system can essentially cause cell death via processes of both apoptosis and autophagy (a lysosomal process)[47,48]. High levels of intracellular calcium are taken up by the mitochondria (Mt) which triggers the opening of MT pores termed "mitochondrial permeability transition pores" (mPTP); such an event leads to increased mitochondrial membrane permeabilization (uptake) and accumulation of Ca^{++} ions[7]. The exposure of the mitochondria to heavy influxes of Ca^{++} ions activates the dephosphorylation of NFAT proteins, which are key regulators of T-cell development and propagation. The NFAT (nuclear factor of activated T-cells) proteins are a family of transcription factors whose activation is regulated by calcineurin, a Ca^{++} dependent phosphatase enzyme[49,50]. NFAT proteins have also been identified as inducers of cytokine gene expression, especially the activation of inteleukin-2 (IL-2). The IL-2 cytokine is a 16 kDa protein secreted by T-lymphocytes, namely, $CD4^{+}$ and $CD8^{+}$ T-cells[51,52]. In turn, these T-cells can stimulate additional immune cell proliferation and development of: 1) helper cells; 2) cytotoxic cells; and 3) regulatory T-cells. This array of different T-cells is responsive to the activation by microbial infections, self-versus non-self/defense responses and induction of programmed cell death (apoptotic) factors. In summary, the activation of opening of Ca^{++} channels can occur in several constituent cell components including, 1) the cell membrane; 2) pore openings in the mitochondria; and lastly in 3) the Ca^{++} channel influx into the endoplasmic reticulum (ER)[53,54]. When all three Ca^{++} associated cell structures and organelles operate in concert, calcium-induced cell death (apoptosis) and/or autophagy ensues resulting in the observed cell toxicity, necrosis, autophagy and cell death.

8. Additional Statements and Conclusion

It was the discovery of ion channels such as the voltage regulated K-voltage regulated channels and the non-selective ion channels like the "Transient Receptor Potential" (TRP) that have advanced the present-day knowledge of channel functions involved in transport, signal transduction and receptor crosstalk. While K-channels are specific only for potassium passage and flow into the cytoplasm, TRP channels are non-selective for the several cations discussed above. Moreover, Kv channels are also pore-forming proteins which can be engaged in 1) the efflux of K^{+} ions across the cell membrane; 2) the balance of action potential; 3) electrical waveforms; and 4) flux frequency[46]. In addition, K^{+} channels are sensitive to voltage changes in the cell membrane action potential and electrical impulses. In contrast, TRP channels display one-way passage and flow of several different cations into cells. It is of further interest that a comparison study of TRP channels TRPM8 and TRPV1 versus KV2.1 channels was performed. It was found that most structural elements from TRP channels and from Kv channels were not sufficiently related to allow for the creation of experimental hybrid channels[48,55] (Table-1). It is of interest that the KV channels were downregulated by GIP in the global mRNA microarray analysis (Table-2) while the TRP channel did not do so. However, it was found that the TRP channels were found to bind GIP TRP channel allosteric sites as found in computer modeling programs (Table-4).

9. Conclusion

It can be ascertained from the reviewed data and information presented in this report, that an alpha-fetoprotein derived peptide (GIP) can interact with specific TRP channel family members to alter Ca^{++} channel influx and the flow of cations into cells. It is further tempting to speculate that GIP could provide the means and transduction signaling for the cellular induction of Ca^{++} induced toxic cell death. Furthermore, biological cells require calcium ions and other ions for the proper functions and regulation of cytoplasmic organelles such as found in the mitochondria and the smooth endoplasmic reticulum (ER). However, if an unregulated and unrestricted flow of Ca^{++} ions is allowed to enter the cancer cell, then this unrestricted flow of Ca^{++} ions into the cell would allow overflow passage into certain cytoplasmic organelles leading to programmed cell death via calcium induced toxicity. In summary, it may be stated that peptide targeted therapy is presently changing the treatment and prognosis of cancer in the clinic. Thus, targeting of ion channels, such as TRPs, could lead to novel approaches in the biomedical field for anti-cancer therapies employing peptide interactions with TRPs and other ion channels[56].

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11. Conflict of Interest

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

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13. Technical Notes: Addendum:

Computer Supporting Data: A search for Pairing of Peptide-to-Protein Interactions: AFP and its derived peptides are bristling with matching amino acid segment sites from cytokines, chemokines and immune system amino acid sequence identities which occur all over the AFP polypeptide molecule[45]. The protein-to-peptide pairing computer software from Serometrix Biotech Company revealed that the GIP peptide contained multiple matched allosteric amino acid pairing interaction sites with cellular and cancer-associated protein molecules; such proteins are the hallmark of diseases specific to cancer and other diseases. This software also focused on the amino acid pairing of small amino acid fragments present on disease cells associated with cancer. Such protein sites (amino acid segments) are potential targets for future therapeutic approaches to cancer treatment.46,47). Such pairing interactions could affect signal transduction pathways by inhibiting (or enhancing) receptor binding by blocking protein-to-peptide interactions. As shown in Table-3, the matched interacting sites with GIP included: 1) growth factors; 2) cell cycle proteins; 3) ubiquitins; 4) apoptosis associated proteins; 5) calcium-linked proteins; and 6) channel proteins. These targeted pinpointed interactions of cellular cycle proteins with cancer often cause cancers to progress to the severe symptoms which are observed clinically delayed in cancer afflicted patient.

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14. References

1. Berridge MJ, Bootman MD, Roderick HL. Calcium signaling: Dynamics, homeostasis and remodeling. Nat. Rev. Mol. Cell. Biol, 2003;4:517-529.
2. Filadi R, Theurey P, Pizzo P. The endoplasmic reticulum-mitochondria coupling in health and disease: Molecules, functions and significance. Cell Calcium, 2017;62:1-15.
3. Galluzzi L, Vitale I, Aaronson SA, et al. Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ, 2018;25:486-541.
4. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science, 1995;267:1456-1462.
5. Dona-Oh M. Calcium's rile in orchestrating cancer cell apoptosis: Mitochondrial centric perspective. Int. J. Mol. Sci, 2023;24:8982-8985.
6. Orrenius S, Goguadze V, Zhivotousky B. Calcium and mitochondria in the regualtion of cell death. Biochem. Biphy. Res. Commun, 2015;460:72-81.
7. Endlichen R, Drahota Z, Stefkova K, Kucera O. The mitochondrial permeability transition pore-current knowledge of its structure, function, regulation and optimized methods for evaluating its functional state. Cells, 2023;12:1273-1283.
8. Zhivotovsky B orrenius. Calcium and cell death mechanisms: A perspective from the cell death community. Cell calcium, 2011;50: 211-221.
9. Verkhrastsky A. Calcium and cell death. Subcell Biochem 2007;45:465-480.
10. Zhang M, Ma Y, Ye X, Zhang N, Pan Z, Wang B. TRP (transient receptor potential) ion channel family: sstructures, biological functions and therapeutic interventions for diseases. Signal transduction and targeted therapy, 2023;8:261-275.
11. Mizejewski GJ. The third domain fragment of alpha-fetoprotein (AFP): Mapping AFP interaction sites with selective and non-selective cation channels. Curr. Top. Peptide &Protein Res, 2016;16:6-82.
12. Mandal SK, Rath SK, Logesh R, Mishra SK, Devkota HP, Das N. Capsicum annuum L and its bioactive constituents: A critical review of ta traditional culinary spice in terms of its modern pharmacological potentials with toxicological issues. Phyto therapy, 2023;37:965-1002.
13. Minke B, Cook B. TRP channels proteins and signal transduction. Physiol Res, 2002;10:1152-1172.
14. Li H, Montell C. TRP and the PDZ Protein, Inad, Form the core complex required for retention of the signalplex in Drosophila Photoreceptor cells. J Cell Biol, 2000;150:1411-1422.
15. Montell C. TRP channels in Drosophila photoreceptor cells. J. Physiol, 2005;567:45-51.
16. Nishida M, Hara Y, Yoshida T, Inoue R, Mori Y. TRP channels:

molecular diversity and physiological function. Microcirculation, 2006;13:535-550.

17. Taberner F, Devesa I, Ferrer-Montiel A. Calcium entry through thermosensory channels. Adv. Exp Med. Biol, 2016;898:265-304.
18. Redondo P, Rosado J. Store-operated calcium entry: unveiling the calcium handling signal plex. Intl Rev Cell Mol Biol, 2015;316:183-226.
19. Zasshi, NY. TRP channels: formation of signal complex and regulation of cellular functions. Nihon Yakurigaki 2003;121:223-232.
20. Goel M, Sinkins W, Keightley A, Kinter M, Schilling W. Proteomic analysis of TRPC5- and TRPC6- binding partners reveals interaction with the plasmalemmal Na (+)/K(+)- ATPase. Pflugers Arch, 2005;451:87-98.
21. Ferrer-Montiel A, Fernandez-Carvajal A, Planells-Cases R, et al. Advances in modulating thermosensory TRP channels. Expert Opin Pat, 2012;22:999-1017.
22. Skryma R, Mariot P, Bourhis XL, Coppenolle FV, Shuba Y, Abeele FV, Legrand G, Humez S, Boilly B and Prevarskaya N. Calcium Currents in LNCaP cells. J. Physiol 527, 2006;71-83.
23. Wonderlin WF, Woodfork KA, Strobl JS. Changes in membrane potential during the progression of MCF-7 human mammary tumor cells through the cell cycle, 1995;165(1):177-185.
24. Marini M, Titiz M, Monteiro de Araujo DS, Geppetti P. TRP channels in cancer: signaling mechanisms and translational approaches. Biomolecules, 2022;13:1557-1567.
25. Arcangeli A, Crociani O, Lastraioli E, Masi a, Pillozzi S, Becchetti A. Targeting Ion channels in Cancer: A novel Frontier in Antineoplastic therapy. Curr Medicin Chem, 2009;16:66-93.
26. Wonderlin WF, Strobl S. Potassium channel, proliferation and G1 progression. J Membr Biol, 1996;154(2):91-107.
27. Tsavaler L, Shapero MhH, Morkowski S, Laus R. TRP-P8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. Cancer Res, 2001;61,3760-3769.
28. Wissenbach U, Niemeyer BA, Fixemer T, Schneidewind A, Trost C, Cavalie A, Reus K, Meese E, Bonkhoff H, Flockerzi V. Expression of CaT- like, a novel calcium selective channel correlates with the malignancy of prostate cancer. J. Biol Chem, 22001;76,19461-19468.
29. Peng JB, Zhuang L, Berger UV, Adam RM, Williams BJ, Brown EM, Hediger MA, Freeman MR. CaT1 expression correlates with tumor grade in prostate cancer. Biochem. Biophys Res Commun, 2000;282:729-734.
30. Mizjewski GJ. Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states. Obstet Gynecol Surv, 2003;58(12):804-826.
31. Mizejewski GJ. Biological role of alpha-fetoprotein in cancer: prospects for anticancer therapy. Expert Rev. Anticancer The, 2002;2(6):709-735.
32. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes and conformational variants. Exp. Biol. Med (Maywood), 2001;226(5):377-408.
33. Mizejewski GJ. Physiology of alpha-fetoprotein as a biomarker for perinatal distress: relevance to adverse pregnancy outcome. Exp Biol Med (Maywood), 2007;232(8):993-1004.
34. Mizejewski GJ. The different biologically active forms of alpha-fetoprotein as functional biomarkers in pregnancy, disease and cancer: A review and update. Advances in Biomarker Sciences & Technology, 2023;2(1):1-11.
35. Gonzclaz-Bugatto F, Foncubierta E, Angeles-Bailen MDL, et al. Maternal and fetal serum transformed alpha-fetoprotein levels in normal pregnancy. J Obstet Gynecol, 2009;35:371-276.
36. Muehlemann M, Miller KD, Dauphinee and Mizejewski GJ. Review of growth inhibitory peptides as biotherapeutic agents for tumor growth, adhesion and metastasis. Cancer Metastasis Rev, 2005;24(3):441467.
37. Mizejewski GJ, Butterstein G. Survey of functional activities of alpha-fetoprotein derived Growth Inhibitory Peptides: review and prospect. Current proteins and petid, 2008;7:73-100.
38. Mizejewski GJ, Muehlemann M, Dauphinee M. Update of alpha-fetoprotein growth-inhibitory peptides as biotherapeutic agents for tumor growth & metastasis. Experimental Chemotherapy, 2006;52:83-90.
39. Mizejewski GJ. An alpha-fetoprotein derived peptide suppresses growth in breast cancer and other malignancies. A review and prospectus med. Res. Archives, 2023;11(7): 1-15.
40. Mizejewski GJ and MacColl R (2003). Alpha-fetoprotein growth inhibitory peptides: potential leads for cancer therapeutics. Mol Cancer Ther, 2003;2(11):1243-1255.
41. Mizejewski GJ, Mirowski M, Garnuszek P, Maurin M, Cohen BD, Poiesz BJ, Posypanova GA, Makarov VA, Severin ES and Severin SE. Targeted delivery of anti-cancer growth inhibitory peptides derived from human alpha-fetoprotein: review of an International Multi-Center Collaborative Study J. Drug Target 2010;18(8):575-588.
42. Mizejewski GJ. Mechanism of cancer growth suppression of alpha-fetoprotein derived growth inhibitory peptide (GIP) comparison of GIP-34 versus GIP-8 (AFPep). Updates and Prospects Cancer, 2011;3(2): 2709-2733.
43. Mizejewski, GJ. Breast cancer and transient receptor potential (TRP) cation channels: Is there a role for non-selective TRP channels as therapeutic cancer targets? A commentary. Intl. J. Can. Res. and Develop 2017;2:4-7.
44. Mizejewski GJ. Potential Use of peptides in the targeting and therapy of solid tumors using a fetal-derived peptide: A review and prospectus. Intl J, Clin. Mil. Oncol Bio Res. Scientia, 2024;3:1-10.
45. Mizejewski GJ. The role of ion channels and chemokines in cancer growth and metastasis: A proposed mode of action using peptides in cancer therapy. Cancers, 2024;16:1531-1545.
46. Watanabe H, Vriens J, Janssens A, Wondergem R, Droogmans G, Milius B. Modulation of TRPV4 gating by intra and extracellular Ca^{++}. Cell Calcium J, 2003;33:486-495.
47. Ji C, Zhang Z, Li Z, She X, Wang X, Li B, Xu X, Song D, Zhang D. Mitochondria-Assocated Endoplasmic Reticulum membranes: Inextricably Linked with the Autophagy process. Oxidative Med. Cell Langev, 2002;2022:7086807
48. Zhou X, Hao W, Shi H, How Y, Xu Q. Calcium homeostasis disruption- a bridge connecting cadmium-induced apoptosis, autophagy and tumorigenesis. Oncol Res. Treat, 2015;38:310-315.
49. Youn HD, Chatila TA, Liu JO. Integration of calcineurin and MEF2 signals by the coactivator p300 during T-cell apoptosis. Embo, 2000;19:4223-4231.
50. Shinasaki F, Kondo E, Akagi T, McKeon F. Suppression of signaling through transcriptiom factor NF-AT by interactions between calcineurin and Bcl-2. Nature, 1997;386:728-731.
51. Kim YD, Choi SC, Oh TY, Chun JS, Jun CD (2007). Eupatilin inhibits T-cell activation by modulation of intracellular calcium flux and NF-kappaB and NF-AT activity. Cell Biochem 2007;108:22-236.
52. Ray JP, Staron MM, Shyer JA, et al. The Interleukin-2-

mTORc1 Kinase Axis Defines the Signaling, Differentiation and Metabolism of T Helper 1 and Follicular B Helper T cells. Immunity, 2015;43:690-702.

53. Sena LA, Li S, Jairaman A, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 2013;38:225-236.
54. Singh RP, Birge RB, Chandra D. Meeting report: International Symposium of mitochondria, cell death and human diseases: Jawaharlal Nehru University, New Delhi, India. Cell Commun Signal 2023;21:127-135.
55. Kalia J, Swartz KJ. Exploring structure-function relationships between TRP and Kv Channels. Sci Reports, 2013;3:1523-1526.
56. Arcangeli A, Crociani O, Masi A, Pillozzi S. Targeting Ion Channels in Cancer: A novel frontier in Antineoplastic therapy. Current medicine chemistry, 2009;16:66-93.
57. Kalia J, Swartz KJ. Exploring structure-function relationships between TRP and Kv Channels. Sci Reports, 2013;3:1523-1526.
58. Arcangeli A, Crociani O, Masi A, Pillozzi S. Targeting Ion Channels in Cancer: A novel frontier in Antineoplastic therapy. Current medicine chemistry, 2009;16:66-93.
59. Ahmed AG, Hussein UK, Ahmed AE, Kim KM, Mahmoud HM, Hammouda O, Jang KY. Mustard Seed (Brassica nigra) Extracts Exhibits Antiproliferative Effect against human lung cancer cells through differential regulation of apoptosis, cell cycle, migration and invasion. Molecules, 2020;25:2069-2076.
60. Nachvak SM, Soleimani D, Rahimi M, Azizi A, Moradinazar M, Rouhani MH, Halashi B. Ginger as an anti-colorectal cancer spice: A systematic review of in vitro to clinical evidence. Food Sci Nutr, 2022;11:651-660.
61. Weil MJ, Zhang Y, Nair, MG. Colon cancer proliferating desulfodinigrin in wasabi (Wasabia japonica) Nutr. Cnacer, 2004;48:207-213.
62. Chen YJ, Huang YC, Tsai TH, Liao HF. Effect of wasabi component 6- (Methysulfinyl) hexyl Isothiocyanate and derivates on human pancreatic cancer cells. Evid-based compl. Alternat Med. V, 2014;2014:1-10.
63. Xu S, Zhang L, Cheng X, YU H, Bao J, Lu R. Capsaicin inhibits the metastasis of human papillary thyroid carcinoma BCPAP cells through the modulation of TRPV1 channel. Food Funct, 2018;9:344-354.
64. Szallas A. Capsaicin and cancer: Guilty as charged or innocent until proven guilty? J Temp, 2023;10:35-49.
65. Wondergem R, Ecay TW, Mahieu F, Owsianik G, Nilius B. HGF/SF and menthol increase human glioblastoma cell calcium and migration. Biochem. Biophys. Res. Comm, 2008;372:210-215.
66. Vriens J, Jannssens A, Prenen J, Nilius B, Wondergem R. TRPV channels and modulation by hepatocyte growth factor/scatter factor in human hepatoblastoma (HepG2) cells. Cell calcium, 2004;36:19-28.
67. Guo H, Carlson JA, Slominski A. Role of TRPM in melanocytes and melanoma. Expl Dermatol, 2012;21:650-654.
68. Legrand C, Merlini JM, de Denarclens-Bezencom C. New natural agonists of the transient receptor potential Ankyrin 1 (TRPA1) channel. Scitif. Reports, 2020;10:11238-11241.
69. Buch TRH, Buch EAM, Boekhoff I, Steinritz D. Role of chemosensory TRP channels in Lung Cancer. Pharmaceut, 2018;11:90-93.