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Review Article
Published: 22 Jan, 2019

Journal of Molecular and Cellular Biology Forecast

Protein Binding and Interactions with Alpha-Fetoprotein (AFP): A Review of Multiple AFP Cell Surface Receptors, Intracytoplasmic Binding, and Inter- Molecular Complexing Proteins

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Abstract

Human Alpha-fetoprotein (HAFP), a tumor-associated fetal protein, is well-known clinically as a biomarker for both fetal defects and benign/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 both circulating and cell-bound. Some AFP receptors are located on tumor cells, others have been detected on monocytes, macrophages, inflammatory and lymphoid- associated cells. The intracytoplasmic AFP binding proteins include nuclear receptors, transcription-related factors, cell cycle checkpoint and DNA repair proteins, caspases, and apoptotic-associated proteins. Finally, the AFP intermolecular complexing group 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.

Thus, the results of this review demonstrate that there exists no apparent single or universal receptor or class of AFP binding proteins on normal, benign or malignant cells.

Keywords: Alpha-fetoprotein; Cancer; Tumors; Receptors; Binding Proteins; Growth; Molecular Complexing

Introduction

Alpha-fetoprotein (AFP) is known as an "Oncofetal Protein" due to its dual presence in the embryo/fetal compartments and in adult benign and malignant tumors [1,2]. This characteristic has enabled AFP to serve as a tumor-associated fetal serum biomarker for pregnant, pediatric, and adult patients in clinics throughout the world.

During development, AFP is first synthesized in the fetal yolk sac and liver, then in the gastro-intestinal tract. In the normal newborn/infant, AFP serum levels steeply decline during the first year of life at which time serum levels are drastically reduced approaching those of adults (5-10 ng/ml) [3]. In contrast, adults with various liver disorders such as cirrhosis, viral infections, and cancer (hepatomas, germ cell tumors) display highly elevated levels of serum AFP [4]. The synthesis of AFP in adults occur mainly in the liver. However, some AFP analyses may be influenced by the presence of free and bound forms of AFP both in the circulation as well as histochemically in the cytoplasm of cells.

Objectives of the Present Report

The objectives of the present report are several-fold. First, this treatise will serve to review the two distinct functional forms of HAFP and their structure and function in order to provide the reader with a background overview. Second, the multiple protein interactions that occur with AFP both cell bound and in the blood vascular system will be reviewed. Such protein-to-AFP interactions will be shown to encompass a) cell surface receptors; b) intracytoplasmic binding proteins; and c) intermolecular complexing proteins. Thirdly, the types of proteins involved at these locations will be parsed into their individual names, anatomical locales and cell types, and their cell function and/or activity and presented in a tabular format. In this manner, the structure/function overview

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Received Date: *12 Dec 2018*
Accepted Date: *18 Jan 2019*
Published Date: *22 Jan 2019*

Citation: *Mizejewski GJ. Protein Binding and Interactions with Alpha-Fetoprotein (AFP): A Review of Multiple AFP Cell Surface Receptors, Intracytoplasmic Binding, and Inter-Molecular Complexing Proteins. J Mol Cell Biol Forecast. 2019; 2(1): 1016.*

ISSN 2643-7953

Table 1: The names and properties of selected cell surface receptors/protein groups for alpha-fetoprotein are listed below.

Name of the receptor group	Receptor Name	Location and/or cell type	Cell function and/or activity	Refs
A) MUCIN Receptors	1. MUC-1 2. MUC-2 3. MUC-4 4. MUC-5B	Colon, breast, trachea, bronchi, testis, cervix, pancreas, sublingual, esophagus	Activates ERbeta receptor, catenin, VWD domain, cys- rich, cell signaling tumor, growth, metastasis	[40,41,85]
B) Scavenger Receptors	1. Mannose eceptor 2. CD36 3. LOX-1 4. SRB1	Stromal cells, blood platelets, RBCs, monocytes, macrophages, lymphoid, bone marrow cells	Platelet aggregation, binds fatty acids, and ECM proteins, binds LDL and HDL, HIV gp 120 proteins, binds cholesterol	[42-45]
C) Chemokine Receptors	1) CXCR4 2) CCR5 3) CXCR7	Peripheral mononuclear cells, macrophages, T-cells, monocytes; lymphoid blast cells	Transmit the mediation of cell migration, immune response, inflammatory reactions, tumor cell receptors	[46,47,49,85]
D) Lysophospholipid Receptors	1) EDGE 2) ORGI 3) P2Y 4) GPR	Lymphoid and dendritic cells, hematopoietic cells, ovary, skin, brain, muscle cells	Transduces multiple cell effects as neural development, angiogenesis, morphogenesis, carcino- genesis, inflammatory responses, lipid signaling	[50-56]
E) Non-Selective and Selective Cation Channels	1) K-Voltage gated 2) Transcient Receptor Potential	Heart, lung, brain, epithelial cells, smooth muscle, T-cells, pancreas, macrophages, Lymphocytes	K-ion Voltage gated, outward K channels, Ca++ sensing, non-selective sensing of spices, heat and cold receptors	[57-61,86]
F) Metastasis-associated proteins	1) adhesion/ contact 2) MMP/ADAM 3) GF Receptors 4) Growth Factors	Stromal and tumor cells, ECM cells, platelets, neurons, endothelial cells	Cell adhesion, contact, migration, cell shape, membrane degradation, invasiveness, growth factor receptor signaling	[62-66]

K=Potassium; SR=Scavenger Receptor; ADAM=A Disintegrin and Metalloprotease; GPR=G-Coupled Protein Receptor; ORG1 Receptor=GPR68; P2Y=Purinergic Receptor; MUC=Mucin; EDGE=Endothelial Differentiation Gene Product.

of HAFP will provide the reader with the necessary background to more fully understand the function of AFP at each intravascular and cellular location.

Overview of AFP Structure and Function

Human (H) AFP is a secreted single chain polypeptide of 609 amino acid which includes a 19 amino acid leader sequence. HAFP has a molecular mass of 69kD, an isoelectric point range of pH 4.7 to 5.1, and a 3-5% carbohydrate glycan component depending on its isoform [5]. HAFP has been classified as a member of the albuminoid gene family together with albumin, alpha-albumin, vitamin-D binding protein, and the AFP-related gene (ARG) product [6]. This oncofetal protein displays a three- domain secondary structure form resulting from multiple intermolecular loops determined by 16 disulfide bridges [5]. By means of x-ray crystallography, the disulfide loops were observed to produce a helical V- or U- shaped tertiary molecular structure.

The tertiary form exhibited an external hydrophilic surface (for increased serum solubility) together with multiple internal hydrophobic molecular clefts which enhance ligand binding. HAFP can also assume an intermediate folding transition state known as a molten globule form [7].

HAFP has been established as a serum carrier protein capable of binding and transporting a multitude of hydrophobic ligands. Such ligands include bilirubin, fatty acids, gossypol, retinoids, flavonoids, steroids, dyes, heavy metals, phytoestrogens, dioxins, and various medicinal drugs [8]. Much of the binding to the hydrophobic ligands occurs in the third domain of AFP, while the second domain is largely involved in interactions with apoptotic proteins and with cell adhesion proteins of the extracellular matrix and interstitium. However, the second domain can also bind fatty acids and bilirubin. While the second and third domains of HAFP share amino acid commonalities to other mammalian forms of AFP, it is the first domain amino acid sequences that distinguishes HAFP from other mammalian species [9].

HAFP is recognized as a regulator of growth during development (pregnancy). Because of this property, it was assumed that AFP was required for growth of the embryo/fetus; however, experimental murine AFP gene knockout models demonstrated that AFP was not a requirement for full term delivery, at least in rodents [10]. Moreover, in the rodent knockout model, second generation females were found to be sterile. The sterility of the female offspring was found to be attributed to anovulation due to an AFP regulatory absence in the hypothalamic-pituitary axis [11]. It has yet to be ascertained whether these regulatory actions would apply to human development. It is known, however, that human AFP deficiencies and or absence during pregnancy reveal that full-term pregnancies could still occur. Thus, the human clinical studies of AFP deficiency in newborns/infants showed both unremarkable fetal development and normal birth outcomes [12]. The females in such clinical human pregnancies cases have yet to reach the age of fertility/maturity, so the pedigree of fertility history in those females have yet to be determined [13]. Thus, the HAFP molecule has been shown to function as a maintenance agent and biologic response modifier of fetal growth during pregnancy and as a growth regulatory factor in benign and malignant tumors.

The Functional Forms of AFP

AFP is known to be the most abundant protein during embryogenesis and fetal life and is present in newborns and infants. AFP is produced by several organs during development including yolk sac and fetal liver, stomach, and pancreas [3]. Functional AFP has 2 major forms in mammals aside from its various isotypes. These forms are as follows: A) a secreted and blood/lymph circulating form of 69kD and B) a cytoplasmic-bound form (67kD) lacking an amino-terminal leader sequence [14,15]. The functional forms are detected by two different methods; A) Form–1 is measured in the blood by various immunoassays; and B) Form–2 is detected by immunohistochemical analysis [16]. Both forms are regulatory proteins which enhance or

Table 2: The names and properties of intracytoplasmic binding/interacting proteins with alpha- fetoprotein are listed below.

Name of the protein binding family	Binding protein Name	Location and/or cell type	Cell function and/or activity	Refs
A) Nuclear receptors with dimerization heptads (Leucine Zipper)	1. Retinoic acid	Hepatomas, human breast cancer cytosols, immature uterus, MCF-7 BC cells, serum, rat zajdela cells	RAR mediates gene expression, induces biological effects of GADD153 and transcription factors; influences growth and apoptosis	[25,26,67-70]
	2. RXR receptor			
	3. c-erbA receptor			
	4. T3R receptor			
B) Cysteine – (cys) aspartic acid protease family (caspases)	Caspase-3	Human hepatomas, Bel 7402 hepatomas, human liver cancer cells cultures hepatoma clinical extracts	Regulation of cell death, polymerase cleavage, protease activity, blocks apoptosis, carcinogenesis	[26,75]
	Caspase-6			
	Caspase-9			
C) Cell cycle associated protein family	1) Cyclins	Mitotic dividing cells, human hepatoma tissues, cancer patient extracts, rat and mouse hepatomas, MCF-7 BC cells	G1-to-S Phase transitions, checkpoint suppression, regulation of Cyclins and Cyclin dependent Kinases, modulate ubiquitin ligase pathways	[87-89]
	2) Cyclin depend. Kinases			
	3) Cell cycle			
	4) Ubiquiteris			
D) DNA-repair proteins	1) BRACA-1,2	Human benign and cancer cell cultures, MCF-7, MDA-MB-231 rat mammary tumors	DNA damage sensing and repair; genomic stability, chromosome aberrations	[70,71]
	2) FANC-1, 2			
	3) Nibrin			
	4) ATM/ATR			
E) Karyophilic transcription factors	1) PTEN	HEPG2 cells, HLE cells, human HCC cells, nude mouse xenographs	Induces CXCR4 expression, AKT phosphorylation, promotes cell migration, activation of AKT/mTOR signals	[67-70]
	2) AKT/mTOR			
	3) GADD153			
	4) Fn14/PI3K			
F) Apoptosis-related proteins	1) IXAP	HEPG2 cells, Raji cells, MCF-7 cells, Jurkat cells	Increases Bcl-2 proteins FAS-dependent and TNFR-dependent cell signaling and cell death	[92-93]
	2) FAS/FASL			
	3) TRAIL-R			
	4) Bcl2			

RXR=Retinoic-X-Receptor; T3R=Triodothyonine Receptor; BRACA=Breast Cancer Antigen; RAR=Retinoic Acid Receptor; ATM=Ataxia Telangrectasia Mutated; ATR=Ataxia Telangiectasia Regulated; Hepg2=Cultured Hepatoma Cells; Akt=Alpha Serine/Threonine Protein Kinase; Mtor=Mammalian Target of Rapamycin (FK506 Binding Protein); FAS=Tumor Neurosis Factor Receptor SF6; 6ADD153=Growth Arrest And DNA-Damage Inducible Protein.

inhibit cell growth, proliferation, migration, and signal transduction and transcription. Thus, the presence of both AFP forms include: 1) The classical full-length AFP polypeptide of 609 amino acids (69kD) which includes a 19 amino acid "Leader Signal Sequence" at the NH2 – terminus together with a single glycosylation site on an aspartate at amino acid #293 on the second domain [17]. This first form is found in the serum of cancer patients, being secreted from the tumors; it is also found in embryo extracts and in fetal serum [1]. 2) The second functional form of AFP is a non- secreted, intracytoplasmic entity with a molecular mass of 67kD or less. It lacks both the Leader signal sequence (amino-terminal end) and the single glycosylation site [14]. This form is called cytoplasmic AFP (CyAFP). The CyAFP intracellular form can occur in malignant cells, benign tumor cells, and non-malignant (lymphoid-associated) cells. The CyAFP has been reported to reside in hepatoma (liver cancer) cells, ascites fluid cells, spinal fluid, immature rodent uterus, MCF-7 human breast cancer cultured cells, and in serum and cytosols from breast cancer patients [15,18]. It has been demonstrated that AFP is both an endocrine and autocrine growth factor which enters tumor and non-tumor cells by receptor-mediated endocytosis. After AFP receptor binding and subsequent activation, cell signal transduction occurs which can affect cell cycle progression, growth, motility, adhesion, migration, proliferation and cell-to-cell contact. In comparison, non-secreted AFP plays a role in regulating expression of KRAS, cyclic AMP, Protein-Kinase A, and cytoplasmic Ca++ levels interacting with both growth factor/receptors and with transcription factors [14].

At present, HAFP is a well-established growth promoting entity; however, several intermediate transition forms of the fetal protein have been shown to inhibit growth [19,20]. Thus, HAFP is able to serve as a dual regulator of growth, capable of both enhancement and inhibition. The capability of both up-and-down modulation of growth, as a dose-dependent function of AFP, has been demonstrated in a multitude of cell types including placental, ovarian, uterine, lymphoid, epidermal, endothelial, breast and liver and in a multitude of benign and cancer cells [21-23]. AFP can apparently regulate growth by several mechanisms which affect apoptosis, autophagy, cytoplasmic signaling modulation, intermolecular complexing, and receptor blockage and desensitization [24,25]. Much of the growth enhancing activities of AFP are enacted through: a) AFP cell surface receptor-mediated signal transduction; and b) intracellular AFP binding to various cytoplasmic enzymes, transcription-related factors, cell cycle and repair proteins, and nuclear receptors [25,26].

The AFP Cell Surface Receptor

From the above discussion, it seems apparent that there does not exist just one universal cell surface receptor for AFP; this would include both normal and cancer cells whether of human or animal origin (Table 1; #1). Studies of a multiplicity of cell surface receptors have increasingly been reported in the biomedical literature for the last 20 years or more. The initial reports suggesting the presence of a receptor were derived from AFP uptake studies in fetal cells by means of endocytosis in both *in vitro* and *in vivo* cell

Table 3: The names and properties of intermolecular proteins that complex with alpha-fetoprotein are listed below.

Group name of complexing protein	Name of complexing protein itself	Location and/or cell type	Cell function and/or activity of AFP-protein complex	Refs
A) Immunoglobulin family	1. IgM IgG	Serum circulating blood protein complexes	Biomarker present in human hepatomas, cirrhosis, and chronic hepatitis patients	77-80
B) Estrogen binding protein	42 KD protein	Intracellular MCF-7 cells, human breast cancer cells (patients)	Cytosolic protein bound to AFP in BC patients extracts 4mKcl-release	15, 81
C) Circulating AFP- bound with proteins	Actin, osteonectin receptor, TGF-beta	Blood circulating protein- to-protein complexes	Serum of post-menopausal breast cancer patients;	5, 82
D) Hetero-dimerization partners	1. AFP itself 2. Dimer 3. Trimers 4. Various cytosolic proteins	AFP domains 1 and 3; dimerization in Raji cells; Soluable AFP isolation procedures form dimers	Formation of AFP polymers in stability studies; AFP can undergo self-aggregation to form oligomers	71, 72
E) Antigen-presenting proteins, innate immunity	Mannose receptor, T-cell receptor MHC class-II protein	Macrophages, dendritic cells, fibroblasts, endothelial, placental cells	AFP-antigen binding to MHC proteins for antigen presentation	4, 45, 94, 95

BC=Breast Cancer; IgM, IgG (Ig=Immunoglobulin); TGF=Transforming Growth Factor; MHC=Major Histocompatibility Complex.

models [27,28]. Subsequent reports demonstrated that the uptake and endocytosis occurred in both normal fetal cells and in tumor cells (rhabdomyosarcomas) [29,30]. Experimental evidence further confirmed that the entry of AFP was via the process of receptor-mediated endocytosis [31,32]. A series of subsequent studies showed that the AFP property of cell uptake and entry were further present in multiple neoplastic cells such as breast/mammary carcinomas, hepatomas, lymphomas, neuroblastomas, gastric tumors, and T- and B- cell malignancies [33-35]. These studies and others strongly supported the presence of multiple AFP cell surface receptors as integral plasma transmembrane proteins. The overall expression of AFP receptors by malignant cells appeared to favor those of immature and incompletely differentiated cells.

In the middle 1980's and early 1990's, reports of the presence of AFP receptors were demonstrated by multiple investigators in mouse T-cell lymphomas, human monocytes, activated human T-lymphocytes, lymphoblastoid Raji cells, and MCF-7 human breast cancer cells (see above). In 1991, a human breast cancer tumor cell membrane receptor was reported in a doctoral thesis with subsequent reports describing isolation of 62 and 67 kD PNA-reactive receptors associated with a higher molecular weight (~200kD) moiety [36-38]. However, this tumor AFP receptor termed RECAF, has yet to be cloned, and its full identification and complete characterization (other than an antibody product) have yet to be reported [39]. In more recent studies, an AFP breast tumor AFP receptor was proposed as a mucin receptor using "*in silico*" computer modeling software methods [40]. The mucins identified and proposed were MUC-1, MUC-2, MU-4, and MUC-5B (Table 1, Group 1). Mucins are composed of complex, multimeric-branched structures with heavy carbohydrate content and molecular weights ranging from 47 to 519 kD. Some mucins, such as MUC-4 display molecular weight of 61 and 65 kD similar to the PNA-reactive receptors described above. Such mucin-like receptors were also reported to be present on monocytes, macrophages, leukemic, lymphoid cells, and tumors of lymphoreticular origin [41].

A second group of AFP receptors, termed the scavenger receptors, whose presence were reported by French investigators in the early 1980's in studies associated with an autocrine AFP/receptor complex signaling and receptor re-cycling process [42] (Table 1, Group 2). Although first reported in lymphoid cells, the presence of the autocrine stimulation signaling process was further confirmed in those of malignant cells. The French group identified two lymphoid cell membrane receptors of 18 and 31 kD, termed scavenger receptors that bound AFP. These scavenger receptors were later detected by "*in silico*" computer software and confirmed by *in vitro* cell-based assays [43,44]. These scavenger receptors have since been identified as the mamnose receptor (CD206), CD36, LOX1, SRA1, and SRB1 in cell-based assays [45]. The scavenger receptors (SRs) represent a class of single or double-pass integral transmembrane glycoproteins bearing pattern recognition domain binding properties.

SRs transmit through G-protein coupled receptor signal transduction cascades following cell-surface ligand binding, receptor clustering, and endocytosis-mediated uptake [43]. Some of the eight classes of SRs bind both chemically-altered low density lipoproteins (LDLs) and high density lipoproteins (HDLs), fatty acids, denatured AFP and albumin, poly-anionic compounds, nucleotides, amyloids, and phospholipids [44]. Many SRs exhibit epidermal growth factor (EGF) repeats, amino acid-rich repeats, and ECM like regions on their domains.

A third group of AFP receptors have been identified and confirmed as members of the chemokine receptor family (Table 1, Group 3). CCR5 chemokine receptor binding to AFP was first reported in 2002 by Eurasian investigators; the receptor was localized to the surface of primary macrophages involved in HIV transfections [46,47]. The binding of AFP to the surface of monocytes from AIDS patients had been previously reported to interfere with the process of HIV-cell membrane fusion and subsequent transfection. It was suggested that the presence of AFP binding to receptor interference with HIV during pregnancy could aid in explaining why 90% of instances of HIV transmission to the baby occurs after term pregnancies in the postnatal and neonatal stages of infancy [48]. Later studies employing "*in silico*" software showed that AFP was capable of interacting/binding not only to CCR5, but also CXCR4 and CCR2, all of which are involved in HIV transfection. It further demonstrated that an AA sequence of AFP third domain folded polypeptide structure (AA#400-480) resembled the structure of various chemokine ligand (Group-II) members; these ligands include GROa, MIP-1B, EOTAXIN, RANTES and MCP-1 [49]. Thus, AFP may pose as a chemokine decoy ligand mimicking protein (a bait protein). It is noteworthy that the ligands for CCR5 include those of RANTES, EOTAXIN, and MIP-1B.

A fourth group of cell surface transmembrane proteins capable of binding to HAFP are the receptors for Lysophospholipids (LPLs), a family of plasma membrane derived inflammation-associated bioactive lipid signaling molecules (Table 1, #4). The LPLs include sphingosine-1-phosphate, Lysophosphatidic acid, and their derivatives [50,51]. These low molecular weight molecules, 250-750 Daltons, consist of a single acyl carbon chain (palmitic, oleic acid) attached to a polar head group. These bioactive paracrine lipid inflammatory mediators are present on circulating cells such as platelets, macrophages, monocytes, and associated cells and only exist in vertebrates [52,53].

The LPLs bind to four different classes of G-protein coupled receptors which include; 1) edge receptors; 2) ORG1 receptors; 3) the P2Y purinergic receptors; and 4) the GPR receptors [54,55]. The LPL receptors are involved in developmental, physiological, immunological, and pathological processes in mammals. The edge receptor class is expressed in lymphoid, dendritic, and hematopoietic cells/tissues; while the ORG1 receptors are on cells engaged in activities such as cell migration/invasion, apoptosis, angiogenesis; similarly also in smooth muscle stimulation, and pH dependent cell signaling [56]. The P2Y receptors are involved in Ca++ signaling, cyclic AMP activities, neurulation, and cell migration and mobility [54,55]. Finally, the GRP receptor group takes part in adenyl cyclase activation, Ca++ mobilization, meiosis, and nerve growth activities [52].

The fifth group of receptor-associated proteins interacting with HAFP includes the selective and non-selective cell transmembrane cation channels (Table 1, #5). This class of cell surface proteins encompass both the potassium voltage-gated (KCN) cation channels and the transient receptor potential (TRP) non-selective channels [57-59]. Both of these channel protein types were identified and localized by "*in silico*" computer software and then confirmed in AFP-peptide treated cells by electrophysiological and RNA-microarray analysis in MCF-7 human breast cancer cell cultures. The KCN proteins identified were subfamily members A, B, D, J, and Q; whereas the TRP subfamily included the canonical (C), vanilloid (V), and the melastatin (M) members [57,60]. The KCN channel current (conductance) changes occur in cells involved in the G1 to S-phase progression in the cell cycle. In TRP channels, membrane changes measured by patch clamp methods, were found to activate MCF-7 cells following AFP-peptide treatments. This caused a decrease in membrane resistance in both channel types and induced a membrane de-polarization and activation of KCN channels that increased Ca++ flow into the cells [57]. Overall, AFP-derived peptides were found capable of stabilizing the cell membrane potential at -30 to -45 mv [57-61].

The sixth group of AFP cell surface interacting proteins are the metastases- associated cell surface proteins involved in cell adherence, attachment, cell-to-cell contact, migration, and invasion (Table 1, #6). The cells bearing such proteins are those contained in the extracellular matrix (ECM), intercellular spaces, stroma, and interstitium [62-66]. Such cells function in cell gap junction establishment, calcium-dependent mobilization and storage, homophilic and heterophilic cell-to-cell adhesion, anchorage- dependent cell death (anoikis), interaction with the ECM proteins, integrin signaling, basement membrane degradation, and collagen proteolysis [62]. The proteins involved in these cell activities have been classified as belonging to four major groups which include; 1) cell adhesive and contact proteins such as cadherins, connectins, connexins, desmogleins, and desmocollins; 2) the metalloproteinases, ADAMS disintegrins, integrins, and annexins; 3) the cell surface growth factor receptors; and 4) growth factors and regulators [62,63,66]. When the proteins components of these four groups were identified, it became evident that AFP is intimately involved in metastatic-directed activities encompassing all the earmarks of cell spreading such as cell migration, mobility, cell shape changes, invasiveness, and basement membrane degradation.

The AFP Intracytoplasmic Binding/ Interacting Proteins

Reports of cytoplasmic protein interactions and/or binding with HAFP have recently emerged in greater numbers in the biomedical literature (Table 2). The non-secreted form of AFP (CyAFP) is a highly active moiety during developmental, postnatal, and cancer stages in mammals especially man [14]. These biological activities include nuclear receptor binding, and interaction with the cysteine-aspartic acid caspase family members, cell cycle associated protein interactions, DNA- repair/checkpoint proteins, karyophilic transcription associated factors, and apoptotic- related proteins [25,26,67-70]. HAFP contains a dimerization domain in the last half of the third domain carboxyterminal polypeptide which enables AFP to attach to cytoplasmic nuclear receptors and transcription factor via a leucine zipper [71-73].

Thus, CyAFP has the capability to form molecular heterocomplexes with cytoplasmic- based enzymatic fragments, limited proteolysis products, and recombinant proteins. More recently, the cytoplasmic form of AFP has been found to affect, influence, and regulate cytoplasmic proteins/factors such as PTEN, AkT/mTOR, GADD153, FN14, PI3K, and several apoptotic proteins such as XIAP, FASL, FAS, TRAIL receptor and Bcl_2 [37-70,74] (Table 2, #1-5).

Examples of the CyAFP interaction with the above list of cytoplasmic proteins/factor are readily found in the biomedical literature. In several reports, Li et al. provided direct evidence that CyAFP functions as a regulator in the phosphatidylinositol- 3-kinase (PI3K)/AKT pathway of Bel 7402 hepatoma cell growth [67,70], The Li et al. group demonstrated that CyAFP colocalized and interacted with the tumor suppressor PTEN (phosphatase and tensin homolog) using the method of fluorescence resonance energy transfer (FRET) analysis. The investigators subsequently utilized the AFP mRNA knockdown technique together with the coadministration of ATRA to inhibit CyAFP expression resulting in enhancement of PTEN levels, a decrease in phosphorylated AKT, and a reduction in cell growth. These results demonstrated that CyAFP was involved in the regulation of both hepatoma cell growth and the process of tumorigenesis. A further experimental study of CyAFP in hepatoma cell growth involved the use of the "growth arrest and DNA damage-inducible protein 1533" termed GADD153, a factor involved in cancer and stress-related apoptosis [67]. By means of microarray analysis, Li et al. examined expression of the GADD153 gene induced by ATRA in studies of cell apoptosis and growth. In this study, investigators found that CyAFP was able to form a complex and interact with RAR in HEPG2 hepatomas producing AFP; however, such complexing was not found in non-AFP-producing hepatoma HLE cells. In the above examples, the direct molecular interaction of AFP with receptors and transcription factors was proven by a) RNA knockdown; b) colocalization studies and c) FRET analysis with interaction distances measured at 12.6 Angstroms. Thus, these research techniques and methodology studies document that CyAFP is indeed an intracellular signaling protein in the cytoplasm of cancer and other cells.

The AFP Intermolecular Complexing Proteins (ICP)

The intermolecular complexing proteins (ICP) which interact with AFP involve a less recognized activity of the oncofetal protein. The ICP form of AFP can occur both in the blood vascular system as well as the interstitual and intracytoplasmic compartments (Table 3, #A to E). A blood circulating form of AFP complexed to a molecular moiety was first reported by Norgaad Petersen in 1976 [76]. The protein complexed to AFP was proposed to be an Immunoglobulin-like molecule identified by electrophoretic methodology. Since then,

the unknown protein has been identified as an IgM moiety and the AFP/IgM complex has been employed as an immunoassay in human clinical studies [77-80] (Table 3A). Another complex form was identified in the blood circulation by Sarcione in 1972 in the serum of post-menopausal women as an AFP-to-protein complex; AFP was released from the complex by treatment with 4m KCL [15] (Table 3B, 3C). The identity of the unknown protein moiety was not reported. However, an estrogen binding moiety to AFP of 42kD was further identified in the cytostatic extracts of malignant breast cells isolated from breast cancer patients [81,82]. Other candidates for complexing to AFP have included IgG, actin, TGF-Beta, osteonectin, and protease substrates [5]. Furthermore, AFP itself can dimerize on itself to produce dimers, trimers, and oligomers (Table 3D). Finally, AFP fragments and/or peptides are involved in antigen presentation to major histocompatibility complex proteins in the adaptive immune response (Table 3E).

Concluding Remarks

It becomes evident from the preceding discussion that HAFP display activities with a plethora of cell surface receptors, cytoplasmic binding proteins, and circulating inter-molecular complexes. A previous proposed concept of a universal tumor receptor for HAFP must come under introspection in view of the multiple documented reports of AFP receptors and binding proteins reported in the biomedical literature. One such cell surface receptor was found not only on tumor cells but on monocytes, macrophages, and cells of the leukemic stem cells, and lymphoid series [83]. Because HAFP is an established growth promoting protein, it is logical that a fetal protein would be active as growth factor at the cell surface, and in cytoplasmic and circulating vascular compartments. However, as stated above, AFP is uniquely equipped to promote growth, but can also inhibit growth in certain instances of microenvironmental stress due to oxidative, pH, osmotic, metabolic, signal transduction, excessive ligand concentrations [22], and in fetal structural defects [2,3,16]. Such situations would permit AFP to temporarily inhibit growth until damage is repaired and growth could be resumed during pregnancy. Thus, AFP appears to serve as a multi-tasking molecular Swiss Army Knife during embryo/fetal development.

Acknowledgement

The author extends his gratitude and thankfulness to Ms. Jennifer Wright in the typing and processing of this manuscript.

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