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Gerald J. Mizejewski*

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Review Article

The Association of Pregnancy, Alpha-fetoprotein, and Fetal Defects with Breast Cancer Risk: A Review and Commentary

Gerald J. Mizejewski

Division of Translational Medicine, Molecular Diagnostics Laboratory Wadsworth Center, New York State Department of Health Biggs Laboratory, Empire State Plaza Albany, NY, USA 12237

***Corresponding Author:** Gerald J. Mizejewski, Division of Translational Medicine, Molecular Diagnostics Laboratory Wadsworth Center, New York State Department of Health Biggs Laboratory, Empire State Plaza Albany, NY, USA 12237.

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Abstract:

Human alpha-fetoprotein (HAFP) during pregnancy has long been utilized in the obstetric clinic as a pregnancy gestational age-dependent biomarker for birth defects such as neural tube defects and aneuploidies. Within the last two decades, the HAFP polypeptide has also been reported to transition into intermediate protein folding forms depending on levels of ligand concentrations in fetal microenvironments. A conformational change or transformation of the AFP polypeptide produces a molten globule form of the fetal protein known as "transformed AFP (TrAFP). This HAFP transformation exposes a short peptide amino acid sequence on the full-length AFP which functions as a growth-inhibiting factor able to inhibit and prevent the growth of cancer cell microfoci in fetal and maternal tissues including the breast. In past studies, it was reported that a reduced risk of postmenopausal breast cancer later in life was associated with elevated AFP levels during pregnancy. Recent studies have further reported that HAFP can interact, bind, and disable mutated DNA-damage repair proteins in a cell's growth cycle which progresses to mitosis; this function prevents replication of cells with damaged DNA. Based on the above studies, the present review and commentary examine whether HAFP, present in transient forms during pregnancy, constitutes a prime factor which could contribute to a reduced maternal breast cancer risk later in the mother's life.

Keywords: alpha-fetoprotein; breast cancer; dna repair; afp peptides; cell cycle; growth suppression; maternal serum; chromosome instability

1.Introduction

Mammalian alpha-fetoprotein (HAFP) is a fetal glycoprotein present during both ontogenic and oncogenic growth [1,2]. HAFP is a single-chain glycoprotein consisting of a molecular mass of 69kD, 610 amino acids, and 3% carbohydrate content [1]. AFP is known as a tumor-associated fetal protein termed an "oncofetal protein" belonging to the albuminoid gene family consisting of albumin, gc-globulin and alpha-albumin. HAFP constitutes a single polypeptide chain which exhibits a triplicate domain structure configured by intramolecular loops dictated by disulfide bridging; this configuration results in a helical V- or U-shaped protein structure first demonstrated and reported by Luft and his associates [3]. Over the last several decades, AFP has been reported and recognized as a regulator of both fetal and tumor growth, acting either as a growth enhancer or a growth inhibitor depending on its immediate surrounding ligand concentrations in microenvironments of the mammalian fetus [4].

During fetal development, AFP is first synthesized in the yolk sac, then in the fetal liver and gastrointestinal tract [2]. AFP has been utilized in the clinical laboratory both as a tumor marker and as a biomarker for fetal defects and perinatal distress [5]. Although HAFP is produced and secreted by the fetus during pregnancy, it can be further re-expressed in adult cancers such as teratomas, hepatomas, and yolk sac tumors of the ovary [6,7]. Thus, in the obstetric clinic, HAFP has been employed as a gestational age-dependent fetal defect marker serving as a pregnancy screening agent for neural tube defects, anencephaly, down syndrome, and other chromosome trisomies; in adults, AFP has been employed as a tumor biomarker for hepatomas and other cancers. In recent years, full-length AFP has been determined to be a cell growth bioregulator and homeostatic agent for both fetal and tumor cells and can regulate growth in both adult cancer proliferation and during fetal development [7,8].

II. Protein Folding and Intermediate Forms:

1) HAFP Protein Folding and the Molten Globule Form:

During folding and unfolding of newly synthesized polypeptides in the endoplasmic reticulum of cells, proteins can pass through multiple transient or intermediate forms during the folding process before attaining their final tertiary-folded structure [9]. During the folding stages of proteins destined for Golgi secretion and transport, hydrophobic amino acid residues are tucked into a protein's interior molecular crevices, while hydrophilic residues predominate on the protein's exterior surfaces; this process ensures increased solubility for vascular passage in the serum of blood vessels [10,11].

HAFP has been reported (see above) to be a serum protein capable of assuming a molten globular configuration; this transition occurs following exposure to stress/shock environments and excessive on- and off-loading of carrier ligands destined for target tissues [12,13]. This transition configuration of a protein can occur following ligand activation of a "hot spot" on the protein chain; the intrinsic hot spots are characterized as being buried within the protein molecule, tightly packed, and susceptible to ligand activation [14]. The third domain of the HAFP molecule further contains a growth inhibitory peptide segment consisting of 34 amino acids (AA) that lies buried in the protein's native full-length, compactly folded form [16]. This buried AA segment on AFP has been known to become accessible following a molecular conformational change involving an apparent rotational hinge in the full-length structure of the AFP protein [15]. The encrypted and buried growth inhibitory peptide (GIP) AA segment on HAFP is not detectable using present-day commercial polyclonal or monoclonal antibodies. Still, recent publications have only just described it (see below). As discussed above, the hidden occult site has been demonstrated to be decrypted or exposed by the partial unfolding of the native full-length AFP protein following exposure of the fetal protein to stress/shock micro-environments of the fetus [16]. This occult 34 amino acid buried segment has now been separately isolated and produced as a stand-alone (single) synthetic peptide being purified, characterized, and assayed for biological activity [17-20].

2) Denatured Intermediate Forms of AFP

As described above, a functional transient form of full-length AFP has been described as a slightly denatured intermediate stage of proteins known as a "molten globule form" (MGF) first described for AFP by Uversky and others [9, 21-23]. The MGF of a protein was originally discovered and ascribed to proteins in the presence of a lowered pH state under isoelectric assay conditions. Overall, MGFs are particularly susceptible to exposure to high ligand concentrations which induces the protein (i.e., AFP) to undergo a conformational change which transitions the protein into the MGF; [24] such changes can result in exposure of hidden and occult AA segments present in molecular crevices of protein formed because of the final tertiary folding of the protein within the cell's ER. As discussed above, a conformational change in a tertiary folded protein can reveal and expose multiple hydrophobic and amphipathic amino acids segments that had been previously buried in the inner folded portion of the protein before the conformational change. Thus, microenvironmental exposure to cells and tissues is capable of inducing stress and/or shock conditions in various organ systems of the body's extracellular milieux. Such stress/shock conditions which cause the conformational changes in proteins can include many types of different physical conditions. Such stresses include: oxidative, osmotic, ionic, temperature, and pH changes, in addition to exposure to high concentrations of fatty acids, steroids, hormones, and growth factors [25,26]. Such microenvironmental stresses occur regularly at the placental interface and in fetal, newborn, juvenile, and adult stages during mammalian ontogeny.

3) Transformed alpha-fetoprotein (TrAFP)

1) As described above, HAFP has been shown to display multiple molecular forms, complexes, and variants [1,2]. Recently, such AFP binding and interacting forms have been reported to demonstrate binding and/or interactions with: A) cell surface receptors, B) intracytoplasmic binding agents, and C) inter-molecular complexing proteins such as with IgG [27]. In addition, a slightly denatured intermediate form of AFP has been reported in the biomedical literature as a pregnancy variant form of AFP (10). This structurally altered functional form of AFP, termed "transformed AFP" (TrAFP), has been identified, studied, and assayed in maternal and fetal serum in clinical pregnancy studies [28].

The novel pregnancy biomarker variant form, termed "Transformed alpha-fetoprotein (TrAFP)" first appeared in the clinical literature during the years 2007 to 2009 [28]. TrAFP is a molten globule, slightly denatured form of AFP found in both nonhumans and other mammals [9,10]. The existence of the TrAFP form was reported from multiple clinical pregnancy reports and observations of a conformationally altered form of AFP following exposure to high concentrations of estrogens and fatty acids [12,14]. This altered tertiary form of full-length HAFP revealed a concealed or buried peptide segment stretch consisting of an intrinsic 34-amino acid peptide sequence later termed the "Growth Inhibitory Peptide (GIP)" [4,13,14]. The growth inhibitory activity or function of this peptide segment on full-length AFP stands in dire contrast to the well-known function of the HAFP polypeptide as an overall growth-enhancer protein [28] (see below).

2) Rabbit antibodies to TrAFP have been produced against TrAFP in the author's (GJM) lab and were not found to cross-react with full-length AFP as determined by commercial radioisotopic, fluorescent, and ELISA AFP assays. In contrast, rabbit antibodies to TrAFP were found to react only with full-length HAFP when the 34 AA GIP segment was exposed as observed in pregnancy maternal serum (28). Such unveiling of the peptide can only occur on maternal serum HAFP polypeptide surface following exposure to fetal stress/shock environments; such events occur at the uterine/placental interface. At this interface, high concentrations of estrogens and polyunsaturated fatty acids accumulate and reside [12]. Fetal liver produced HAFP has been reported to be transferred from fetal serum to the amnionic sac compartment of the placenta, where it further diffuses into the maternal circulation from the amniotic fluid compartment [29,30]. As mentioned, a maternal serum antibody assay kit for TrAFP was developed and employed to quantify TrAFP levels in the maternal sera from second and third trimester pregnancies. Measurement of TrAFP levels was studied and reported by doctors in an obstetric clinic to assess fetal well-being and to predict adverse perinatal outcomes before birth [31-33]. In the clinic, the presence of TrAFP levels in maternal serum was found helpful in predicting risks of fetal distress and demise in multiple third trimester pregnancies; these conditions included: 1) fetal growth restriction/intrauterine growth retardation; 2) fetal chronic hypoxic stress; 3) threatened pre-term labor; and 4) fetal hemodynamic re-distribution [28,31,32]. These reports support the potential use of maternal serum TrAFP as a candidate late pregnancy biomarker for perinatal distress conditions. Such a biomarker could be useful in assessing and/or predicting risks of adverse perinatal outcomes and heightened fetal distress in the third trimester gestation period prior to birth in at risk pregnancies.

III. Growth Inhibitory Peptide: The Exposed Peptide Sequence Following AFP Conformational Transformation:

1) Transformation and Future Functions:

The 34 amino acid peptide segment exposed on the full length HAFP polypeptide following the ligand-induced conformational change has been referred to as the Growth Inhibitory Peptide (GIP-34) as described above. This transient exposed peptide segment, derived from HAFP, has been determined to inhibit cancer cell growth regulation and proliferation. Thus, GIP has been studied for its potential anti-cancer properties among its many other varied functions. The conformational transformation process of full-length AFP involves a molecular conformational change that exposes the buried peptide segment in full-length AFP. This change allows the peptide, now on the surface of full-length AFP, to interact with fetal cells transiently to exert its biological effects, such as repairing cell structure, modulating signal transduction, and regulating immune responses. Furthermore, the peptide's ability to revert back into the intact AFP protein form provides a dynamic and reversible action to cease interaction with impaired cell targets after repairs are made. This could involve repairing cellular damage or modulating various cellular functions such as signal transduction and receptor crosstalk. Thus, aside from pregnancy studies, GIP has been researched for its ability to inhibit the growth of cancer cells, making it a potential therapeutic agent in clinical oncology [15,16]. Other mechanistic reports demonstrated the involvement of cell cycle arrest and the prevention of cell cycle inhibitor degradation by ubiquitin [34]. Therefore, it could be proposed that, a growth inhibitory peptide fragment of AFP present only during pregnancy, could be made available to treat cancer in adult patients [34].

2) AFP and the Immune State of Pregnancy

The proposed concept that normal human pregnancy is actually a controlled state of inflammation has been validated in the biomedical literature [35]. The human conceptus has classically been viewed as a foreign (non-self) object in the mother's body and has long been considered a tissue allograft residing in the maternal uterus. Investigators have since described the conceptus as being produced and present in an immunologically privileged site situated in juxtaposition to the uterine/placental cell's immunotolerant barrier. This barrier is located in direct contact with maternal uterine tissue containing natural killer (NK) cells of the maternal innate immune system [36]. In turn, the maternal NK cells secrete various cytokines that attract maternal immune-activated lymphocytes to the placental boundary; these in turn, cause the maternal cells to view the conceptus foreign cell clusters as "tissue inflammatory cells sites" rather than foreign cells and tissues. Such lymphocytes then act to block all off intruder cells from the maternal tissues at the placental/uterine interface [37]. Thereafter, the conceptus cells are viewed by maternal immune cells only as sites of tissue inflammation and not foreign (non-self) intruder cells. Obviously, any fetal protein that mimics the many lymphokines/cytokines of the immune system (in structure and function) would have a definite advantage in sustaining and maintaining the foreign tissue as site of inflammation. AFP is a protein consisting of a series of lengthwise successive structural modular peptide cassettes on full-length AFP that mimic immune system cytokines and immunoregulatory peptides such as lymphokines [36,38].

3) The GIP peptide, isolated from full- length AFP, may also play a role in suppressing both the cell-mediated and humoral branches of the immune system [36]. This suppression could be crucial in pregnancy, where the maternal immune system needs to be adjusted, modulated, and reconditioned to prevent rejection of the foreign fetus. By modulating both serum antibodies (the humoral immunity) and cell-mediated responses, GIP could aid in maintaining immune tolerance. This ensures that the maternal immune system does not attack the fetal cells, which are actually genetically distinct from cells of the host mother. The regulation of the immune system by GIP could be crucial in ensuring a successful pregnancy by possibly reducing and preventing maternal immune reactions against the fetus [39]. Understanding the mechanisms by which GIP and/or intact AFP modulate the immune system could lead to novel therapies for conditions where immune modulation is necessary, such as in organ transplantation and autoimmune disease treatments.

Alpha-fetoprotein Topic	Biological Roles, Components, Variants and Forms	References
1) Alpha-fetoprotein structural forms, variants, derivates, gene family	- Carbohydrate and pH isoforms - Conformational variants - mRNA expressed variants - Peptic fragments, 3 domains - Member of albuminoid gene family	1-4, 5-8
2) Alpha-fetoprotein as a Molten Globular protein form and denatured intermediate form	- Low pH induced AFP form - A ligand induced form - Due to estrogen & fatty acids - Present at placental interface	9,11,15,16,21-23
3) Alpha-fetoprotein as a Transformed protein (TrAFP)	- A slightly denatured form - A transient form - Unfolded tertiary AFP form - A cryptic form of AFP	12-14, 27,28,21,32
4) Transformed alpha-fetoprotein as a fetal distress biomarker	- Risk predictor in late pregnancy - Fetal growth retardation - Fetal chronic hypoxic stress - Threatened preterm labor - Hemodynamic redistribution	9,10,28,29

5) Alpha-fetoprotein derived peptides (GIP) (Growth Inhibitory Peptides) functional activities, and properties	- Development of an AFP-derived peptide termed "Growth Inhibitory Peptide" (GIP) - Bioassay development, physical properties - Studies of cell entry and uptake - Participates in crosstalk and signal transduction within cytoplasm - Therapeutic agent for cancer	9,10,12-14,16-19
6) Alpha-fetoprotein, immunity, and pregnancy	- Inflammation, monocytes - NK (Natural Killer) cells - NK, cytokines, lymphocytes - Placental uterine interface - Self versus non-self	36-38
7) Studies have shown that high levels of pregnancy AFP can reduce breast cancer risk	Reports from American, Danish, and French scientists have confirmed such studies of the reduction of breast cancer risk	40-42, 43-48
8) Alpha-fetoprotein can bind and interact with proteins of the cell cycle DNA damage and repair response	Short amino acid sequence stretches on AFP 3rd domain can bind and interact with DNA repair proteins to present and down regulate damaged DNA from being replicated	49-57

Table 1: Summary studies of Alpha-fetoprotein (AFP) structure and transient/variant forms of AFP implicated in reducing breast cancer risk.

IV. Breast Cancer Prevention by Biochemical Means:

1) Cancer Chemoprevention

Reports from the decades of the late 1970s and 1980s indicated that birth defects, multiple births, and familial associations were feasible factors involved in the reduction of future breast cancer risk to the mother [40-42]. The reported epidemiologic observations that high AFP levels in pregnant women reduce the subsequent risk of both pre-and postmenopausal breast cancer might be well related to the growth regulatory properties of AFP [43]. An initial study by Richardson et al. [44] indicated that a reduced risk of postmenopausal breast cancer was associated with elevated third-trimester AFP levels in young pregnant women. This initial study by Richardson et al. utilized stored frozen maternal serum samples and compared them to present-day breast cancer serum samples from afflicted women. Richardson reported that a reduced risk of future postmenopausal breast cancer showed a causal relationship associated with high serum AFP (SAFP) levels in women younger than 30 years at first pregnancy. Richardson further reported that elevated steroid hormones have a lesser albeit positive effect in similar stored frozen samples [45]. Utilizing blood samples stored for at least 20 years, Richardson et al. re-assayed frozen/stored maternal SAFP samples from women 20 years later together with diagnostically confirmed serum samples from women with breast cancer. In a follow-up study, a Danish report by Melbye et al. (46) confirmed and extended Richardson's findings and included previous second trimester AFP blood samples from premenopausal women. The report by Melbye's Danish investigators, using the country of Denmark's national medical records as a resource, confirmed and extended the earlier studies of Richardson. Further Danish studies included second trimester SAFP levels in premenopausal women aged 38 [44,46]. Thus, both of the above studies concluded that high levels of maternal serum AFP during second and third pregnancy samples were associated with a lowered incidence of future breast cancer risk; this latter association was particularly strong for pregnancy serum samples from younger maternal age women.

A later study by Vatten et al. further confirmed both the Ricardson and Melbye studies concerning serum AFP and future breast cancer risk. They extended their findings to include cord serum AFP, ethnicity, and hypertension [47,48]. Overall, multiple reports have provided evidence that elevated SAFP levels during second and third trimesters, and even at term, protected women against a breast cancer risk later in life. As in earlier studies, the protective effect was even more effective at younger maternal age pregnancies.

The above results provide the impetus to suggest that AFP and its derived peptide segments could be employed not only for cancer therapy, but also be considered for chemoprevention of breast and possibly other cancer as well [43]. This chemoprotective effect of AFP during pregnancy was further observed in women bearing multiple pregnancies (twins), and in pregnancy patients presenting with pre-eclampsia [40-42]. All such situations share the commonality of elevated serum AFP levels, which may contain sufficient quantities of conformationally induced (transformed) AFP variants to affect growth suppression of cancer microfoci in maternal breast tissues [28-34]. Cancer cell microfoci in the tissues of young adult and middle-age population has long been known [1].

2) Effect of AFP on DNA-damage Repair Proteins:

There appears to be a further explanation for the breast cancer protective effect involving elevated levels of serum AFP. Another possible answer may lie in the clinical presentation of gene and chromosome instability disorders such as Fanconi's anemia (FA), Ataxia telangiectasia (AT), and BRCA1 and BRCA2 gene products, all known to have genetically unstable DNA repair protein disorders [49]. All these disorders demonstrate elevated SAFP levels in infants, juveniles, and adults. The FANCD2 and FANCN1 proteins are capable of interacting with BRCA1 and BRCA2 proteins forming multi-protein complexes that normally monitor DNA damage during cell cycle progression [50, 51]. However, in the G2 phase of the cell cycle, aberrant FA cells harboring unrepaired damaged DNA are capable of interacting with mutated checkpoint regulatory protein components that that are capable of overriding G2-to-mitosis transition arrest. Usually, the checkpoint 1 (CHES 1) proteins will halt cell cycle progression at the G2 phase in the presence of unrepaired DNA; this step prevents damaged DNA cells from entering into mitotic cell replication of cells [52, 53]. In FA patients, cells containing

damaged DNA-repair proteins allow mitosis to occur, thus, propagating corrupt DNA-containing cells throughout the body, including breast cells and tissues.

It has further been demonstrated by both "in silico" (computer) and experimental studies that the amino acid sequence stretches on AFP 3rd domain (AFP-3D) are capable of binding and/or interacting with proteins of the cell cycle DNA damage and repair response [53]. In that prior report, it was demonstrated that certain short amino acid sequences on the AFP-3D are capable of interacting with ataxia telangiectasia mutated (ATM) proteins such as, AT/RAD3 related (ATR) proteins, and Fanconi anemia complementation group (FANC) proteins (i.e., FANCD2). These disorders exhibit mutations (damages) in cell cycle checkpoint kinase proteins involved in cell cycle arrest and DNA repair. Fanconi anemia is a disorder also involved in DNA repair in which aberrant cell proteins can override cell cycle checkpoint arrest, allowing cell mitosis to proceed. Thus, damaged or mutated regulatory proteins are able to bypass the G2-to-mitosis checkpoint phase of the cell cycle premitting replication of cells with damaged DNA [56]. As described above, short amino acid stretches present on AFP-3D can bind and interact with DNA-repair proteins from cultured MCF-7 breast cancer cells. Thereafter, it was demonstrated that purified 34-mer GIP peptide was also capable of down-regulating the expression of DNA repair proteins (CHES1, FANCD2) as evidenced by mRNA microarray analysis in cultured MCF-7 breast cancer cells [57].

V. Conclusion:

It is tempting to speculate that during pregnancy, AFP and its derived peptides, could contribute to the reduction of breast cancer risk in women later in life. It is presently proposed that microfoci clusters of breast cancer cells in pregnant women could possibly be affected by three possible mechanisms working singly or in concert. These mechanisms are: 1) growth suppression, inhibition , and elimination of microfoci breast cancer cells by TrAFP and/or AFP-derived growth inhibitory peptides (); 2) AFP-3rd domain amino acid sequence stretches that bind and interact with maternal breast cancer cells carrying damaged DNA-repair proteins exemplified by ATM, RAD3, BRCA1, BRCA2, and FANCD2; and 3) the interaction of AFP-derived growth inhibitory peptides that down-regulate the mRNA in DNA-repair system in breast cancer cells. Thus, there appears to be at least three different mechanisms of AFP, its various forms, and exposed peptides that might act singly or be additive in reducing the microfoci in breast cancer cells. Such activities could reduce future breast cancer risk later in life as observed from previously pregnant young women.

Unfortunately, it could be predicted that such protection would not be afforded to: A) never pregnant women; B) women conceiving late in their reproductive years, and C) in women with abortion histories. Thus, women who undergo at least one full-term pregnancy before age 30 or thereabouts, will receive a nine-month dose of transformed AFP together with the GIP-34 peptide. These events occur following fetal-placental transfusion of AFP, TrAFP, and encrypted peptide into the serum of all women during pregnancy.

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Conflict of Interest:

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

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