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REVIEW

Alpha-fetoprotein: a renaissance

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Abstract Alpha-fetoprotein (AFP) is a major mammalian embryo-specific and tumor-associated protein that is also present in small quantities in adults at normal conditions. Discovery of the phenomenon of AFP biosynthesis in carcinogenesis by G. Abelev and Yu. Tatarinov 50 years ago, in 1963, provoked intensive studies of this protein. AFPs of some mammalian species were isolated, purified and physico-chemically and immunochemically characterized. Despite the significant success in study of AFP, its three-dimensional structure, mechanisms of receptor binding along with a structure of the receptor itself and, what is the most important, its biological role in embryo- and carcinogenesis remain still obscure. Due to difficulties linked with methodological limitations, research of AFP was to some extent extinguished by the 1990s. However, over the last decade a growing number of investigations of AFP and its usage as a tumor-specific biomarker have been observed. This was caused by the use of new technologies, primarily, computer-based and genetic engineering approaches in studying of this very important oncodevelopmental protein. Our review summarizes efforts of different scientific groups throughout the world in studying AFP for 50 years with emphasis on detailed description of recent achievements in this field.

Keywords AFP · Alpha-fetoprotein · Structure and function · Biologically active peptides · Hepatocellular carcinoma · Onco-biomarker

Introduction

Fifty years ago, in 1963, a discovery of an antigen specific for mouse chemically induced hepatoma by *Garry I. Abelev* [1] and for human hepatocellular carcinoma (HCC) by *Yuri S. Tatarinov* [2] was first reported. This antigen was not revealed in extracts of normal adult kidney, spleen and blood serum, but was detected in mouse and human embryonic liver extracts, amniotic fluid, and the blood serum. This antigen was shown to electrophoretically belong to α-globulin fraction. It became clear that the liver cancer cells produced and secreted into the blood serum an embryo-specific α-globulin that was initially named α_F in mice and ESA-globulin in human. The newly revealed antigen was identical to the protein discovered by *C. Bergstrand and B. Czar* in human fetus in 1956 [3]. Later, in 1970, this antigen was named "alpha-fetoprotein" (AFP) and has been long recognized as the first oncodevelopmental biomarker. Currently, AFP is considered as a "golden standard" among tumor-specific molecular biomarkers [4].

Immediately after its discovery as a tumor-associated antigen, AFP became an attractive object for intensive studies by different groups of investigators throughout the world (Table 1). At the first stages of investigations different immunochemical methods were widely exploited to assess level of AFP during embryonic development and tumor growth and to reveal sites of AFP biosynthesis [5–8]. Data obtained independently by *A. Terentiev* [9, 10] and *K. Kithier* [11] with the use of the method of immune-precipitation allowed distinguishing AFP from fetuin, another embryo-specific protein. Also, immunological relationships between human and animal AFPs along with responses to injection of homologous or heterologous AFP in adult animals were studied by the

Dedicated to the memory of Professor Yuri S. Tatarinov, the honorary person, who died last year and whose 85-year anniversary we would celebrate in September, 2013. Professor Yu. S. Tatarinov was born on September 2, 1928 and died on January 29, 2012. He was Head of Biochemistry Department at N.I. Pirogov Russian National Research Medical University from 1972 to 1999 and founded the scientific school that trained several generations of scientific followers to whom authors of the present review belong.

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Table 1 The main scientific groups carried out studies in the field of alpha-fetoprotein

Names of group leaders	Institution	City and country	Major data obtained	Years of data publication
Bergstrand C.G. and Czar B.	Karolinska Hospital	Stockholm, Sweden	Electrophoretic revealing of a new embryo-specific protein in serum of human fetus	1956
Abelev G.I.	N.F. Gamaleya Research Institute of Epidemiology and Microbiology,	Moscow, Russia	Discovery of AFP in mouse chemically induced hepatoma	1963–2008
	N.N. Blokhin Research Oncological Center		Development of methods of isolation and purification	
			Study of regulation of gene expression and epitopic structure of AFP	
Tatarinov Yu.S.	Astrakhan State Medical Institute,	Astrakhan, Russia	Discovery of AFP in human hepatocellular carcinoma (HCC)	1963–2005
	N.I. Pirogov Russian National Research Medical University	Moscow, Russia	Immunochemical characteristics	
			Carbohydrate heterogeneity and lectin-binding and estrogen-binding ability of human AFP	
Gitlin D. and Boesman M.	University of Pittsburgh School of Medicine	Pittsburgh, Pennsylvania, USA	Revealing yolk sac and fetal liver as sites of biosynthesis of AFP	1967
Hirai H. and Nishi S.	Hokkaido University	Sapporo, Hokkaido, Japan	Immunological cross-reactions between AFPs of different species	1967–1973
	University of Wisconsin	Madison, Wisconsin, USA	Localization of estrogen-binding site in chimeric human–rat proteins. Immunosuppressive activity	1991–1999
Uriel J.	Institut de Recherches Scientifiques sur le Cancer (CNRS)	Villejuif, France	Estrogen-binding ability of rat, mouse and human AFP	1972–1976
			Revealing receptors for AFP in different cell types including human MCF-7 breast cancer and T-lymphoma cell lines. Binding of fatty acids to AFP	1983–1996
Ruoslahti E. and Seppala M.	University of Helsinki, University Central Hospital	Helsinki, Finland	Development of RIA to reveal very low amounts (5–7 μ/ml) of AFP in adult human blood serum. Study of homology between AFP and SA. Bilirubin-binding ability	1971–1979
			Fetal patho-physiology of AFP	
Terentiev A.A. and Moldogazieva N.T.	N.I. Pirogov Russian National Research Medical University	Astrakhan, Russia	Differentiation from fetuin, another embryo-specific protein	1968–1986
		Moscow, Russia	Development of methods AFP isolation and purification. Study of estrogen-binding ability of human AFP	1988–1992
			Structural and functional mapping of AFP. Modeling of 3D structure of HAFP-DES complex. Study of biological activity conformational dynamics of AFP-derived heptapeptide $AFP_{14–20}$	1997–2012
Dugaiczyk A.	University of California	Riverside, California, USA	Structure, polymorphism and repeated DNA elements in alpha-fetoprotein gene. Evolution of albuminoid gene family	1982–1996
Nunez E.A.	INSERM	Paris, France	Fatty acid-binding capability. Conformational changes in rodent and human AFP	1989–1992

Table 1 (continued)

Names of group leaders	Institution	City and country	Major data obtained	Years of data publication
H. Taga and K. Taketa	Okayama University Medical School	Okayama, Japan	Study of structure of carbohydrate moiety of human AFP, its isoforms and lectin-binding capacity	1989–1998
Uversky V.N.	Institute of Protein Research University of South Florida	Pushchino, Moscow Region, Russia. Florida, USA	Formation of a molten globule form (MGF) by AFP. Study on influence of pH and organic solvents on AFP conformation with the use of biophysical methods	1995–1999
Mizejewski G.J. and Jacobson H.I.	Wadsworth Center, New York State Department of Health	Albany, New York, USA	Immunosuppressive activity of AFP. Inhibition of estrogen-dependent proliferation of immature mouse uterine cells and MCF-7 human breast cancer and human prostate cells both by intact and recombinant AFP	1983–1995
			Study of biological activity of growth inhibitory peptides (GIP). Mapping of structure-function peptide sites	1997–2013
Dudich I.V. and Dudich E.I	Institute of Immunological Engineering	Lyubuchany Moscow Region, Russia	Study of induction of caspase-3-mediated apoptosis by high concentration of AFP. Engineering and study of regulatory activity of recombinant AFP	1997–2012
Butterfield L.H.	University of California, University of Pittsburgh Cancer Institute	Los Angeles, California, USA Pittsburgh, Pennsylvania, USA	Revealing HLA-restricted epitopic nonapeptide segments in AFP able to recognize and activate $CD8^+$ T-lymphocytes in patients with HCC. Basis for cancer immunotherapy1	1999–2012
Behboudi S. and Alisa A.	Institute of Hepatology University College London	London, UK	HLA-DR-restricted epitopic peptide segments in AFP able to recognize $CD4^+$ T-lymphocytes circulating in the blood of patients with HCC	2005–2010
Szpirer J. and Szpirer C.	Institut de Biologie et de Médecine Moléculaires	Bruxelles, Belgium	Study of the role of AFP in sexual differentiation of hypothalamo-pituitary system and female fertility	2002–2006
Li G.	Peking University Health Science Center	Beijing, China	Study of intracellular effectors of signaling pathway stimulated by AFP. Inhibition of TRAIL-induced apoptosis	2002–2011
Severin S.E. and Severin E.S.	Moscow Research Institute of Medical Ecology	Moscow, Russia	Usage of recombinant AFP and its domain III as vectors for targeted receptor-mediated delivery of antitumor drugs to tumor tissue	2000–2012

group of *H. Hirai* [12, 13]. Breakdown of immunological tolerance by immunization with heterologous AFP was demonstrated.

Development of new highly sensitive methods such as enzyme-linked immunoassay and radio-immunoassay (RIA) allowed determining very low amounts of alpha-fetoprotein in the blood serum of normal human adults. These new approaches with about 1000-fold higher sensitivity than the immune-precipitation method have been exploited by *E. Ruoslahti and M. Seppala* to discover AFP at concentrations as low as 5–7 ng/ml in adult human blood serum [14, 15].

Human and some animal AFPs (including those of mouse, rat, rabbit, guinea pig, etc.) have been isolated, purified, and physico-chemically and immunochemically characterized (for more details see reviews [16, 17]). To date (February, 2013) primary structure of full-length AFPs has been reported for thirteen mammalian species (UniprotKB/Swiss-Prot knowledge base). However, the biological role of AFP during embryonic development, in carcinogenesis and, possibly, in normal adults along with mechanisms underlying its functioning are not fully elucidated.

Over the last decade, attempts to evaluate the biological role of AFP by identification of its functionally important sites with the use of computer-based amino acid sequence alignment have been undertaken [18]. Comparison of primary structures of AFP and some physiologically active proteins has led to revealing similarity between peptide segments of AFP and functionally important sites of those proteins and to prediction of multiple functions of AFP. As a result, multimodularity and polyfunctionality of AFP was suggested (for more details see review [19]). Some peptide fragments of AFP have been synthesized and tested for biological activity. Localization of functionally important sites followed by determination of their amino acid sequences and types of biological activity has provided valuable information for structural–functional mapping of AFP (Fig. 1).

Moreover, development of new computer-based technologies has allowed generating a model of three-dimensional (3D) structure of AFP that has not been experimentally elucidated yet. Such a model will contribute to studying mechanisms of interaction of AFP with low molecular weight ligands and other proteins. Experimental data on AFP–ligand and AFP–protein interactions will be described below. Also, genetic engineering approaches have been used to obtain recombinant forms of AFP and its domains for studying their functions and developing novel therapeutic agents with antitumor activity on their basis [20, 21].

Studies on structure of AFP

Structure of AFP was studied with the use of experimental methods from 1970s through 1980s [22–24]. AFP was shown to be a glycoprotein with the molecular weight ranging from 68 to 73 kDa in dependence on carbohydrate content and biological origin. Being a secreted protein, AFP is synthesized as a precursor that undergoes post-translational processing with cleavage of a signal peptide. The amino acid sequence of immature AFP polypeptide chain was deduced from its mRNA nucleotide sequence and the translation product was shown to contain 609 residues [22]. Initial studies showed that the signal peptide contains 19 residues, and the mature protein is composed of 590 residues. However, later the *N*-terminal amino acid residue of mature AFP molecule isolated both from cultured human hepatoma HepG2 cells and embryonic tissues was shown to be arginine instead of threonine and, therefore, the mature human AFP molecule was demonstrated to contain 591 residues [23, 24].

AFP gene is a member of the family of albuminoid genes that are localized in tandem arrangement on mouse chromosome 5 or human chromosome 4 to form multigene cluster (region 4q11–q13) [25, 26]. Except AFP itself, translational products of these genes include serum albumin (SA), vitamin D-binding protein (VTDB), and alpha-albumin (afamin). Members of the albuminoid gene family are evolutionary closely related and originate from common ancestor [26].

All members of the family also demonstrate similar structural and physico-chemical properties. There, molecular weight varies from 66 kDa (for albumin) to 82 kDa (for afamin) and these variations are, mainly, attributed to differences in carbohydrate content of the proteins [27–29]. They also exhibit considerable similarity in their primary structure with characteristic distribution of cysteine residues; for example, human AFP and SA share 40 % identity with highly conserved cysteine residues including double cysteines [30].

Proteins of albuminoid gene family are also characterized by similar α-helical secondary structure (up to 65–67 % α-helices in AFP and about 50 % in serum albumin) with lack of β-pleated sheets. These proteins exhibit similar spatial organization and consist of three homologous domains (I–III) each of which, in turn, consists of two globular subdomains (IA, IB, IIA, IIB, IIIA, IIIB) linked by 15 regular-arranged disulfide bonds [31, 32]. The three-domain structure of AFP was demonstrated by electron microscopy, image processing and circular dichroism that revealed existence of U-shaped structure with three regions of electron density mass: one on the top and two others on edges of the molecule.

All domains have similar secondary structure and contain 68, 55, and 71 % α-helices; but they differ by parameters of tertiary structure [31]. Domains I and III have rigid tightly packed conformation and join to each other by relatively flexible and proteolytically sensitive domain II. *C*-terminal part of domain II can be considered as a “hinge region” responsible for conformational flexibility of other domains, and therefore, it may promote interaction between AFP and low molecular weight ligands and other proteins [33]. Domains I and II of human AFP include 192 residues each (residues 19–210 and 211–402, respectively); domain III consists of 199 residues (402–601). The highest identity degree between amino acid sequences of human AFP and SA is found within domain III (46 %), whereas the lowest one—within domain I (31 %) [own unpublished data].

There is also high similarity degree between amino acid sequences of AFP from different mammalian species. For example, primary structures of human and mouse AFPs share 66 % identity [34]. The highest identity degree is observed between domains III (72 %) and the lowest one between domains I (59 %) of AFPs of these species. Interestingly, different domains within the same protein (either AFP or SA) from the same species exhibit lower similarity (from 18 to 25 % identity in human) than the same domains of different proteins (see above). These data suggested the way of evolution from primordial gene that first expanded to give tripartite ancestral gene that, in turn, was duplicated to give *AFP* and *ALB* genes [35].

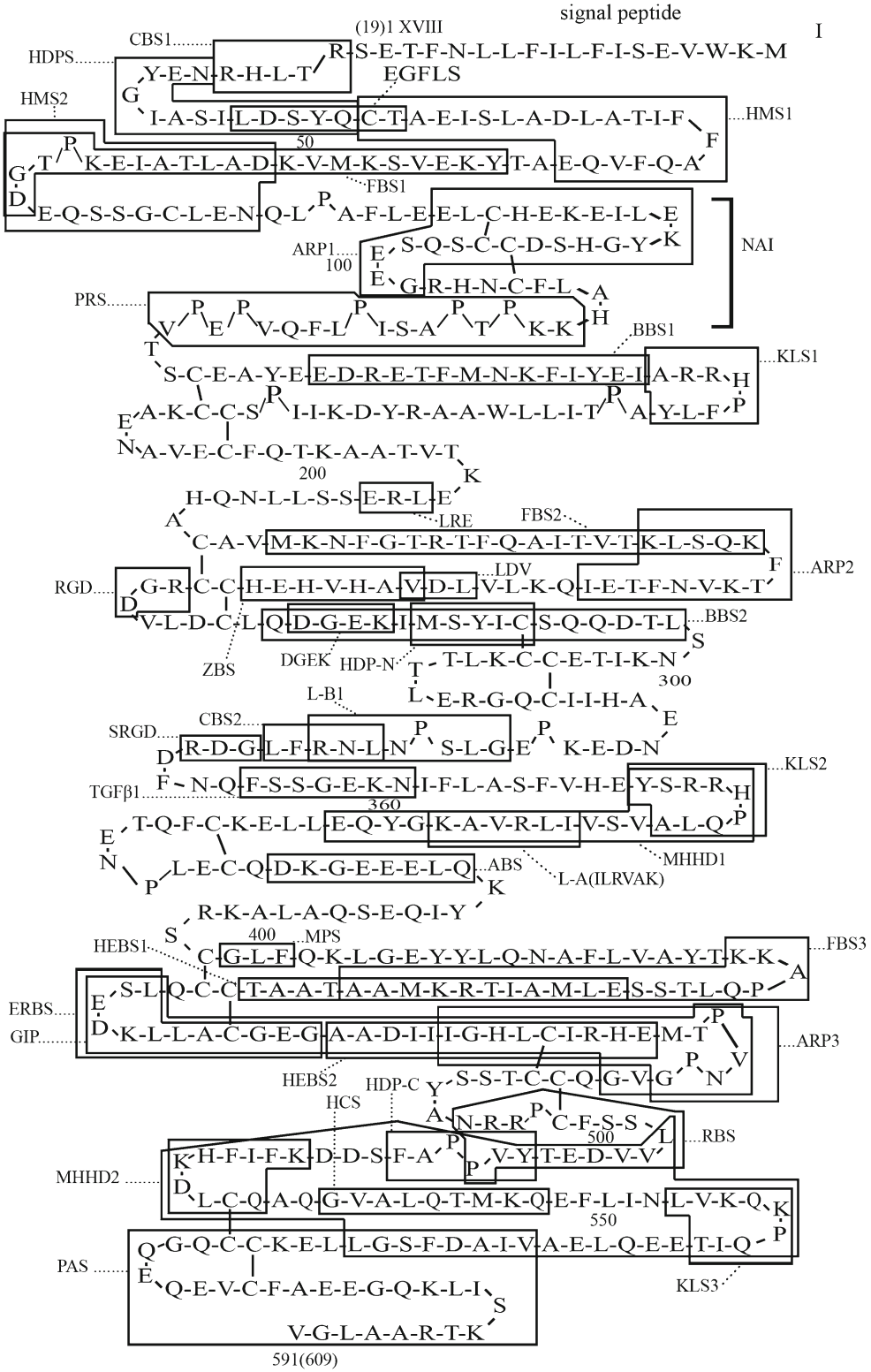

Fig. 1 Map of primary structure of human AFP. Signal peptide and functionally important sites are indicated. *I–XVIII* signal peptide; *EGFLS* epidermal growth factor like segment ($AFP_{14–20}$); *TGFβ1* segment of transforming growth factor-β1; *GIP* growth inhibitory peptide; *FBS1, FBS2, and FBS3* fatty acid-binding sites 1, 2, and 3; *HEBS1* major human estrogen-binding site; *HEBS2* secondary human estrogen-binding site; *ERBS* estrogen-receptor-binding site; *HDPS* homeodomain proteins segment; *CBS1 and CBS2* cycline-binding segments 1 and 2; *BBS1 and BBS2* bilirubin-binding sites 1 and 2; *ABS* actin-binding site; *HMS1 and HMS2* heavy metal-binding sites 1 and 2; *NAI* non albumin identity site; *PRS* proline rich segment; *KLS1, KLS2, and KLS3* kinetensin-like segments 1, 2, and 3; *LRE, LDV, RGD, DGEK, and ILRVAK* integrin-binding sites; *SRGD* segment reverse to RGD; *MHHD1 and MHHD2* motifs of hetero- and homodimerization 1 and 2; *HCS* histocompatability class II segment; *MPS* milk peptide segment; *PAS* plasminogen activator segment; *ZBS* zink-binding site; *L-B1* laminin B1; *L-A* laminin A; *ARP1, ARP2, ARP3* apoptosis related polypeptides 1, 2, and 3

Conformational changes in AFP under different conditions have been also studied. High concentrations of fatty acids were shown to cause conformational and functional changes in human and rodent AFPs [36, 37]. Incubation with high concentrations of unsaturated fatty acids leads to loss of ability to interact with polyclonal antibodies to AFP. Also, high concentrations of estrogens cause conformational changes in AFP and its domain III and increase in ability of AFP to stimulate growth of estrogen-dependent human breast (MCF-7) and prostate cancer [38, 39].

Detailed mechanisms of conformational changes in AFP were studied by *V. Uversky* and colleagues during the second half of the 1990s. They showed that these changes may be induced by decrease in pH value and may lead to exposure of hydrophobic ligand-binding regions in AFP globule [40]. Besides, irreversible conformational changes in AFP were caused by removal of low molecular weight ligands or influence of organic solvents, such as 9.5 M urea and hexane, and were accompanied by loss of unique compact tertiary structure with retaining secondary structure elements. These changes were detected by methods of circular dichroims, fluorescence spectroscopy and scanning microcalorimetry [41, 42].

Conformation of AFP became corresponding to molten globule form (MGF) that usually appears under mild denaturing conditions and is common for numerous proteins. AFP has been proposed to be secreted into the blood in native compact conformation, but to exist in the cytoplasm in MGF. In this form, a protein is usually characterized by high degree of internal mobility including rotational isomerism of side chains. Probably, the molten globule state contributes to adaptive response of a protein to variety of intracellular conditions. Also, transition from native conformation to MGF may facilitate functioning of AFP including its interaction with cell surface receptors and low molecular weight ligands with subsequent cellular uptake of the complex formed.

Content of carbohydrates in AFP varies from 3 to 5 %, whereas serum albumin contains only 0.5 %, vitamin D-binding protein and afamin have 5 and 21 % of carbohydrates, respectively. Carbohydrate moieties of the family members include glucose, galactose, mannose, *N*-acetylglucosamine, and sialic acids [43]. For example, branched oligosaccharide chain of human AFP contains D-glucose, D-mannose, and two sialic acid residues. Human AFP has been shown to contain only one glycosylation site (N233), whereas mouse AFP has three such sites: N232, N310, and T483. In rat AFP carbohydrate components may be attached to N232, S96, and N310.

AFPs isolated from both embryonic and tumor tissues of the same species have the same amino acid sequence; they are also immunologically identical. However, numerous studies demonstrated that AFP exhibits molecular microheterogeneity that is determined by difference in structure of carbohydrate moieties of AFP from different tissues and diseases [44]. This provides existence of AFP isoforms that differ from each other by their p*I* values and lectin-binding capacities [45, 46]. Tissue and tumor specificity of various glycoforms of AFP is determined by specific set of enzymes involved in glycosylation reactions [47, 48].

Concanavalin A, Lens culinaris agglutinin A, erythroagglutinating phytohemagglutinin and Allomyrina dichotoma lectin are lectins primarily used to study carbohydrate structures of glycoproteins by affinity chromatography or affinity electrophoresis. The group of *H. Taga and K. Taketa* developed combination of two-dimensional mixed-lectin (or gradient) agarose gel-affinity electrophoresis with sequential digestion of carbohydrate moieties with sialidase, beta-galactosidase and beta-*N*-acetylhexosaminidase for the determination of sugar chain structure of different isoforms of AFP [43, 49–52]. Five glycoforms, AFP-P2, AFP-P3, AFP-P4, AFP-P5, and AFP-P6 differed from each other by type of sialylation (mono- or di-) of galactose with biantennary structures attached at different manners to mannose were identified in cord blood [50]. Changes in carbohydrate structures of AFP from patients with benign and malignant diseases were also shown. At the early stage of HCC the addition of fucose to the reducing end *N*-acetylglucosamine was shown. Increase in heterogeneity of galactose and *N*-acetylglucosamine residues in cases of both advanced HCC and AFP-producing extrahepatic malignancies was observed. More than 94 % of the AFP carbohydrate structures found in patients with benign and malignant liver diseases were biantennary oligosaccharides [51].

Investigations of AFP functions

In a number of experimental models in vitro and in vivo, AFP from different biological species has been shown to exhibit various types of biological activity. Growing data demonstrate multi-functionality of AFP that is provided by its multi-modular structure. Below, we briefly describe the main types of biological activity of AFP that suggest its putative functions during embryonic development and cancer growth.

Binding of hydrophobic ligands It has been long demonstrated that AFP binds and, possibly, transfers variety of hydrophobic ligands including fatty acids, estrogens, phytoestrogens, bilirubin, retinoids, flavonoids, dyes, some drugs, and metal ions [53–69]. In particular, many embryonic and tumor cells can accumulate unsaturated fatty acids bound to AFP [53–59]. A suggestion has been made that AFP may deliver fatty acids (predominantly, polyunsaturated ones, such as arachidonic, $C_{20:4}$, and docosahexaenoic, $C_{22:6}$) to proliferating cells that require increased energy supply and intermediate products of β-oxidation of fatty acids.

Estrogen-binding activity of AFP is of crucial importance because this ability may represent a significant regulatory mechanism during embryonic development, since AFP may be involved in regulation of concentrations of free, i.e. active, forms of hormones in vivo. This might protect fetal tissues from circulating maternal estrogens and prevent degradation of hormone molecules. The most successful results in studying the estrogen-binding activity were obtained by the group of French researchers led by *J. Uriel*, who demonstrated high-affinity binding of both free and immobilized

estrogens to rodent AFPs [64–69]. However, the estrogen-binding ability of human AFP was not demonstrated until the end of 1980s.

Data obtained by our group, led by *Yuri Tatarinov* with the use of method of affinity chromatography demonstrated ability of human AFP isolated from maternal and embryonic tissues by mild butanol extraction to bind immobilized estrone and synthetic estrogen analogue—diethylstilbestrol (DES) [70–73]. Preliminary incubation of AFP with free estrogens and other steroids, except DES, did not reduce ability of AFP to bind immobilized hormones. However, pre-incubation of AFP with free DES was accompanied by almost two-fold reduction in binding to immobilized estrogens. These data suggested ability of both free and immobilized DES to bind to human AFP. Length of a spacer used for hormone immobilization and a mode of immobilization, i.e. spatial orientation of the hormone molecule, were crucial for estrogen binding. Possibly, interaction between AFP and estrogens in vivo requires some messenger molecule. Usage of organic solvent, butanol, for protein extraction caused dissociation of AFP–hormone and/or AFP–fatty acid complexes and release of estrogen-binding sites. We suggested conformational changes in AFP molecule caused by removal of fatty acids and followed by exposure of hydrophobic estrogen-binding sites.

Recently, we generated computer-based model of 3D structure of human AFP on the basis of homology with human SA and VTDB to elucidate molecular mechanisms underlying estrogen-binding affinity of human AFP [74]. The modeling was based on experimentally solved 3D structures of SA and VTDB extracted from PDB database. Visualization of the model showed its U-shaped structure with a cavity formed by domains I and III (Fig. 2). So, the structure of our model is consistent with the experimental data obtained earlier by *A. Luft* and *F. Loscheider* [41] by combining electron microscopy with laser-assisted optical system, which allowed producing three regions of relative mass density in human and bovine AFP. Secondary structure elements corresponded to α-helices and random coils with no β-structures. Amount of α-helices was close to experimental data and constituted about 69 % from the total polypeptide chain length. Low RMSD values calculated for hypothetical estrogen-binding site during molecular dynamics (MD) simulation indicated reasonable good quality for this part of the model and allowed performing DES docking to the model.

MD simulation was also performed for human AFP–DES complex to obtain dynamical picture of conformational changes along with detailed mechanisms of interaction between AFP and the hormone molecule (Fig. 3). Two sets of amino acid residues were suggested to take part in binding of DES to human AFP: (1) hydrophobic side chains of residues L138, M448, and M548 that interact with aromatic rings of

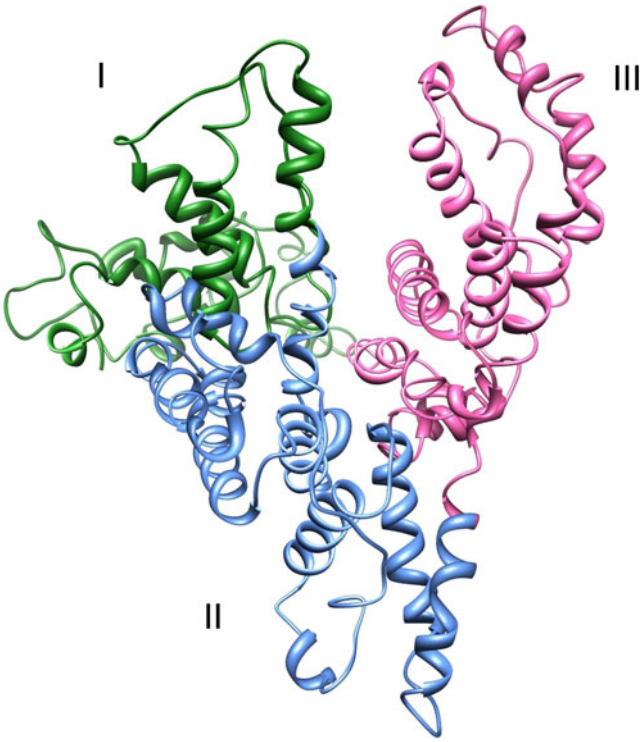

Fig. 2 Model of tree-dimensional structure of human AFP generated on the basis of homology with serum albumin. Domains I, II and III are indicated. Secondary structure elements are represented by alpha-helices and irregular structures

DES; and (2) hydrogen bonding with participation of OH-groups of the hormone and polar atoms of S445, R452, and E551 side chains.

Immunosuppressive activity It has been long experimentally demonstrated that AFP is able to interact with macrophages to decrease their phagocytic activity and expression of Ia antigen [75, 76]. Also, AFP inhibits activity of natural killer (NK) cells [77, 78], reduces proliferation of T-lymphocytes stimulated by ConA and PHA with reduction of amount of total and $CD4^+$ thymocytes [79–81], and also induces

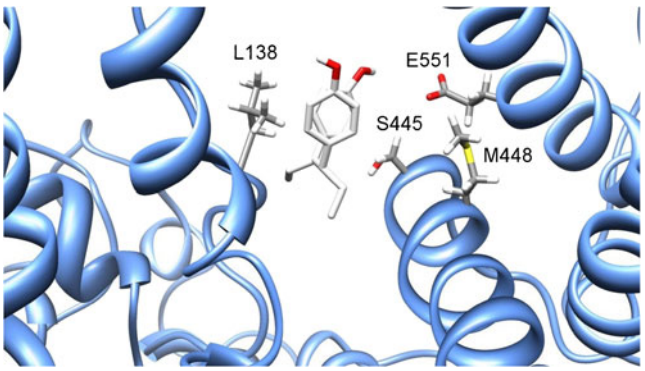

Fig. 3 Estrogen-biding region of human AFP determined by docking of DES to 3D model of HAFP. Amino acid residues that participate in complex formation are shown

activity of T-suppressor cells [82]. Some authors suggested that immunosuppressive activity of AFP is not its intrinsic property and depends on the presence of low molecular weight ligands or carbohydrate moieties. For instance, only one of seven molecular variants of AFP that differ from each other by content of sialic acids and p*I* value exhibited immunosuppressive activity [83]. This isoform (with p*I*=5.1) constitutes 6 % of total AFP amount and contains 1 mol/mol sialic acids. However, most other data demonstrate immunosuppressive activity of both native and recombinant AFP to be provided by structure of its protein moiety irrespectively to presence of ligands and/or carbohydrate components [84].

Treatment with recombinant human AFP reduced lymphocyte reactivity and the extent of neuroinflammation in mice with experimental autoimmune encephalomyelitis, due to involvement of AFP in immune cell apoptosis [85]. AFP increased expression of Bax, Bid, Bad, and ApaF genes in peripheral blood lymphocytes as well as caspase-3, Fas, FasL, and TNF-related apoptosis inducing ligand (TRAIL) in infiltrating immune cells. The induction of apoptotic markers was accompanied with increased Foxp3 expression in lymph node cells along with accumulation of $CD4^+$ regulatory T-cells.

Existence of two types of linear antigenic determinants in human AFP was reported. The first type is the immunodominant sites that belong to major histocompatibility complex (MHC) class I antigens and the second one—minor sub-dominant sites. 13 and 17 linear epitopes localized in domains I and II, respectively, were found in human recombinant AFP with the use of a set of 36 rat monoclonal antibodies (MAB) by immunoenzyme assay [86]. One of these epitopic peptide segments with sequence CKAENAVE (residues 193–200) was recognized by MAB AFY6. Interestingly, the linear epitopic sites in native AFP were hidden and became exposed after deglycosylation procedures. Exposure of the epitopic sites led to interaction of AFP with immunoglobulins in patients with primary liver cancer, liver cirrhosis, and chronic hepatitis and to induction of spontaneous immune response in these patients.

Studies performed by the group of *L. Butterfield* identified four immunodominant HLA-restricted human AFP-derived peptides that stimulate specific T-cell response. These peptides may be recognized by $CD8^+$ T-lymphocytes. AFP-producing dendritic cells obtained by genetic engineering were able to generate AFP-specific immune response in culture of autologous human T-lymphocytes and in transgenic mice HLA-A2.1/Kb [87]. The nonapeptide segment GVALQTMKQ in the third domain of AFP (residues 542–550) activated T-lymphocytes to stimulate their cytotoxicity and cytokine releasing ability [88].

Dendritic cells activated by nonapeptide LLNQHACAV from the second domain of human AFP (residues 218–226) were also able to stimulate T-lymphocytes. Increased level of cytokines IL-12 and TNF-α along with appearance of cytotoxic T-lymphocytes were observed. Lysis of HLA-A2-positive HepG2 and T2 hepatoma cells along with all three line HCC cells was demonstrated [89]. $CD8^+$ T-cell response to human leukocyte antigen class I-restricted immunodominant peptides derived from AFP in patients with HCC was proposed to be used for the cancer immunotherapy [90–94].

Another group led by *A. Alisa* and *S. Behboudi* reported revealing of HLA-DR-restricted linear epitopic segments in AFP able to recognize $CD4^+$ T-lymphocytes circulating in the blood of patients with HCC. Also, association of $CD4^+$ or $CD8^+$ T-cell response with AFP concentrations and stage of HCC were demonstrated. In particular, $CD4^+$ T-cell response was shown to correlate, mainly, with low serum AFP concentrations and with early stage of the disease [95, 96]. AFP-specific $CD4^+$ T-cells may produce IFN-γ, IL-2, TNF-α and TGF-β [97]. Also, this group of researchers demonstrated the $CD4^+$ T-cell response to be detected only in HCC patients, whereas the $CD8^+$ response was detectable in both HCC and healthy donors. Moreover, these results suggest the anti-AFP $CD4^+$ T-cell (Th1) response to be specific for the early tumor stage or liver cirrhosis, whereas the anti-AFP $CD8^+$ T-cell (Tc1) response was revealed in patients with more severe liver cirrhosis [98].

Our group also studied immunomodulating activity of AFP and AFP-derived synthetic peptides in cultured lymphocytes [99–101]. Using a method of indirect immunofluorescence and commercially available monoclonal antibodies labeled by fluorescein isothiocyanate (FITC), we demonstrated influence of human AFP on expression of antigens of cluster of differentiation on the surface of lymphocytes from patients with atopic bronchial asthma and rheumatoid arthritis. Human AFP caused almost two-fold reduction in number of $CD16^+$ lymphocytes and decrease expression of CD25, CD71, and HLA-DR antigens. However, it slightly increased expression of apoptopic marker CD95. Human AFP-derived synthetic peptide with sequence LDSYQCT (residues 14–20) designated as $AFP_{14–20}$ has similar effect and significantly decreased expression of HLA-DR antigen and induced expression of CD95 antigen [99]. In reaction of lymphocytic blast-transformation AFP and the heptapeptide $AFP_{14–20}$ caused moderate stimulation of proliferation of intact lymphocytes and marked inhibition of proliferation of PHA-activated lymphocytes at concentrations of 10^{-7} to 10^{-9} M [100, 101].

In a culture of human immortalized myelogenous leukemia K-562 cell line, the heptapeptide caused 1.5–2.0-fold increase in cytotoxic activity of NK-cells. These data suggest ability of $AFP_{14–20}$ to increase antiviral and anti-microbial activity of the lymphocytes. Also, this peptide in dose-dependent manner inhibits proliferation of lymphocytes from patients with acute and chronic lymphocytic leukemia with low sensitivity to antitumor agent, cytosar, increasing anti-proliferative activity of this drug [102]. Thus, $AFP_{14–20}$

was proposed to be one of the biologically active sites of human AFP.

Computer-based models of heptapeptide LDSYQCT and its analogs obtained by point amino acid substitutions were constructed and MD simulation studies were performed. Differences in biological activity of AFP-derived peptides were shown to be provided not only by physico-chemical, but also by conformational and dynamics properties of their amino acid residues [103, 104]. Correlation between biological activity of the peptides and their conformational dynamics that, in turn, depends on inter- and intramolecular interactions, was also demonstrated. Peptides LDSYQCT, PYECE, YECE, and YVCE with relatively rigid conformation were shown to exhibit similar activity in influencing on expression of the CD antigens on the surface of T-lymphocytes in patients with immunopathologies [105]. On the contrary, peptides with relatively flexible backbone (LYVCS, SYKCE, YACS, and YQCE) have no effects on expression of cell surface antigens on T-lymphocytes. These data may be used as a basis for the development of new therapeutic agents with immunomodulatory activity.

Dual regulation of cell proliferation Synthesis of AFP during embryonic development and its high levels in fetal serum suggest its capability to stimulate tissue growth. Indeed, an ability of AFP to stimulate proliferation and differentiation of variety of cell types including lymphoid and epidermal cells, fibroblasts, hepatocytes, and ovarian and uterine cells has long been demonstrated [106–108].

AFP has been shown to be a dual regulator of cell proliferation and tissue growth exhibiting both stimulatory and inhibitory effects. AFP exhibited stimulatory effect at low concentrations, and in estrogen-resistant tissues, both normal and tumor [107, 109–111]. In HCC, estrogen-resistant tumor, intact AFP significantly decreased expression of cyclin-dependent kinase inhibitor p27(kip1,) both at mRNA and protein levels, and increased expression of proliferating cell nuclear antigen (PCNA) [112]. Stimulation of cell proliferation is accompanied by inhibition of apoptosis [111].

Inhibitory effect of AFP is exhibited on estrogen-sensitive cell lines. Inhibition in vitro and in vivo of estrogen-dependent proliferation of immature mouse uterine cells and MCF-7 human breast cancer and prostate cancer cell lines was studied by groups of *G. Mizejewski* and *H. Jacobson* [113–116]. Inhibition of estrogen-sensitive tumor growth by AFP was also confirmed by epidemiological studies that demonstrated significant reduction in risk of mammary cancer during pregnancy, and this effect persisted for several years after delivery [115]. Recombinant human AFP also inhibited growth of estrogen-dependent MCF-7 tumor [117–119]. However, in the case of estrogen-independent MDA-MB-231 tumor cells, these researchers did not observe antitumor activity of human AFP.

Also they identified a structural motif of 34 amino acid residues in length (446–479) responsible for suppression of proliferation of estrogen-dependent cells [120–124]. In contrast to intact AFP, this peptide did not require pre-incubation with hormone to exhibit biological activity and it was defined as growth-inhibitory peptide (GIP, also either P149 or P447). Immunochemical studies showed that antibodies against this peptide does not recognize it within the native AFP, but may do that in conformationally changed protein [120]. This segment was suggested to be sterically hidden in native AFP, but it became exposed under high estradiol concentrations. In a model in vivo both P149 and the intact AFP molecule decreased fetotoxicity of estrogens and insulin [125]. Defects in perinatal development and fetus death decreased by 50 and 63–73 %, respectively.

This peptide was chemically synthesized and tested for biological activity, and was shown to inhibit estrogen-dependent proliferation of mouse immature uterine cells, and this activity was retained in chemically modified peptides obtained by formation of *S*-methyl-cysteine or *S*-(2-aminoethyl)-cysteine. Activity of the derivatives was 31 and 29 %, respectively, and was comparable to that of intact peptide (45 %) [122, 123]. Other analogs of P149, in which two cysteine residues were replaced with alanine, glycine, or serine, have also been synthesized. The highest biological activity (37 %) was exhibited by alanine-containing peptide [124]. The glycine-containing peptide was two-times less active (17 %), whereas the serine-containing peptide has no biological activity. In estrogen-resistant prostate tumor growth inhibition all peptides were inactive [126].

Subsequent studies revealed that octapeptide fragment of P149, EMTPVNPG (residues 471–478), exhibited the highest biological activity [127]. It caused the more potent inhibition of mouse uterine cell proliferation (49 %) than the P149 itself (45 %) and the intact AFP molecule (35 %). Later, two proline residues were replaced with two 4-hydroxyprolines, and the resultant octapeptide EMTOVNOG became more hydrophilic than the initial peptide [128]. Also, cyclo-EMTOVNOG with more rigid conformation than the initial octapeptide was obtained. Both peptides exhibited the same biological activity similar to that of intact octapeptide P149, and G-protein-coupled membrane receptor-mediated mechanism was suggested. MD simulation supported this suggestion [129].

Proliferation of MCF-7 cells was significantly decreased by addition of 2 μg/ml cyclized nonapeptide from human AFP (EKTOVNOGN or cP) [130]. cP did not increase neither cell death rate nor number of binding sites for estradiol, nor the endogenous aromatase activity of MCF-7 cells. cP also inhibited proliferation of estrogen-dependent ZR75-1 cells, but had no effect on estrogen-independent MDA-MB-231 cells. Over-expression of cyclin-dependent kinase inhibitor p21(Cip1) detected after cP treatment suggests its participation in inhibition of MCF-7 cell proliferation. The cyclic peptide

was also able to increase antitumor effect of tamoxifen and was proposed to be used a basis to develop a new therapeutic agent [131–134].

Using mutant mice with lack of *AFP* gene another group of investigators led by *J. Szpirer* and *C. Szpirer* reported data that argue the role AFP in regulation of tissue growth during embryonic development [135]. Intercross mating of heterozygous mutants ($Afp^{+/-}$) gave rise to offspring of three types: wild ($Afp^{+/+}$), heterozygous ($Afp^{+/-}$), and homozygous ($Afp^{-/-}$) types. Genotyping procedures revealed AFP mRNA in liver extracts and amniotic fluid of wild type and heterozygous embryos, but not in homozygous ones. All embryos were viable and normally developed. Nevertheless, homozygous mutant females were sterile. They had normal estrogen level, but were deficient in progesterone synthesis, and this indicated impairment in ovulation. Elevation of luteinizing and follicle-stimulating hormone ratio (LH/FSH) indicated that this is due to disruption in function of hypothalamo-pituitary system.

AFP was not shown to be synthesized in brain, but it may be found there in considerable amounts due to receptor-mediated uptake by nervous cells [136]. Estrogens bound to AFP may penetrate into nervous cells and regulate level of gonadotropic hormones by negative feedback mechanism. Disruption of AFP synthesis observed in $Afp^{-/-}$ mice resulted in dysregulation of LH and FSH synthesis [137]. On the basis of these findings authors made suggestion that AFP is required for sexual differentiation of hypothalamo-pituitary system during embryonic development.

Putative mechanisms of AFP-initiated signal transduction The detailed mechanisms of regulation of cell proliferation and tumor growth by AFP remain unknown. However, specific binding of AFP to receptors located on the surface of normal and tumor cells has long been demonstrated. A number of experimental data evidence existence of receptors for AFP on the surface of human lung, stomach, ovary and prostate tumor cells, mouse T-lymphoma YAC-1 cells, and rat Morris hepatoma 777 and rhabdosarcoma cells and also on the surface of normal cells of lung, heart, endothelial, reproductive and immune tissues [138–149]. Presence of AFP and its receptor in normal human placenta at term suggests a possible receptor-mediated mechanism for placental transport of AFP between the fetal and maternal circulations [149].

Receptors for AFP were first demonstrated for human MCF-7 breast carcinoma cells *by* the group of *J. Uriel* [138–142]. Penetration of AFP-receptor complex into mouse T-lymphoma cells at 37ºC with subsequent departure being non-degraded was shown [140]. The Scatchard analysis revealed three types of receptors: the first one with high affinity (K_d=2.2×10^{-9} M) and low capacity (700 binding sites per cell), the second one with average values of affinity (K_d=8.6×10^{-7} M) and capacity (210,000 sites per cell) and the third one with low affinity (K_d=5.7×10^{-6} M) and high capacity (910,000 sites per cell).

Adult normal thymocytes and T-lymphocytes in mice were not able to uptake AFP. However, mouse fetal and newborn thymocytes demonstrated ability to specifically bind and to absorb radiolabelled AFP. Human peripheral blood monocytes and PHA-stimulated T-lymphocytes absorbed AFP, whereas non-proliferating T-lymphocytes had no such ability. Only one type of receptor-binding sites with K_d=3.0×10^{-7}M (about 88,000 sites per cell) was revealed on the surface of normal proliferating T-lymphocytes [143]. Receptors for AFP were identified on the surface of human U937 and THP monocyte cell lines [144]. About 50 % of radiolabelled AFP penetrated into cells in 60 min at room temperature. The Scatchard analysis showed availability of two types of binding sites with K_d=5.0×10^{-11}M (49 sites per cell) and 2.5×10^{-7}M (7,800 sites per cell). SDS-electrophoresis in polyacrylamide gel in reducing conditions demonstrated the molecular weight of the receptors to vary from 62 to 65 kDa. Two types of binding sites for AFP differing in affinity with K_d=2.7×10^{-9} and 8.9×10^{-8} M (12,810 and 119,700 sites per cell, respectively) were revealed on the surface of AFP-stimulated NIH 3 T3 tumor cells [148].

As a whole, up to three types of receptors for AFP have been revealed on cell surfaces: the first one with high specificity and low binding capacity, the second one with low affinity and high capacity, and the third one with the average values of affinity and capacity. These receptor types, evidently, have different functional significance. This is concluded from the fact that saturation of high-affinity receptors occurs at physiological concentrations of AFP (10 ng/ml), whereas saturation of low-affinity receptors occurs at high concentrations of AFP. However, as it has been mentioned above, structure of these receptors along with mechanisms of AFP-receptor binding remains unstudied.

R. Moro and co-workers with the use of monoclonal antibodies directed against alpha-fetoprotein receptor proposed that the latter may serve as wide-spread cancer biomarker [146]. Recently, *G. Mizejewski* using data obtained from computer modeling, proteolytic fragmentation/ cleavage patterns, and amino acid sequence analysis along with protein binding analysis proposed a family of multi-ligand-binding receptors that meet the most requirements for AFP receptor [150]. This receptor family was initially identified as the scavenger receptors which belong to single- and double-pass integral transmembrane proteins. Also, a widespread epithelial cell surface transmembrane protein, mucin, was proposed to be an AFP receptor [151]. However, these data do not support the concept that the AFP receptor is a "universal" tumor receptor and/or biomarker. Instead, mucin was proposed to bind to AFP and to engage signal transduction initiated by AFP.

Experimental data obtained in culture of proliferating HeLa and BEL-7402 cells by the group of *Gang Li* evidenced ability of AFP to stimulate phosphorylation and, thereby, activation of extracellular receptor kinases ERK1 and ERK2 that are key intracellular effectors of receptor tyrosine kinase (RTK)-mediated signaling [152, 153]. At concentration of 20 μg/ml AFP-stimulated proliferation of hepatocellular carcinoma BEL-7402 cells, and this was accompanied by over-expression of proto-oncogenes *c-fos*, *c-jun*, *and N-ras*.

Protein products of *c-fos* and *c-jun* have been recognized to be components of transcription factor AP-1 that is stimulated by mitogen-activated protein kinases (MAPKs) and induces further transcription of a number of genes that control synthesis of proteins associated with cell proliferation and differentiation. *N-ras* controls biosynthesis of p21(ras), an intracellular effector of signaling pathway mediated by RTKs. Also, in cultured HeLa cells AFP has been shown to stimulate cell proliferation and to increase by 96 and 81 % biosynthesis of p21 (ras) and mutant tumor suppressor p53, respectively [153].

At the same concentration (20 μg/ml) AFP was able to stimulate expression of receptors Fas and TRAILR (TNF-related apoptosis inducing ligand receptor) in Jurkat T-lymphoma cell line as well as their ligands FasL and TRAIL in human hepatoma BEL-7402 cell line [154]. Co-incubation of Jurkat and BEL-7402 cells stimulated biosynthesis of Fas and suppressed FasL along with induction of caspase-3 expression. Addition of AFP to the culture medium was accompanied by suppression of Fas and induction of FasL in BEL-7402 cells and suppression of caspase-3 in both cell types. A conclusion was made that AFP triggers proliferation of hepatoma cells by escaping from immune surveillance through altering the expression of Fas/FasL and TRAIL/TRAILR.

Regulation of cell apoptosis Some time later, the same group of investigators led by *Gang Li* showed that caspase-3-mediated cascade is the main pathway in TRAIL-induced apoptosis that is abolished by AFP [155]. Caspase-3 co-localized and interacted with AFP in the cytoplasm and translocated into the nucleus of BEL-7402 cells that being co-treated with all-*trans* retinoic acid (ATRA) and TRAIL undergo apoptosis. AFP complexed with caspase-3 and blocked transduction of apoptotic signal into human hepatoma cells. Knock-down of *AFP* gene increased the sensitivity of BEL-7402 cells to TRAIL, and thereby, triggered caspase-3 signaling. ATRA- or TRAIL-resistance in AFP-producing hepatoma cells was attributed to high level of cytoplasmic AFP. Also, capability of intracellular AFP to bind RAR and to block RA-RAR signaling with demonstration of its co-repressor-like role was proposed [156].

Cytoplasmic AFP has also been shown to function as a regulator in the phosphatidylinositol-3-kinase (PI3K)/AKT pathway in human hepatocellular carcinoma cells with the use of fluorescence resonance energy transfer (FRET) technique. Co-localization and interaction of AFP with phosphatase and tensin homolog deleted on chromosome 10 (PTEN) in the cytoplasm of AFP-producing BEL-7402 and HepG2 cells was demonstrated [157]. PTEN is a dual lipid and protein phosphatase; loss of its function leads to accumulation of PI3K and activation of AKT, and this is resulted in protein phosphorylation and cell cycle progression, survival and migration.

These results confirmed data showed that AFP-positive human stomach cancer is characterized by significantly lower apoptotic index than AFP-negative ones and this might evidence inhibition of apoptosis by AFP [158].

On the contrary, high concentrations of AFP (0.1–0.2-mg/ml) were demonstrated to induce cell apoptosis evaluated by morphological changes such as cell shrinking, DNA fragmentation and changes in kinetic parameters. Responsibility of AFP for resistance of hepatoma HepG2 cells to cytotoxic effect of TNF-α was reported by the group of *E. Dudich and I. Dudich* [159]. TNF-α-induced cell death is caused by inhibition of nuclear factor NF-κB that induces expression of genes that encode synthesis of cell survival factors, such as Bc1-XL and XIAP. *AFP* gene seems to be a target for NF-κB because inhibition of the latter is accompanied by suppression of AFP synthesis [160].

Induction of apoptosis by AFP involved Ca^{2+} and protein kinase-independent mechanism, which also did not require synthesis of RNA and a protein. Addition of AFP to cultured tumor cells caused release of cytochrome *c* from mitochondria into cytoplasm, with subsequent formation of apoptosome complex and activation of caspases-3 and 9 [161]. An injectable recombinant human AFP was proposed for the potential treatment of myasthenia gravis, multiple sclerosis, rheumatoid arthritis, autoimmune uveitis and psoriasis [162].

Clinical usage of AFP

The first studies on AFP after its discovery as an oncofetal protein were, as it was mentioned before, devoted to evaluation of its level during embryonic development and pathological conditions along with elucidation of sites of its biosynthesis. The investigations carried out during 1960s through 1970s showed that in mammalian species AFP is synthesized, mainly, by yolk sack and the fetal liver [8]. By 12–14-th week of gestation AFP concentration in the fetal blood serum reaches up to 10 mg/ml. After delivery, blood level of AFP decreases to 0.1 mg/ml and in normal adults it may be found only at very low concentrations (5–10 ng/ml) [14, 15]. Increased levels of AFP in maternal serum during pregnancy were observed in defects of neural tube development and

spina bifida whereas decreased level—in Dawn syndrome [163, 164].

In HCC, concentrations of AFP may vary from 1 μ/ml up to 10 mg/ml whereas in teratocarcinoma it reaches 1–2 μ/ml [165]. Despite AFP is considered as a "golden standard" among tumor-specific biomarkers, it is characterized by poor sensitivity and specificity. That is why additional biomarkers have been proposed for the early detection of HCC. High concentrations of AFP are observed in 70–80 % patients with low-differentiated HCC, and about 57 % of them are shown to contain AFP mRNA circulating in the blood serum. Detection of circulating mRNA of AFP became a valuable parameter not only for diagnostic, but also for prognostic purposes. Also, technologies are developed to use micro-RNA and RNA interference to suppress *AFP* gene and to inhibit cell proliferation and tumor growth [166, 167].

Comparative proteomic analysis of gene expression in tumor and non-tumor tissues in HCC patients demonstrated six molecular markers that may be exploited for assessment of liver destruction [168]. Correlation between over-expression of these proteins and concentration of AFP in the blood serum was observed. Also, global analysis of 213 genes in 21 AFP-producing cancer cell lines showed correlation between high blood level of AFP and over-expression of hepato-specific genes that encode growth factors, coagulation factors, proteins involved in angiogenesis, Wnt- and MAPK-mediated signaling along with lipid and iron metabolism [169]. AFP seems to be functionally linked with glypican-3, heparansulfate proteoglycan, which is expressed in HCC. Both AFP and glypican-3 (GPC-3) were shown to be involved in interaction with mutant p53 which is over-expressed in patients with HCC [170]. These data along with synergistic action of AFP with growth factors evidence possibility of coordinated and co-operated action of the regulatory proteins in tumorigenesis.

AFP glycoforms in combination with other proteins have been also proposed to be biomarkers for early detection of HCC. In particular, levels of lens culinaris agglutinin-reactive AFP (AFP-L3) and des-gamma carboxyprothrombin (DCP) were significantly higher in patients with HCC than in those without HCC [171, 172]. In addition to AFP-L3 and DCP other biomarkers such as glypican-3, osteopontin, squamous cell carcinoma antigen–immunoglobulin M complexes, alpha-1-fucosidase, chromogranin A, human hepatocyte growth factor and insulin-like growth factor in combination with imaging techniques were proposed to increase the sensitivity and specificity in the early diagnosis and in prognosis of HCC [173, 174].

By the group of *S. Severin* and *E. Severin* an ability of human AFP to specifically penetrate into tumor cells through receptor-mediated mechanism along with capability to bind cytotoxic agents were exploited as a basis for the development a new chemotherapeutic strategy. Native AFP and its recombinant domain III were used as a vector for targeted delivery of antitumor drugs to tumor cells [20, 21]. Domain III of AFP was expressed in *E .coli* in the form of inclusion bodies and, after refolding, it was shown to specifically bind to cancer cells [175]. A procedure of refolding of hydrophobic cysteine-rich proteins on immobilized metal-chelating chromatography on silica matrix instead of agarose was developed to increase the yield of the protein of 98 % purity [176].

Usage of intact AFP molecule as a vector for targeted delivery of cytotoxic drugs is restricted by low amount of sites for binding of hydrophobic ligands. Besides, usage of free cytotoxic drugs for chemotherapeutic purposes is restricted by development of multidrug resistance. That is why a technology based on placement of cytotoxic agents inside nanoparticles covalently linked to recombinant domain III of AFP through formation of amide bond with the help of carbodiimide was recently developed [21]. This technology was used for transportation of paclitaxel to MCF-7 breast cancer cells with high level of expression of AFP receptors (300 thousand per cell). The cytotoxic effect of the drug placed inside nanoparticles bound to AFP domain III as a vector was higher than that of either free drug or nanoparticles with drug, but not bound to the vector.

Further, GIP, the 34 amino acid fragment of human AFP domain III responsible for inhibition of estrogen-dependent tumors, was used as a vector molecule [177]. This work was performed by Russian, American, and Polish scientific groups in collaboration. The cytotoxic agent, doxorubicin (DOX), was conjugated with GIP that provided its targeted delivery to MCF-7 cells. Usage of radioactive or fluorescent label evidenced specific binding and uptake of GIP–DOX complexes by tumor cells.

Conclusion and perspectives

Alpha-fetoprotein represents the most prominent onco-biomarker used for the early diagnosis of HCC and monitoring of the tumor progression and metastasis, assessment of the cancer prognosis and successfulness of antitumor therapeutic measures. Assessment of glycosylated forms of human AFP is a perspective tool for differential diagnostics of HCC and chronic hepatic diseases. AFP mRNA was proposed to be used for evaluation of amount of circulating hepatoma cells to monitor tumor progression and metastasis.

Global genomic and proteomic analysis of protein interaction networks with participation of AFP allows revealing its interaction partners in tumor and non-tumor tissues. Tumor-associated proteins have been demonstrated to possess large amount of linkages in protein–protein networks and are located in a network hubs. Co-localization and co-expression of AFP with a number of other proteins involved in tumorigenesis may evidence their interaction and functioning in a

cooperative and coordinated manner. Analysis of molecular networks gives new perspectives for diagnosis and classification of tumor diseases along with determination of potential targets for novel antitumor drugs. Intramolecular effectors of AFP-initiated signaling pathways may also serve as targets for novel therapeutic agents.

Structural and functional mapping of AFP performed with the use of experimental and computer-based technologies represents a new approach for determination of its functionally important sites. To date, a large number of linear structural motifs are revealed in AFP to demonstrate its multi-modular and poly-functional character. Generation of the computer-based model of 3D structure of AFP was performed with perspectives to be used in study of mechanisms of ligand- or receptor-binding.

References

1. Abelev GI, Perova SD, Khramkova NI, Postnikova ZA, Irlin IS. Production of embryonic alpha-globulin by the transplantable mouse hepatomas. Transplantation. 1963;1:174–80.
2. Tatarinov YuS. Detection of embryospecific alpha-globulin in serum of patients with primary liver cancer . In I-st All-Union Biochem Cong Abstr Book. 1963;2:274. Moscow-Leningrad.
3. Bergstrand CG, Czar B. Demonstration of a new protein fraction in serum from the human fetus. Scan J Clin Lab Invest. 1956;8:174–6.
4. Debruyne EN, Delange JR. Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. Clin Chim Acta. 2008;395:19–26.
5. Tatarinov YS. Content of embryo-specific alpha-globulin in the blood serum of human fetus, newborns and adults. Vorp Med Khim. 1965;11:20–4.
6. Abelev GI. Alpha-fetoprotein in ontogenesis and its association with malignant tumors. Adv Cancer Res. 1971;14:295–358.
7. Ruoslahti E, Seppala M. Alpha-fetoprotein in cancer and fetal development. Adv Cancer Res. 1979;29:275–346.
8. Gitlin D, Boesman M. Sites of serum alpha-fetoprotein synthesis in the human and in the rat. J Clin Invest. 1967;46:1010–6.
9. Terentiev AA. Comparative studies of physico-chemical properties of human, bovine and sheep embryo-specific alpha-globulins. Proc ASMU 1968; XVI:336–41. Astrakhan.
10. Terentiev AA. Comparative study of fetuin and bovine embryo-specific alpha-globulin. Bull Exp Biol Med. 1969;7:42–5.
11. Kithier K, Masopust J, Rádl J. Fetal alpha-globulin of bone serum differing from fetuin. Biochim Biophys Acta. 1968;160:135–7.
12. Nishi S, Watabe H, Hirai H. Production of antibody to homologous alpha-fetoprotein in rabbits, rats and horses by immunization with human alpha-fetoprotein. J Immunol. 1967;109:957–60.
13. Nishi S, Watabe H, Hirai H. Species cross-reaction of alpha-fetoprotein and break down of the tolerance to alpha-fetoprotein by immunization with heterologous alpha-fetoprotein. Tumor Res. 1973;8:17–22.
14. Ruoslahti E, Seppala M. Studies of carcino-fetal proteinsIII. Development of radio-immunoassay for alpha-fetoprotein. Demonstration of alpha-fetoprotein in serum of healthy human adults. Intern J Cancer. 1971;8:374–83.
15. Ruoslahti E, Seppala M. α-Foetoprotein in normal human serum. Nature. 1972;235:161–2.
16. Deutsch HF. Chemistry and biology of alpha-fetoprotein. Adv Cancer Res. 1991;56:253–312.
17. Mizejewski GJ. The phylogeny of alpha-fetoprotein in vertebrates: survey of biochemical and physiological data. Crit Rev Eucaryot Gene Expr. 1995;5:281–316.
18. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes and conformational variants. Exp Biol Med. 2001;226:377–408.
19. Terentiev AA, Moldogazieva NT. Structural and functional mapping of alpha-fetoprotein. Biochemistry (Mosc). 2006;71:120–32.
20. Lutsenko SV, Feldman NB, Finakova GV, Gukasova NV, Petukhov SV, Posypanova GA, et al. Study of antitumor activity of alpha-fetoprotein and epidermal growth factor conjugates in vitro and in vivo. Tumor Biol. 2000;21:367–74.
21. Godovannyi AV, Vorontsov EA, Gukasova NV, Pozdnyakova NV, Vasilenko EA, Yabbarov NG, et al. Targeted delivery of paclitaxel-loaded recombinant alpha-fetoprotein fragment-conjugated nanoparticles to tumor cells. Dokl Biochem Biophys. 2011;439:158–60.
22. Morinaga T, Sakai M, Wegmann TG, Namaoki T. Primary structure of human alpha-fetoprotein and its mRNA. Proc Natl Acad Sci U S A. 1983;80:4604–8.
23. Pucci P, Siliciano R, Malorni A, Marino G, Tecce MF, Ceccarini C, et al. Human alpha-fetoprotein primary structure: a mass spectrometric study. Biochemistry. 1991;30:5061–6.
24. Dudich I, Tokhtamysheva N, Semenkova L, Dudich E, Hellman Y, Korpela T. Isolation and structural and functional characterization of two stable peptic fragments of human alpha-fetoprotein. Biochemistry. 1999;38:10406–14.
25. Song Y-H, Naumova AK, Liebhaber SA, Cooke NE. Physical and meiotic mapping of the region of human chromosome 4q11-q13 encompassing the vitamin D-binding protein DBP/Gc-globulin and albumin multigene cluster. Genome Res. 1999;9:581–7.
26. Beatie WG, Dugaiczyk A. Structure and evolution of human alpha-fetoprotein deduced from partial sequence of cloned cDNA. Gene. 1982;20:415–22.
27. Ruoslahti E, Terry WD. Alpha-fetoprotein and serum albumin show sequence homology. Nature. 1976;260:804–5.
28. Cooke NE, David EV. Serum vitamin-D-binding protein is a third member of the albumin and alpha-fetoprotein gene family. J Clin Invest. 1985;76:2420–4.
29. Lichtenstein HS, Lyons DE, Wurfer MM, Johnson DA, MacGinley MD, Leidli JC, et al. Afamin is a new member of the albumin, alpha-fetoprotein and vitamin D-binding protein gene family. J Biol Chem. 1994;269:18149–54.
30. Terentiev AA. Structural prerequisition for participation of alpha-fetoprotein and transforming growth factor (TGF-beta) in cell cycle regulation. Med Immunol. 2004;6:256.
31. Luft AJ, Loscheider FL. Structural analysis of human and bovine α-fetoprotein by electron microscopy, image processing and circular dichroism. Biochemistry. 1983;22:5971–8.
32. He X-M, Carter D. Atomic structure and chemistry of human serum albumin. Nature. 1992;358:209–15.
33. Zizkovsky V, Strop P, Korcakova J, Havranova M, Mikes F. Fluorescence spectroscopy, fluorescence polarization and circular dichroism in studies of pH-dependent changes in the alpha-fetoprotein molecule. Ann N Y Acad Sci. 1983;417:49–56.
34. Barker ME. Evolution of alpha-fetoprotein: sequence comparisons among AFP species and with albumin species. Tumor Biol. 1988;9:123–36.
35. Gorin MB, Cooper DL, Eiferman F, van de Rijn P, Tilghman SM. The evolution of alpha-fetoprotein and albumin: 1 a comparison of the primary amino acid sequences of mammalian alpha-fetoprotein and albumin. J Biol Chem. 1981;256:1954–9.
36. Valette G, Vranckx R, Martin M-E, Benassayag C, Nunez EA. Conformational changes in rodent and human alpha-fetoprotein:

influence of fatty acids. Biochim Biophys Acta. 1989;997:302–12.
37. Haourigui M, Thobie N, Martin M-E, Benassayag C, Nunez EA. In vivo transient rise in plasma free acids alters the functional properties of alpha-fetoprotein. Biochim Biophys Acta. 1992; 1125:157–65.
38. Festin SM, Bennett JA, Fletcher PW, Jacobson HI, Shaye DD, Andersen TT. The recombinant third domain of human alpha-fetoprotein retains the antiestrothophic activity found in the full-length molecule. Biochim Biophys Acta. 1999;1427:307–14.
39. Carreres G, Dauphinee MJ, Eisele LE, MacColl R, Mizejewski GJ. Anti-prostate cancer and anti-breast cancer activities of two peptides derived from alpha-fetoprotein. Anticancer Res. 2002;22:2817–20.
40. Uversky VN, Kirkitadze MD, Narizhneva NV, Potekhin SA, Tomashevsky AY. Structural properties of alpha-fetoprotein from human cord serum: the protein molecule at low pH possesses all the properties of the molten globule. FEBS Letts. 1995;364:165–7.
41. Uversky VN, Narizhneva NV, Ivanova TV, Tomashevski AY. Rigidity of human alpha-fetoprotein tertiary structure is under ligand control. Biochemistry. 1997;36:13638–45.
42. Uversky VN, Narizhneva NV, Ivanova TV, Kirkitadze MD, Tomashevsky AY. Ligand-free form of human alpha-fetoprotein: evidence for the molten globule state. FEBS Letts. 1997;410:280–4.
43. Taketa K. Characterization of sugar chain structures of human AFP by lectin affinity electrophoresis. Electrophoresis. 1998;19:1774–9.
44. Breborowich J. Microheterogeneity of human alpha-fetoprotein. Tumor Biol. 1988;9:3–14.
45. Krivonosov SK, Zorin NA, Ionova NK, Terentiev AA, Tatarinov YS. Interaction of pregnancy-associated proteins with hemagglutinin and concanavalin A. Byul Eksp Biol Med. 1985;5:568–71.
46. Zorin NA, Krivonosov SK, Zorina RM, Terentiev AA, Ionova NK. Competitive inhibition of interactions between phytohemaglutinin A and blood serum glycoproteins by carbohydrates. Vopr Med Khim. 1986;32:96–8.
47. Aoyagi Y, Saitoh A, Suzuki Y, Igarashi K, Oguro M, Yokota T, et al. Fucosylation index of alpha-fetoprotein, a possible aid in the early recognition of hepatocellular carcinoma in patients with cirrhosis. Hepatology. 1993;17:50–2.
48. Seta N, Gayno S, Jezequel-Guer M, Toueg ML, Erlinger S, Durand G. Sequential study of serum glycoprotein fucosylation in acute hepatitis. J Hepatol. 1997;26:265–71.
49. Taketa K, Ichikawa E, Sakuda H, Iwamasa T, Hayakawa M, Taga H, et al. Lectin reactivity of alpha-fetoprotein in a case of renal cell carcinoma. Tumor Biol. 1989;10:275–80.
50. Taketa K, Fujii Y, Taga H. Characterization of E-PHA-reactive alpha-fetoprotein isoforms by two-dimensional lectin affinity electrophoresis. Electrophoresis. 1993;14:1333–7.
51. Shimizu K, Katoh H, Yamashita F, Tanaka M, Tanikawa K, Taketa K, et al. Comparison of carbohydrate structures of serum alpha-fetoprotein by sequential glycosidase digestion and lectin affinity electrophoresis. Clin Chim Acta. 1996;254:23–40.
52. Taketa K. Multimodal application of lectin affinity electrophoresis of alpha-fetoprotein. Electrophoresis. 1998;19:1774–9.
53. Parmelee DC, Evenson MA, Deutsch HF. The presence of fatty acids in human alpha-fetoprotein. J Biol Chem. 1978;253:2114–9.
54. Berde CB, Nagai M, Deutsch HF. Human alpha-fetoprotein. Fluorescence studies on binding and proximity relationships for fatty acids and bilirubin. J Biol Chem. 1979;254:12609–14.
55. Hsia JC, Er JS, Tan CT, Ester T, Ruoslahti E. α-Fetoprotein binding specificity for arachidonate, bilirubin, docosahexaenoate and palmitate. J Biol Chem. 1980;255:4224–7.
56. Nagai M, Becker JD, Deutsch HF. The fatty acid levels in rat alpha-fetoprotein derived from fetuses, pregnancy and hepatoma sera. Oncodev Biol Med. 1982;3:343–50.
57. Iturralde M, Alava MA, Gonzalez B, Anel A, Pineiro A. Effect of AFP and albumin on the uptake of polyunsaturated fatty acids by rat hepatoma cells and fetal rat hepatocytes. Biochim Biophys Acta. 1991;1086:81–8.
58. Aussel C, Negrel L. Effect of AFP on arachidonic acid metabolism in the preadipocyte cell line OB-17. Prostaglandins Leukotriene Med. 1986;21:69–76.
59. Ruoslahti E, Ester T, Seppala M. Binding of bilirubin by bovine and human alpha-fetoprotein. Biochim Biophys Acta. 1979;578: 511–9.
60. Aoyagi Y, Ikenaka T, Ichida F. Alpha-fetoprotein as a carrier protein in plasma and its bilirubin-binding ability. Cancer Res. 1979;39:3571–4.
61. Aoyagi Y, Ikenaka T, Ishida F. Copper (II)-binding ability of human alpha-fetoprotein. Cancer Res. 1978;38:3483–6.
62. Birkenmeier G, Usbeck E, Saro L, Kopperschlager G. Triazine dye binding of human alpha-fetoprotein and albumin. J Chromatogr. 1983;265:27–35.
63. Ruiz-Gutierrez V, Moreno R, Moreda W, Copado MA, Rodriguez-Burgoz A. Detection of squalene in alpha-fetoprotein and fetal serum albumin from bovine. J Prot Chem. 2001;20:19–23.
64. Soloff MS, Swartz SK, Pearlmutter F, Kithier K. Binding of 17-β-estradiol by variants of alpha-fetoprotein in rat amniotic fluid. Biochim Biophys Acta. 1972;427:644–51.
65. Uriel J, de Nechaut B, Dupiers M. Estrogen-binding properties of rat, mouse and man fetospecific serum protein. Demonstration by immuno-autoradiographic methods. Biochem Biophys Res Commun. 1972;46:1175–80.
66. Uriel J, Bouillon D, Dupiers M. Affinity chromatography of human, rat and mouse alpha-fetoprotein on estradiol-sepharose adsorbents. FEBS Lett. 1975;53:305–8.
67. Uriel J, Bouillon D, Aussel C, Dupiers M. Alpha-fetoprotein: the major high affinity estrogen binder in rat uterine cytosols. Proc Natl Acad Sci USA. 1976;75:1452–6.
68. Nishi S, Matsue H, Yoshida H, Yamamoto R, Sakai M. Localization of estrogen-binding site of alpha-fetoprotein in chimeric human-rat proteins. Proc Natl Acad Sci USA. 1991;88:3102–5.
69. Milligan SR, Khan O, Nash M. Competitive binding of xenobiotic oestrogens to rat alpha-fetoprotein and sex steroid binding proteins in human and rainbow trout (*Oncorhynchus mykiss*) plasma. J Comp Endocrinol. 1998;112:89–95.
70. Terentiev AA, Moldogazieva NT, Tagirova AK, Tatarinov YS. Affinity chromatography of human alpha-fetoprotein on immobilized estrogens. Byul Eksp Biol Med. 1988;4:422–4.
71. Terentiev AA, Moldogazieva NT, Tagirova AK, Tatarinov YS. Competitive affinity chromatography of human alpha-fetoprotein on immobilized diethylstilbestrol. Byul Eksp Biol Med. 1990;5: 438–40.
72. Tatarinov YS, Terentiev AA, Moldogazieva NT, Tagirova AK. Human alpha-fetoprotein and its purification by chromatography on immobilized estrogens. Tumor Biol. 1991;12:125–30.
73. Terentiev AA, Moldogazieva NT, Tagirova AK, Tatarinov YS. Affinity chromatography of rodent alpha-fetoprotein on immobilized diethylstilbestrol. Byul Eksp Biol Med. 1992;6:588–90.
74. Terentiev AA, Moldogazieva NT, Levtsova OV, Maximenko D, Borozdenko D, Shaitan KV. Modeling of three-dimensional structure of human alpha-fetoprotein complexed with diethylstilbestrol: docking and molecular dynamics simulation study. J Bioinform Comput Biol. 2012;10:1241012.
75. Lu CY, Changelian PS, Unanue ER. Alpha-fetoprotein inhibits macrophage expression of Ia antigens. J Immunol. 1984;132:1722–7.
76. Atemezem A, Mbemba E, Marfaing R, Vaysse J, Pontet M, Saffar L, et al. Human alpha-fetoprotein binds to primary macrophages. Biochem Biophys Res Commun. 2002;296:507.
77. Peck AB, Murgita RA, Wigzell H. Cellular and genetic restrictions in the immunoregulatory activity of α-fetoprotein. III. Role of the MLC-stimulating cell of α-fetoprotein induced suppression of T cell-mediated cytotoxicity. J Immunol. 1982;128:1134–40.

78. Cohen BL, Orn A, Gronvik K-O, Gidlund M, Wigzell H, Murgita RA. Suppression by alpha-fetoprotein of murine natural killer cell activity stimulated in vitro and in vivo by interferon and interleukin 2. Scand J Immunol. 1986;23:211–23.
79. Yachnin S. Demonstration of the inhibitory effect of human AFP on in vitro transformation of human lymphocytes. Proc Nat Acad Sci U S A. 1976;73:2857–60.
80. Yachnin S. The immunosuppressive properties of α-fetoprotein: a brief overview. Ann N Y Acad Sci. 1983;417:105–7.
81. Matsuura E, Kang Y, Katikawa H, Ogata P, Kotani T, Ohtaki S, et al. Modulation of T-cell function by alpha-fetoprotein. An in vivo study on porcine thyroid peroxidase induced experimental autoimmune thyroiditis in transgenic mice producing human alpha-fetoprotein. Tumor Biol. 1999;20:162–71.
82. Alpert E, Dienstag JL, Sepersky S, Littman B, Rocklin R. Immunosupressive characteristic of human AFP: effect on tests of cell mediated immunity and induction of human suppressor cell. Immunol Commun. 1978;7:163–85.
83. Van Oers NS, Cohen BL, Murgita RA. Isolation and characterization of a distinct immunoregulatory isoform of alpha-fetoprotein produced by the normal fetus. J Exp Med. 1989;170:811–25.
84. Semeniuk DJ, Boismenu R, Tam J, Weissenhofer W, Murgita RA. Evidence that immunosuppression is an intrinsic property of the alpha-fetoprotein molecule. Adv Exp Mol Biol. 1995;383:255–69.
85. Irony-Tur-Sinai M, Grigoriadis N, Tsiantoulas D, Touloumi O, Abramsky O, Brenner T. Immunomodulation of EAE by alpha-fetoprotein involves elevation of immune cell apoptosis markers and the transcription factor FoxP3. J Neurol Sci. 2009;279:80–7.
86. Kang G, Matsuura E, Sakamoto T, Sakai M, Nishi B. Analysis of epitopes of mouse monoclonal antibodies against human alpha-fetoprotein. Tumor Biol. 2001;22:254–61.
87. Butterfield LH, Koh A, Meng W, Volhmer CM, Ribas A, Dissette V, et al. Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from alpha-fetoprotein. Cancer Res. 1999;59:3134–42.
88. Butterfield LH, Meng WS, Koh A, Vollmer CM, Ribas A, Dissette VB, et al. T cell responses to HLA-A*0201-restricted peptides derived from human alpha fetoprotein. J Immunol. 2001;166:5300–8.
89. Butterfield LH, Ribas A, Mena WS, Dissette VB, Amarnami S, Vu HT, et al. T-cell responses to HLA-A*0201 immunodominant peptides derived from alpha-fetoprotein in patients with hepatocellular cancer. Clin Cancer Res. 2003;9:5902–8.
90. Butterfield LH, Ribas A, Dissette VB, Lee Y, Yang JQ, De la Rocha P, et al. A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. Clin Cancer Res. 2006;12:2817–25.
91. Liu Y, Daley S, Evdokimova VN, Zdobinski DD, Potter DM, Butterfield LH. Hierarchy of alpha fetoprotein (AFP)-specific T cell responses in subjects with AFP-positive hepatocellular cancer. J Immunol. 2006;177:712–21.
92. Butterfield LH. Recent advances in immunotherapy for hepatocellular cancer. Swiss Med WKLY. 2007;137:83–90.
93. Pardee AD, Butterfield LH. Immunotherapy of hepatocellular carcinoma: unique challenges and clinical opportunities. Oncoimmunol. 2012;1:48–50.
94. Thimme R, Neagu M, Boettler T, Neumann-Haefelin C, Kersting N, Geissler M, et al. Comprehensive analysis of the alpha-fetoprotein-specific CD8+ T-cell responses in patients with hepatocellular carcinoma. Hepatology. 2008;48:1821–33.
95. Alisa A, Ives A, Pathan AA, Navarrete CV, Williams R, Bertoletti A, et al. Analysis of CD4+ T-cell responses to a novel alpha-fetoprotein-derived epitope in hepatocellular carcinoma patients. Clin Cancer Res. 2005;11:6686–94.
96. Ayaru L, Pereira SP, Alisa A, Pathan AA, Williams R, Davidson B, et al. Unmasking of alpha-fetoprotein-specific CD4+ T-cell responses in hepatocellular carcinoma patients undergoing embolization. J Immunol. 2007;178:1914–22.
97. Alisa A, Bouswell S, Pathan AA, Ayaru L, Williams R, Behboudi S. Human CD4+ T-cells recognize an epitope within alpha-fetoprotein sequence and develop into TGF-producing CD4+ T-cells. J Immunol. 2008;180:5109–17.
98. Behboudi S, Alisa A, Boswell S, Anastassiou J, Pathan AA, Williams R. Expansion of anti-AFP Th1 and Tc1 responses in hepatocellular carcinoma occur in different stages of disease. Br J Cancer. 2010;102:748–53.
99. Terentiev AA, Kazimirski AN, Salmasi JM. AFP and apoptosis: synthetic oligopeptide LDSYQCT (AFP_{13-19}) induced expression of CD-95 (FAS, Apo-1). Tumor Biol. 2000;21(Suppl1):110.
100. Terentiev AA, Simonova AV, Arshinova SS, Kulakov VV, Bahus GO, Kudryavtseva EV, et al. Influence of peptide LDSYQCT (sequence 14–20 of human AFP) on proliferative activity of human lymphocytes. Tumor Biol. 2003;24 Suppl 1:54.
101. Terentiev AA, Simonova AV, Arshinova SS, Kulakov VV, Bahus GO, Pavlichenkov AV, et al. Influence of the human AFP-derived peptide LDSYQC(acm)T on proliferative and cytotoxic activity of peripheral blood lymphocytes. Immunologiya. 2004;5:279–81.
102. Salmasi JM, Kazimirski AN, Terentiev AA, Poryadin GV, Kudryavtseva EV, Tatarinov YS. LDSYQCT-peptide synthetic analogue of human alpha-fetoprotein (13–19) increases antiproliferative effect of cytozar on the lymphocytes of chronic leukemia patients. Tumor Biol. 1999;20 Suppl 2:43.
103. Moldogazieva NT, Terentiev AA, Kazimirsky AN, Antonov MY, Shaitan KV. Conformational dynamics of human alpha-fetoprotein-derived heptapeptide LDSYQCT analogs. Biochemistry (Mosc). 2007;72:529–39.
104. Moldogazieva NT, Shaitan KV, Antonov MY, Vinogradova IK, Terentiev AA. Influence of intramolecular interactions on conformational and dynamic properties of analogs of heptapeptide AFP_{14-20}. Biochemistry (Mosc). 2011;76:1321–36.
105. Moldogazieva NT, Terentiev AA, Antonov MY, Kazimirsky AN, Shaitan KV. Correlation between biological activity and conformational and dynamic properties of tetra- and pentapeptide fragments of fetoplacental proteins. Biochemistry (Mosc). 2012;77:469–84.
106. Leffert HI, Sell S. Alpha-fetoprotein biosynthesis during the growth cycle of differentiated fetal rat hepatocytes to primary monolayer culture. J Cell Biol. 1974;61:823–9.
107. Toder V, Bland M, Gold-Gefter L, Nebel J. The effect of alpha-fetoprotein on the growth of placental cells in vitro. Placenta. 1983;4:79–86.
108. Hamel S, Hoskin DW, Hooper DC, Murgita RA. Phenotype and function of bone marrow-derived T and non-T cells activated in vitro by alpha-fetoprotein. In: Mizejewski GJ, Jacobson HI, editors. Biological activities of alpha-fetoprotein. Florida: CRC; 1987. p. 167–77.
109. Wang XW, Xu B. Stimulation of tumor-cell growth by alpha-fetoprotein. Int J Cancer. 1998;75:596–9.
110. Wang XW, Xie H. Alpha-fetoprotein enhances the proliferation of human hepatoma cells in vitro. Life Sci. 1999;64:17–23.
111. Laderoute MP, Pilarski LM. Inhibition of apoptosis by alpha-fetoprotein (AFP) and role of AFP receptors in anti-cellular senescence. Anticancer Res. 1974;14:2429–38.
112. Shehata MA, Nosseir HR, Nagy HM, Farouk G. Cyclin dependent kinase inhibitor p27(kip1) expression and subcellular localization in relation to cell proliferation in heap to cellular carcinoma. Egypt J Immunol. 2006;13:115–30.
113. Mizejewski GJ, Vonnegut M, Jacobson HI. Estradiol-activated alpha-fetoprotein suppresses the uteroptropic response to estrogens. Proc Natl Acad Sci U S A. 1983;80:2733–7.
114. Allen SHG, Bennett JA, Mizejewski GJ, Andersen TT, Ferraris S, Jacobson HI. Purification of human alpha-fetoprotein from human cord serum with demonstration of its antiestrogenic activity. Biochim Biophys Acta. 1993;1202:135–42.

115. Jacobson HI, Thompson VD, Janerich DT. Multiple births and maternal risk of breast cancer. Amer J Epidemiol. 1989;129:865–73.
116. Jacobson HI, Bennett JA, Mizejewski GJ. Inhibition of estrogen-dependent breast cancer growth by a reaction product of alpha-fetoprotein and estradiol. Cancer Res. 1990;50:415–20.
117. Boismenn R, Semeniuk D, Murgita RA. Purification and characterization of human and mouse recombinant alpha-fetoprotein expressed in *Echerichia coli*. Protein Expres Purif. 1997;10:10–26.
118. Bennett JA, Semeniuk DJ, Jacobson HI, Murgita RA. Similarity between natural and recombinant human alpha-fetoprotein as inhibitors of estrogen-dependent breast cancer growth. Breast Cancer Res Treat. 1997;45:169–79.
119. Bennett JA, Zhu S, Pagano-Mirarchi A, Kellom TA, Jacobson HI. Alpha-fetoprotein derived from a human hepatoma prevents growth of estrogen-dependent human breast cancer xenografts. Clin Cancer Res. 1998;4:2877–84.
120. Mizejewski GJ, Dias JA, Hauer CR, Henrikson KP, Gierthy I. Alpha-fetoprotein derived synthetic peptides: assay from an estrogen-modifying regulatory segment. Mol Cell Endocrinol. 1996;118:15–33.
121. Vakharia D, Mizejewski GJ. Human alpha-fetoprotein peptides bind estrogen receptor and estradiol and suppress breast cancer. Breast Cancer Res Treat. 2000;63:41–52.
122. Eisele LE, Mesfin FB, Bennett JA, Andersen TT, Jacobson HI, Soldwedel H, et al. Studies of a growth inhibitory peptide derived from alpha-fetoprotein and some analogs. J Peptide Res. 2001;57:29–38.
123. Eisele LE, Mesfin FB, Bennett JA, Andersen TT, Jacobson HI, Soldwedel H, et al. Studies on analogs of a peptide derived from alpha-fetoprotein having antigrowth properties. J Peptide Res. 2001;57:539–46.
124. MacColl R, Eisele LE, Stack RF, Hauer C, Vakharia DD, Benno A, et al. Interrelationship among biological activity, disulphide bonds, secondary structure and metal ion binding for a chemically synthesized 34-amino-acid peptide derived from α-fetoprotein. Biochim Biophys Acta. 2001;1528:127–34.
125. Butterstein G, Morrison J, Mizejewski GJ. Effect of alpha-fetoprotein and derived peptides on insulin and estrogen-induced fetotoxicity. Fetal Diagn Ther. 2003;18:360–9.
126. Carerres G, Dauphinee MJ, Eisele LE, MacColl R, Mizejewski GJ. Anti-prostate cancer and anti-breast cancer activities of two peptides derived from alpha-fetoprotein. Anticancer Res. 2002;22:2817–20.
127. Mesfin FB, Bennett GA, Jacobson HI, Zhu S, Andersen TT. Alpha-fetoprotein-derived antiestrotrophic octapeptide. Biochim Biophys Acta. 2000;1501:33–43.
128. Mesfin FB, Andersen TT, Jacobson HI, Zhu S, Bennett GA. Development of a synthetic cyclized peptide derived from alpha-fetoprotein that prevents the growth of human breast cancer. J Peptide Res. 2001;58:246–56.
129. Hamsa A, Sarma MH, Sarma RH. Plausible interaction of an alpha-fetoprotein cyclopeptide with the G-protein coupled receptor model GPR30: docking study by molecular dynamics simulated annealing. J Biomol Struct Dyn. 2003;20:751–8.
130. Sierralta WD, Epunan MJ, Reyes JM, Valladares LE, Andersen TT, Bennett JA, et al. A peptide derived from alpha-fetoprotein inhibits the proliferation induced by estradiol in mammary tumor cells in culture. Oncol Rev. 2008;19:229–35.
131. Bennett JA, DeFreest L, Anaka I, Saadati H, Balulad S, Jacobson HI, et al. AFPep: an anti-breast cancer peptide that is orally active. Breast Cancer Res Treat. 2006;98:133–41.
132. Mizejewski GJ, MacColl R. Alpha-fetoprotein growth inhibitory peptides: potential leads for cancer therapeutics. Mol Cancer Ther. 2003;2:1243–55.
133. Mizejewski GJ. Alpha-fetoprotein (AFP)-derived peptides as epitopes for hepatoma immunotherapy: a commentary. Cancer Immunol Immunother. 2009;58:159–70.
134. Mizejewski GJ. Mapping of structure-function peptide sites on the human alpha-fetoprotein amino acid sequence. Atlas Genet Cytogenet Oncol Hematol. 2010;2:331–82.
135. Gabant P, Forrester L, Nicols J, Van Reeth T, De Mees C, Bajac B, et al. Alpha-fetoprotein, the major fetal serum protein, is not essential for embryonic development but is required for female fertility. Proc Natl Acad Sci U S A. 2002;99:12865–70.
136. Dohler KD. The pre- and postnatal influence of hormones and neurotransmitters on sexual differentiation of the mammalian hypothalamus. Int Rev Cytol. 1991;131:1–57.
137. De Mees C, Laes JF, Bakker J, Smitz J, Hennuy B, Van Vooren P, et al. Alpha-fetoprotein controls female fertility and prenatal development of the gonadotropin-releasing hormone pathway through an antiestrogenic action. Mol Cell Biol. 2006;26:2012–8.
138. Uriel J, Trojan J, Moro R, Pineiro A. Intracellular uptake of α-fetoprotein: a marker of neural differentiation. Ann N Y Acad Sci. 1983;417:321–9.
139. Villacampa MJ, Moro R, Naval J, Failly-Crepin C, Lampreave F, Uriel J. Alpha-fetoprotein receptors in a human breast cancer cell line. Biochem Biophys Res Commun. 1984;122:1322–7.
140. Naval J, Villacampa MJ, Goguel AF, Uriel J. Cell-type-specific receptors for alpha-fetoprotein in a mouse T-lymphoma cell line. Proc Natl Acad Sci U S A. 1986;82:3301–4.
141. Uriel J, Naval J, Laborda J. Alpha-Fetoprotein-mediated transfer of arachidonic acid into cultured cloned cells derived from a rat rhabdomyosarcoma. J Biol Chem. 1987;262:3579–85.
142. Torres JM, Laborda J, Naval J, Darracq N, Calvo M, Mishal Z, et al. Expression of alpha-fetoprotein receptors by human T-lymphocytes during blastic transformation. Mol Immunol. 1989;26:851–7.
143. Torres JM, Geuskens M, Uriel J. Activated human T lymphocytes express albumin binding proteins which cross-react with alpha-fetoprotein. Eur J Cell Biol. 1992;57:222–8.
144. Suzuki Y, Zeng CQY, Alpert E. Isolation and partial characterization of a specific alpha-fetoprotein receptor on human monocytes. J Clin Invest. 1992;90:1530–6.
145. Alava MA, Iturralde M, Lampreave F, Pineiro A. Specific uptake of alpha-fetoprotein and albumin by rat Morris 7777 hepatoma cells. Tumor Biol. 1999;20:52–64.
146. Moro R, Tamaoki T, Wegmann TJ, Longnecker BM, Laderoute MP. Monoclonal antibodies directed against a widespread oncofetal antigen: the alpha-fetoprotein receptor. Tumor Biol. 1993;14:116–30.
147. Lorenzo HG, Geuskens M, Macho A, Lachkar S, Verdiere-Sahuque M, Pineiro A, et al. Alpha-fetoprotein binding and uptake by primary cultures of human skeletal muscle. Tumour Biol. 1996;17:251–60.
148. Li MS, Li PF, Yang FY, He SP, Du GG, Li G. The intracellular mechanism of AFP promoting the proliferation of NIH 393 cells. Cell Res. 2002;12:151–6.
149. Newby D, Dalgliesh G, Lyall F, Aitken DA. Alpha-fetoprotein and alpha-fetoprotein receptor expression in the normal human placenta at term. Placenta. 2005;26:190–200.
150. Mizejewski GJ. Review of the putative cell-surface receptors for alpha-fetoprotein: identification of a candidate receptor protein family. Tumor Biol. 2011;32:241–58.
151. Mizejewski GJ. Review of the adenocarcinoma cell surface receptor for human alpha-fetoprotein; proposed identification of a widespread mucin as the tumor cell receptor. Tumor Biol. 2013;34:1317–36.
152. Li MS, Li PF, Du GG, Li G. The promoting molecular mechanism of alpha-fetoprotein on the growth of human hepatoma Bel7402 cell line. World J Gastroenterol. 2002;8:469–75.
153. Li MS, Li PF, Chen Q, Du GG, Li G. Alpha-fetoprotein stimulated the expression of some oncogenes in human hepatocellular carcinoma Bel7402 cells. World J Gastroenterol. 2004;10:819–24.
154. Li M-S, Ma Q-L, Chen Q, Liu X-H, Li P-F, Du G-G, et al. Alpha-fetoprotein triggers hepatoma cells escaping from

immune surveillance through altering the expression of Fas/FasL and tumor necrosis factor related apoptosis-inducing ligand and its receptor of lymphocytes and liver cancer cells. World J Gastroenterol. 2005;11:2564–9.
155. Li M-S, Li H, Li C, Zhou S, Guo L, Liu H, et al. Alpha fetoprotein is a novel protein-binding partner for caspase-3 and blocks the apoptotic signaling pathway in human hepatoma cells. Int J Cancer. 2009;124:2845–54.
156. Li M-S, Li H, Li C, Guo L, Liu H, Zhou S, et al. Cytoplasmic alpha-fetoprotein functions as a co-repressor in RA-RAR signaling to promote the growth of human hepatoma Bel 7402 cells. Cancer Lett. 2009;285:190–9.
157. Li M-S, Li H, Li C, Wang S, Wei J, Liu Z, et al. Alpha-fetoprotein: a new member of intracellular signal molecules in regulation of the PI3K/AKT signaling in human hepatoma cell lines. Int J Cancer. 2011;128:524–32.
158. Goldstein NS, Blue DE, Hankin R, Hunter S, Bayati N, Silverman AL, et al. Serum alpha-fetoprotein levels in patients with chronic hepatitis C: relationships with serum alanine aminotransferase values, histologic activity index and hepatocyte MIB-I scores. Am J Clin Pathol. 1999;111:811–6.
159. Semenkova LN, Dudich EI, Dudich IV, Shingarova LN, Korobko VG. α-Fetoprotein as a TNF-resistance factor for human hepatocarcinoma cell line HepG2. Tumor Biol. 1997;18:30–40.
160. Dudich E, Semenkova L, Dudich I, Gorbatova E, Tokhtamisheva N, Tatulov E, et al. Alpha-fetoprotein causes apoptosis in tumor cells via a pathway independent of CD95, TNFR1 and TNFR2 through activation of caspase-3-like proteases. Eur J Biochem. 1999;266:750–61.
161. Semenkova L, Dudich E, Dudich I, Tokhtamisheva N, Tatulov E, Okruzhnov Y, et al. Alpha-fetoprotein positively regulates cytochrome c-mediated caspase activation and apoptosome complex formation. Eur J Biochem. 2003;270:4388–99.
162. Dudich E. MM-093, a recombinant human alpha-fetoprotein for the potential treatment of rheumatoid arthritis and other autoimmune diseases. Curr Opin Mol Ther. 2007;9:603–10.
163. Seppala M. Fetal pathophysiology of human alpha-fetoprotein. Ann NY Acad Sci. 1975;259:59–73.
164. Brock DJ, Scrimgeour JB, Nelson MM. Amniotic fluid alpha-fetoprotein measurements in the prenatal diagnosis of central nervous system disorders. Genetics. 1975;7:163–9.
165. Petropoulos C, Andrews G, Tamaoki T, Fausto N. Alpha-fetoprotein and albumin mRNA levels in liver regeneration and carcinogenesis. J Biol Chem. 1983;258:4901–6.
166. Won YS, Lee SW. Targeted retardation of hepatocarcinoma cells by specific replacement of alpha-fetoprotein RNA. J Biotechnol. 2007;129:614–9.
167. Zhang H, Bai ZL, Chen J, Wang Z, Li J. Alpha-fetoprotein-specific transfer factors downregulate alpha-fetoprotein expression and specifically induce apoptosis in Bel7402 alpha-fetoprotein-positive hepatocarcinoma cells. Hepatol Res. 2007;37:557–67.
168. Lee NP, Chen L, Lin MC, Tsang FH, Yeung C, Poon RT, et al. Proteomic expression signature distinguishes cancerous and nonmalignant tissues in hepatocellular carcinoma. J Proteome Res. 2009;8:1293–303.
169. Saito S, Ojima H, Ichikawa H, Hirohashi S, Kondo T. Molecular background of alpha-fetoprotein in liver cancer cells as revealed by global RNA expression analysis. Cancer Sci. 2008;99:2402–9.
170. Wang XY, Degos F, Dubois S. Glypican-3 expression in hepatocellular tumors: diagnostic value for preneoplastic lesions and hepatocellular carcinomas. Hum Pathol. 2006;37:1435–41.
171. Spangenberg HC, Thimme R, Blum HE. Serum markers of hepatocellular carcinoma. Semin Lver Dis. 2006;26:385–90.
172. Durazo FA, Blatt LM, Corey WG, Lin JH, Han S, Saab S, et al. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol. 2008;23:1541–8.
173. Bertino G, Neri S, Bruno CM, Ardini AM, Calvagno GS, Malaguarnera M, et al. Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. Minerva Med. 2011;102:363–71.
174. Bertino G, Ardini A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellualar carcinoma serum markers. Semin Oncol. 2012;39:410–33.
175. Sharapova OA, Pozdniakova NV, Laurinavichiute DK, Iurkova MS, Posypanova GA, Andronova SM, et al. Purification and characterization of recombinant human alpha-fetoprotein fragment, corresponding to the C-terminal structural domain. Bioorg Khim. 2010;36:760–8.
176. Sharapova OA, Yurkova MS, Laurinavichyute DK, Andronova SM, Fedorov AN, Severin SE, et al. Efficient refolding of a hydrophobic protein with multiple S–S bonds by on-resin immobilized metal affinity chromatography. J Chromat A. 2011;1218:5115–9.
177. Mizejewski GJ, Mirowski M, Garnuszek P, Maurin M, Cohen BD, Poiesz BJ, et al. Targeted delivery of anti-cancer growth inhibitory peptides derived from human α-fetoprotein: review of an International Multi-Center Collaborative Study. J Drug Target. 2010;18:575–88.