ALPHA-FETOPROTEIN: UNFAMILIAR ASPECTS OF A FAMILIAR ONCOFETAL-PROTEIN

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ABSTRACT

The alpha-fetoprotein (AFP) is a biochemical marker of hepatocellular carcinoma and hepatic inflammatory diseases. Serum AFP is also known to be elevated in patients of yolk sac tumors. AFPs are single-chain glycoproteins. Since the cells that produce the protein modify the glycan chain post-transcriptionally, it offers a unique opportunity to differentiate the source of the AFP synthesized. Lectin affinity electrophoresis of AFP can be carried out on samples and separated AFP bands detected by antibody-affinity blotting. While these tests are designed to clarify the diagnosis, in practical experience, they often produce contradictory findings. Nevertheless, together with good interpersonal skills and communication, knowledge, judicious and appropriate use of technology we will be in a better position to relieve and to comfort. More specific tools such as the sub-fractionation techniques of the AFP molecule and greater understanding of its in vitro and in vivo characteristics would further refine the uses of AFP. The humble AFP may have far reaching screening, diagnostic, therapeutic, prognostic and preventive applications in the future.

Keywords: Alpha-fetoprotein, chromosome 4q11-13, hepatocellular carcinoma, isochromosome 12p, lectin affinity, oncofetal-protein, tumor marker, yolk sac tumors.

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INTRODUCTION

AFP as a tumor marker and a prenatal screening test. It is well known and a widely used tool. Advances in immunochemistry, genetics and new understanding of the AFP molecule have opened new avenues to help to improve the sensitivity and specificity of the AFP test as well as introduce novel uses. This paper explores some of these advances and practical implications.

HISTORICAL BACKGROUND

In 1956, a fetal sera component was first detected as a postalbumin migrating protein, using paper electrophoretic techniques, by Bergstrand and Czar[1]; Masopust and Kotal assigned to the then unknown protein the name "fetoprotein"[2] and Gitlin and co-workers[3] devised the name "alpha-fetoprotein" (AFP) for the electrophoretic a1-migrating human fetal protein.

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CLINICAL SIGNIFICANCE

In 1965 Tatarinov described a protein in the sera of subjects with liver tumors[4]. AFP production is oddly reactivated in liver tumors and germ cell tumors[5], to a lesser degree after chemical and mechanical liver injuries followed by regeneration e.g. acute viral hepatitis. It is for this reason that AFP is often used as a biochemical marker of hepatocellular carcinoma and hepatic inflammatory diseases from screening to diagnostic and monitoring of treatment and recurrence.

Serum AFP is also known to be elevated in patients of yolk sac tumors and similarly has diagnostic, therapeutic and prognostic implications. Other rare tumors have been reported to also produce AFP, including some renal cell cancers, lung cancers, colon cancers, pancreatic cancers and those from primitive gut origin. The clinical role of measurements of the protein is less well established in these diseases.

In the early 1970s, Brock and co-workers reported elevated AFP levels in human amniotic fluid[6] and in maternal serum[7] that correlated with the presence of neural tube defects in the fetus. Since then, amniotic and maternal serum AFP levels, reading together with other biochemical markers, have also been increasingly used to screen for spinal bifida, anencephaly, hydrocephalus and trisomy-21 in utero.

Other less common fetal or obstetrical causes of a positive result are: congenital Finnish nephrosis, Turner syndrome with cystic hygromas, gastrointestinal obstructions, missed abortion, imminent or actual fetal demise, severe Rh problems, and esophageal or duodenal atresia, skin defects and other conditions associated with fetal edema.

FUNCTION OF AFP

Many proteins are known to serve as precursor molecules and to contain multiple modular sequences or cassette segments generated by proteolytic processing to produce smaller biologically active peptides. Similarly, AFP could serve as circulating protein reservoirs of biological active peptide fragments[8,9,10]. Among the proposed and confirmed biologically active peptide segments on the AFP protein molecule are[10]:

ARP apoptosis-related peptide;
EGFL epidermal growth factor-like segment;
GIP (documented) growth inhibitory peptide;
HCS (confirmed) histocompatability Class II segment;
ILS (GenBank derived) insulin-like segment;
KLS kinesin-like segments;
LPH leucine predicted heptads;
L-A (GenBank derived) laminin-A segment;

L-B1 laminin-B1 segment;
LRE LDV, and RGD, (documented) cell adhesion sequences (one-letter amino acid code);
MPS (documented) milk casein peptide segment;
PAS (GenBank derived) plasminogen activator segment;
PRS proline-rich sequence;
SRGD segment reversed RGD site.

Similar to albumin (ALB), AFP is known to bind and transport ligands including bilirubin, fatty acids, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxins, and various organic drugs[9,10]. Unlike ALB, high concentrations of hydrophobic ligands (i.e., fatty acids, estrogens) have been reported to induce an irreversible conformational change in the tertiary structure of AFP[10,11].

Many of the proposed biological activities of the peptides or whole AFP molecule are likely to be dependent on the cellular type, location, stage of development and other extracellular matrix and environmental influences such as hormonal exposure patterns, toxins and environmental pollution[10]. Among the binding sites confirmed, predicted and proposed on the AFP protein are[10]:

ABS (proposed) actin-binding site;
BBS (predicted), bilirubin-binding sites;
EBS (documented) estrogen-binding site (in rodents);
FBS (confirmed) fatty acid-binding site;
HMS (predicted) heavy metal-binding sites;
RBS (proposed) HAFP receptor-binding site;
NAI (confirmed); non-ALB identity site;
ZBS (proposed) zinc-binding site.

Both full-length AFP and its fragments have been implicated in both apoptosis and tumor cytotoxity[10]. AFP and its derived peptides are able to inhibit proliferation in instances where growth is involved; these include fetal development, cancer cells, liver regeneration, to esoteric capabilities such as frog metamorphosis[12]. Neoplastic growth might be regulated through the presence of an AFP cell surface receptor that undergoes internalization[13]. It has been shown that AFP liganded to a fatty acid is endocytosed via clathrin-coated receptosomes, transported to the endoplasmic reticulum and Golgi complex and released into the cell in an intact form[14].

The second protein domain of the AFP molecule is made up of short peptide sequences common to extracellular matrix (ECM) proteins bearing cellular adhesion motifs (CAMs)[10]. Adhesive macromolecules have potential utility in developmental and disease states involving growth, differentiation, cell migration, and tumor metastasis[15]. Some of the CAM-derived synthetic peptides have been found to block cell differentiation, tumor growth, and angiogenesis. Some ECM proteins (laminin, collagen, and fibronectin) contain multiple biologically active peptide sequences with differing activities specific to cell type[10].

ONTOGENESIS

AFP is the principal protein of mammalian fetal serum analogous to ALB of mammalian adult serum. It is one of the earliest proteins to be synthesised by the embryonic liver. It is synthesized by the yolk sac, hindgut/midgut endoderm, and the foregut hepatic diverticulum at 26 days post-ovulation. At 32–52 days post-ovulation, AFP is also expressed in the mesonephric duct and tubules; and transiently expressed in the pancreas at 40–50 days. Upon parturition, blood AFP levels decrease precipitously and only trace amounts are expressed in the adult liver[16].

TRANSCRIPTION CONTROL

The human AFP gene maps to chromosome 4q11-13. Various experimental models have shown that AFP expression is regulated mainly on the transcriptional level. The AFP gene has a 7 kb regulatory region upstream. Within this region are a tissue-specific promoter, three independent enhancers, and a silencer that is postulated to be responsible for AFP gene expression decrease in the adult liver.

Some transcription factors, including hepatocyte nuclear factors (HNFs), which influence the transcription of most liver-specific genes, have been shown to bind to the promoter gene. However, the mechanisms leading to the drastic changes of AFP synthesis level in the course of ontogenesis and carcinogenesis are not clear. Also, little is known about negative regulators of AFP gene expression in cells of non-hepatic origin and in adult liver[17].

MOLECULAR STRUCTURE

AFP is classified under a member of a three-domain albuminoid gene family that consists of [9,10,11,17]:

- ALB;
- AFP;
- vitamin-D binding protein (DBP); and
- alpha-albumin (aALB).

This family of proteins is structurally characterized by cysteine residues that are folded into layers that form loops dictated by disulfide bridging, resulting in a triple domain, U-shaped molecular structure. All 4 proteins are mapped in tandem to chromosome 4 within the 4q11-q22 region, encompassing 15 exons and 14 introns. These proteins are highly homologous in primary structure. All of them are synthesized in liver and secreted into blood serum, providing delivery of their bound ligands to different tissues[10].

Mammalian AFPs are single-chain glycoproteins with molecular masses ranging from 66 to 72 kDa and a 3%–5% carbohydrate (glycan) content[16]. The sugar chains are not genetically encoded, but are dependent on the set of glycosylation enzymes present in the endoplasmic reticulum (ER) and the Golgi complex of the host cell.

The clinical significance is that the protein is modified post-transcriptionally by the cells that produces the protein, offering a unique opportunity to differentiate the source of the AFP synthesized as the glycosylation process is unique for a particular organic and under various pathological conditions[10]. Differentiating the source of the AFP would logically be of diagnostic value in differentiating the various possible cause of the raised serum or histological AFP result.

LECTIN AFFINITY

Lectins are a group of specific glycoproteins present in animal as well as plant cells that can be used as differentiating markers to study cancers cell lines. This biochemical affinity of lectins depends on the process of cellular glycosylation. Alterations in glycosylation play an important role in the metastatic behavior of tumor cells. Carbohydrate residues of the membrane glycoproteins can be detected using lectins due to their binding specificity to carbohydrates[18].

Lectin affinity electrophoresis of serum AFP can be carried out on samples and separated AFP bands detected by antibody-affinity blotting. The following major bands have been identified by determination of 'kinetic constants'[19]:

AFP-L1, -L2 and -L3 with lens culinaris agglutinin A (LCA-A);
AFP-C1 and -C2 with concanavalin A (Con-A);
AFP-P1, -P2, -P3, -P4 and -P5 with erythroagglutinating phytohemagglutinin; and
AFP-A1, -A2 and -A3 with allomyrina dichotoma lectin.

AFP bands with the lowest number have either low or no affinity and those with higher numbers have higher affinities for the respective lectins.

AFP from cord blood and chronic liver disease is characterized by the predominance of AFP-C2, AFP-L1, AFP-P2 and AFP-A3. Hepatocellular carcinomas are differentiated from the benign liver disease by increased proportions of AFP-L3 and AFP-P4. Extrahepatic tumors are known to have low affinity to Con A, medium affinity to LCA-A, high affinity to erythroagglutinating phytohemagglutinin and low affinity to allomyrina dichotoma lectin, with slow electrophoretic migration in yolk sac tumours; symbolically represented by AFP-C1, AFP-L2, AFP-P5 and AFP-A1 (slow-migrating AFP-Als in yolk sac tumor).

These test can be used to help to increase the sensitivity and specificity of AFP as a marker of hepatocellular carcinoma where AFP levels are only slightly elevated, differentiate between benign liver conditions and hepatocellular carcinoma and between extrahepatic tumors, including germ cell tumors, and hepatocellular carcinoma[19].

PRACTICAL ISSUES

The liver is a common target destination of many metastatic tumors, including adenocarcinomas and germ cell tumor. The increasing use of minimally invasive biopsy techniques means that many of the histological diagnoses are vague and limited especially if the tumors are poorly differentiated. Definitive histological diagnosis is not always possible even with the best available scientific advances today. Epidemiological profile, clinical history and physical examination, biophysical and functional imaging, biochemical tumor markers and genetic profiling would play crucial roles in the determination of the primary source of the tumor in the absence of crucial histological confirmation.

AFP blood level is a well-known marker for hepatocellular carcinoma that is widely used in clinical practice today. Because of this lack of specificity, the poorly differentiated liver tumor associated with a raised AFP level, on its own merit only, is not enough to make a diagnosis of hepatocellular carcinoma, particularly in a young person. Other screening tumor markers such as CEA, CA125, CA19-9 should be read together with the serum AFP results. Imaging modalities include, ultrasound, CT scan, MRI and PET scans. Hep Par 1, a monoclonal antibody with expression confined primarily to benign and malignant hepatocytes, is commercially available. A host of other histoimmunochemical stains, AE1/AE3, CAM 5.2, B72.3, monoclonal carcinoembryonic antigen (mCEA), polyclonal CEA (pCEA), factor XIIIa, inhibin, CD10, villin, MOC-31, cytokeratin (CK) 7, CK 19, and CK 20 are also helpful to distinguish the differential primary tumor source. These, and other newly proposed markers[20], would further contribute to delineate the plan of treatment and prognostication, but require care in interpretation and further validation. For tumor sufferers with raised AFP, AFP subfractionation using lectin affinity is commercially available for LCA-A in Singapore, but not for other lectins. Less familiar is the use of AFP levels in germ cell tumors. Further investigations to confirm germ cell tumors include serum bHCG levels and LDH levels, together with the detection of isochromosome 12p[21] using FISH technique, is also available locally.

While these tests are designed to clarify the diagnosis, in practical experience it often produces contradictory findings. Eventually, a presumptive 'best diagnosis' must still be made regardless of whether any confirmatory test results can be obtained. The relative success, failure or diseases progression with the presumptive treatment might also contribute to inform the physician regarding the correct diagnosis. This application of the hypothetical deductive diagnostic approach is already well familiarized among family physicians who practice it on a daily basis. Knowing how best to advise and communicate with our specialist colleagues with respect to ordering test and interpreting the results can play a crucial role as part of the team treating our patients.

Suffering from a tumor of unknown origin can be a frustrating and frightening experience for the patient and family. Finding the source of the tumor can determine the most appropriate treatment and prognostic judgment and also offer some comfort to the patient and his or her family.

Besides intimate knowledge of the ideas, concerns and expectations of the patient and his or her family in the community, sound knowledge of the latest scientific methods are also ultimately essential in our practice. Besides good old empathy, our knowledge of such cutting edge technology would give patients and their families confidence in putting themselves under our care. Together with good interpersonal skills and communication, knowledge, judicious and appropriate use of technology, we can relieve often and comfort always.

FUTURE POTENTIAL APPLICATIONS

AFP as a prenatal tool and tumor maker has already been widely and effectively used for screening, diagnosis, prognostication and monitoring of disease progress and treatment. More specific tools such as the sub-fractionation techniques described and greater understanding of its in vitro and in vivo characteristics would further refine these uses of the AFP analysis.

Studies involving uptake of this oncofetal-protein have culminated in radioimaging reports as well as the use of AFP as a chemotherapeutic drug conjugate and immunotherapeutic agents. Therapeutic utilization of AFP and its peptidic fragments as growth-response modifiers[22], gene therapy, vaccination and cancer chemoprevention is not unconceivable[23]. The humble AFP may have far reaching screening, diagnostic, therapeutic, prognostic and preventive applications in the future.

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