UDC 577.112:577.322:576.342:615.36 **https://doi.org/10.15407/biotech14.01.025**

CURRENT CONCEPT OF THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF ALFA-FETOPROTEIN AND THE POSSIBILITIES OF ITS CLINICAL APPLICATION

O. K. GULEVSKYY, YU. S. AKHATOVA

Institute for Problems of Cryobiology and Cryomedicine
of the National Academy of Sciences of Ukraine, Kharkiv

E-mail: Julija_Veselovskaja@meta.ua

Received 05.12.2020
Revised 19.02.2021
Accepted 26.02.2021

This paper was aimed to review the literature data from native and foreign sources accumulated for 40-years period of research of the features of the molecular structure, functions, production and application of human alpha-fetoprotein (AFP), which is known as one of the most studied and increasingly demanded proteins. Results of fundamental studies performed with the use of modern methods, including various types of electrophoresis, chromatography, electron microscopy and immunoassay, in order to characterize the principal physicochemical capacities and localization of free and bound forms of AFP, as well as polypeptide structure, heterogeneity and topography of AFP receptors are highlighted here. The data on the mechanisms of AFP synthesis, its conformational features, binding sites and intracellular metabolism are also presented. The concepts of physiological functions and mechanisms of AFP transport in an organism are presented. Data on AFP isolation from the natural primary products and its production by means of recombinant and synthetic methods are shown. This review also summarizes information on the current possibilities of clinical application of AFP and the prospects for its usage in anticancer therapy for targeted delivery of chemotherapy drugs, with emphasis on the description of the recent progress in this field.

Key words: alpha-fetoprotein, sources of alpha-fetoprotein production, application of alpha-fetoprotein, targeted delivery of chemotherapy drugs.

General characteristics

Alpha-fetoprotein (AFP) is a single-chain oncofetal glycoprotein with a molecular weight of about 70 kDa, including something like 600 amino acids and 4% carbohydrates. In terms of its structure and physicochemical properties, AFP is homologous to the main transport protein of blood serum of adults — albumin. AFP is synthesized by the cells of the visceral endoderm of the yolk sac, the embryonic liver and in a small amount in the gastrointestinal tract. Then it enters the blood of the fetus, amniotic fluid, and from them partially into the mother's blood. Normally, AFP can be detected in fetal serum starting from the 4th week of pregnancy [1, 2]. Its concentration attains peaks between 12 and 16 weeks and then decreases gradually until nascency. Since AFP crosses the placenta, it can be found at a fairly high concentration in the maternal serum, reaching a maximum between 32 and 36 weeks of gestation, with AFP levels in the maternal serum differing between primiparous and multiparous (Fig. 1) . Immediately after nascence, the serum AFP level decreases by several orders of magnitude. In the blood of healthy people, the normal concentration of AFP is 0–12 IU/mL [3].

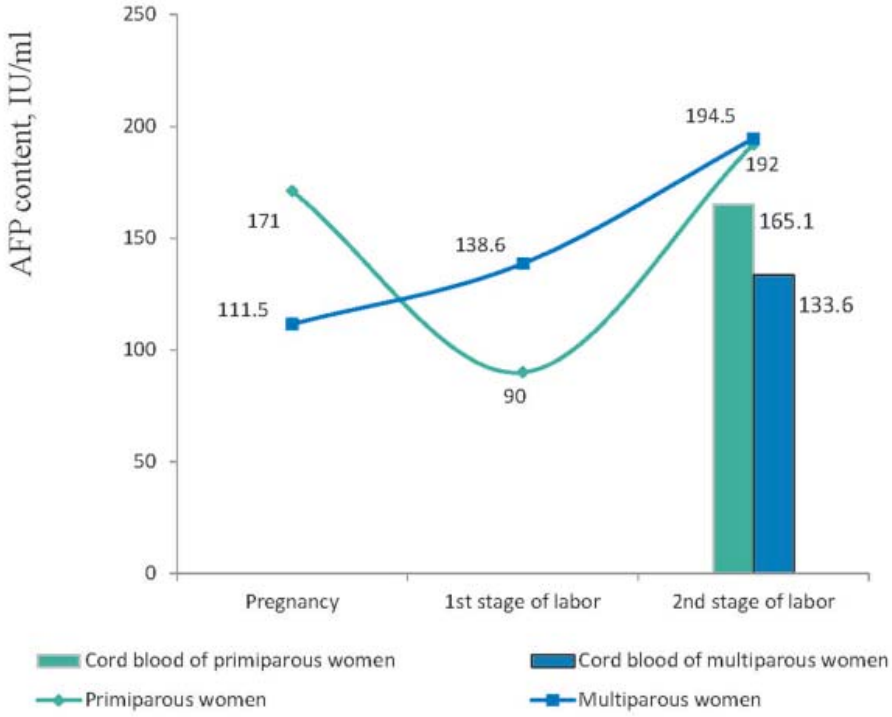

Fig. 1. **The content of AFP in the cord and peripheral blood of pregnant women at the end of pregnancy [4]**

When studying the levels and sites of AFP localization, it was found that it is present in all organs and tissues of the human fetus, in the blood serum of pregnant women and newborns, as well as in the tissues of the placenta, amniotic fluid and cord blood [5, 6].

Intensive studies of the conformational states of AFP carried out in the early 2000s showed that, despite its stability in solution, AFP has sufficient conformational mobility and can form a molten globule [7]. It was shown that in the native AFP molecule, part of its biologically active sites are hidden inside the globule and do not participate in complexation with other molecules. They become available only upon conformational modification of AFP as a result of the action of various physicochemical factors, such as temperature, pH, and salt composition of the medium [7]. The study of conformational changes in AFP under the influence of such factors enables to understand the molecular mechanisms of its functioning, the peculiarities of interaction with ligands in the circulatory channel and intracellular metabolism.

Reception and intracellular AFP pathway

For the first time, the assumption of the existence of specific receptors to AFP (receptor for alpha-fetoprotein, ReCAF) was made by R. Moro in 1981 to explain the absorption of AFP by growing cells under the mechanism of receptor-mediated endocytosis [8]. ReCAFs are membrane-embedded AFP-binding transmembrane glycoproteins. However, such receptors are still poorly known. The problem of AFP receptors seems to be key for understanding not only the transport, but also other possible functions of AFP. Currently, this is one of the topical points in the study of the AFP role in the development process. There are 4 types of membrane receptors for AFP with molecular weights of 18, 31, 60, and 62–67 kDa [9, 10]. The first two are "scavenger receptors" and are located mainly on the cells of the vascular endothelium. Their role is to remove denatured and modified AFP molecules and albumin from the bloodstream. The third is also found on the surface of the endothelium. It is a sialoglycoprotein and its properties are close to the fourth, classical ReCAF, which is found on monocytes, lymphocytes, some cells of the reproductive system and a number of tumors [9].

Due to the fact that AFP belongs to the class of transport proteins, a significant proportion of it, both in serum and in tissues and cells, is in a bound state, i.e. in the form of complexes with transported ligands and/or with receptor molecules. There are data in the literature on the intracellular distribution and state of AFP in different types of cells, in

particular, in hematopoietic stem cells (HSC) [11]. It is reported that labeled AFP is detected in the cytoplasm, Golgi complex, endoplasmic reticulum and mitochondria, and exists in the cytoplasm in two forms — free and bound. During native electrophoresis of cytoplasmic protein fractions, labeled AFP was detected in the form of several protein bands with different molecular weights: 65–68 kDa (free AFP), 125–130, 210–220, and 255–260 kDa (bound AFP) (Fig. 2) [11].

This indicates that upon leaving the endosome into the cytoplasm, AFP interacts with cytoplasmic distributor proteins (p52, p62, p67, p182, p55, and p147, depending on the cell type) (Fig. 2, *a*, *b*). The authors emphasize the fact that this interaction is reversible, since during SDS electrophoresis of the same protein fractions of the HSC cytoplasm, only one fraction of labeled AFP was detected — free (Fig.*2, c*).

It has also been shown by electron microscopy that covalently bound AFP conjugates with horseradish peroxidase in the process of endocytosis are first found in clathrin vesicles, then in endosomes and folded membranes of the central region of the Golgi apparatus, while egg albumin after internalization (the degree of its internalization is very low) was found in lysosomes. After internalization, the main part of AFP is not degraded and is re-released into the extracellular environment, i.e, AFP is recirculated [3]. In addition, it is known that AFP is able to bind not only to cells, but also to structures of the extracellular matrix [12].

AFP functions

At the level of the organism, AFP is a multifunctional protein with selective cellular stimulating and inhibitory activity. Its main function is transport namely transfer of low molecular weight substances to tissues and cells of the embryo. AFP binds and transfers such ligands as bilirubin, fatty acids, steroids, retinoids, flavonoids, phytoestrogens, dyes, heavy metals, dioxin, as well as various drugs [12] At the same time, some hydrophobic ligands, such as fatty acids or estrogens, cause conformational changes in the protein tertiary structure.

AFP has an exceptionally high affinity for polyunsaturated fatty acids (PUFA), which is necessary for building cell membranes and a special class of biologically active substances — eicosanoids. Due to the fact that PUFAs are not synthesized in the body, but are part of the cell membranes, they are used in the synthesis of prostaglandins and are especially important for the formation of nervous tissue, selective binding of PUFAs in the placenta and their transfer from the mother's blood to the blood and embryonic cells is one of the key AFP

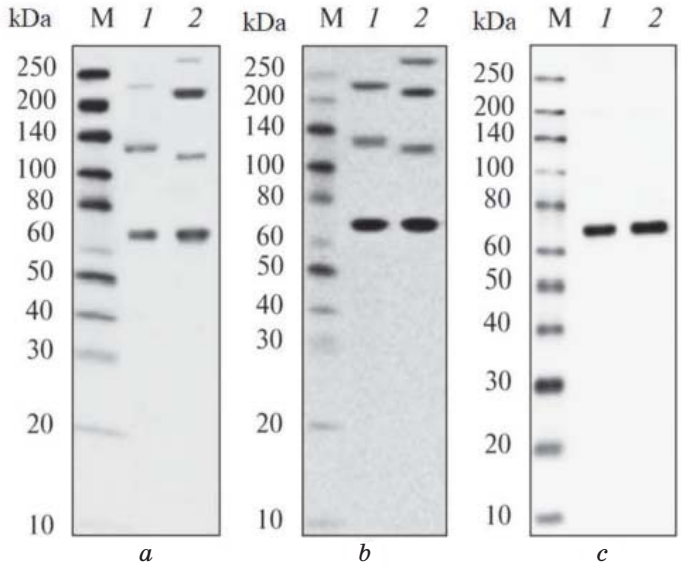

Fig. 2. **Interaction of 125I-labeled AFP with AFP-binding proteins/receptors in the cytoplasm of bone marrow HSCs [11]:**
a — detection of 125I-labeled AFP in the cytoplasm of bone marrow HSCs during native electrophoresis of the protein fraction of the cytoplasm; *b* — detection of the interaction of 125I-labeled AFP with AFP-binding proteins/cytoplasmic receptors in vitro during native electrophoresis; *c* — detection of 125I-labeled AFP in the cytoplasm during SDS-electrophoresis of the cytoplasmic protein fraction;
M — protein markers; *1* — CD34+ 133-117-135+ HSC; *2* — CD34+ 133-117+ 135-HSC

functions. The affinity of AFP for PUFA is 105 times higher than that of albumin [2].

In the embryonic period, AFP plays obviously an important role in the regulation of growth and differentiation of fetal tissues, in protecting the fetus and mother from the oncoming attack of the immune systems, in limiting the effect of steroid hormones, in particular maternal estrogens, on the fetus [12]. Among the probable functions, AFP is immunosuppressive, i.e., suppression of immune responses to antigens in a developing fetus. Since new proteins (antigens) appear in the development process, antibodies to them will also arise, which will lead to a number of complications. Therefore, the embryo's own immune system is suppressed, and AFP is possibly involved in providing this suppression [12]. This is supported by numerous studies confirming that AFP suppresses the expression of the molecules of the major histocompatibility complex and thereby prevents the presentation of antigens by macrophages. This is one of the main mechanisms responsible for the immunological incompetence of the developing fetus.

The effect of AFP on specific immunity has also been elucidated [2]:

- AFP inhibits the production of antibodies and the maturation of cytotoxic T-lymphocytes to T-dependent antigens without affecting the activity of mature T– and B-lymphocytes. The main target of the fetal protein is proliferating T-helpers corresponding to cytotoxic T-lymphocytes in the main histocompatibility complex.
- AFP suppresses the proliferative response of lymphocytes to mitogen.
- AFP increases the activity of specific T-suppressors.
- AFP reduces the phagocytic ability of macrophages.
- Depending on the experimental conditions, AFP can increase or decrease the production of prostaglandin E2 in macrophages, while indomethacin (an inhibitor of cyclooxygenase), together with AFP, loses the ability to suppress the synthesis of prostaglandin E2.
- A consequence of the stimulating effect of AFP on the production of prostaglandin E2 by monocytes is that AFP significantly suppresses the synthesis of TNF-α by activated monocytes (by 58%) and IL-1β (by 67%).
- AFP reduces the activity of natural killer cells.

Thus, AFP has definitely multidirectional immunomodulatory effects. In high doses, comparable to the concentrations in fetal blood, it inhibits lymphocyte proliferation, as demonstrated in cell cultures [2]. At the same time, no interactions of AFP with IL-2 supporting the proliferation of T cells were revealed, which made it possible to draw a conclusion about the direct effect of AFP on T cells.

On the one hand these data is indicative of the mother's immune system participation in pregnancy control even in the case of complete genetic compatibility of the mother and fetus, and on the other hand of AFP important role in this process [12]. When considering AFP from the standpoint of its immunosuppressive properties Clark et al. put forward the fetoembryonic hypothesis of the body's defense against autoimmune influences [13]. The gist of the hypothesis is that developing humans and gametes are protected by soluble immunosuppressive glycoproteins found in amniotic fluid and ergastoplasm known as glycodelin-A (GdA) and glycode lin-S (GdS), respectively. Structural analysis of their nucleoligosaccharide sequences suggests that GdA and GdS have very unusual carbohydrate functional group sequences that allow them to exhibit immunosuppressive properties. AFP has similar GdA and GdS sequences in its structure.

A study was carried out aimed at elucidating the role of AFP in the activation of cytokine synthesis *in vitro*. The production of IL-1β, IL-6 and TNF-β in whole blood cell cultures of 10 healthy subjects was studied after polyclonal activation by lipopolysaccharide in the presence of AFP. As a result of the experiments, it turned out that AFP is intact in relation to the studied cytokines [14].

Much attention is paid to the effect of AFP on the mitotic cycle of cells. The antiproliferative effect of this protein was studied in the works of the 70s–80s.The authors note the dependence of the effect on the AFP dose, purification degree, type of the cells and medium composition [15]. In the works of the 90s.much attention is paid to the synergistic (amplifying) properties of AFP. It has been shown that, without having a mitotic effect, AFP increases significantly (by 2–3 times) the effect of epidermal growth factor [16], insulin-like growth factor, and TGF-β [17].

Among other AFP properties, its regulatory role in the metabolism of steroid hormones and the ability to block the binding of antibodies to the acetylcholine receptor are noted [2, 16]. Thus, it was found that AFP negatively affects the metabolism and properties of estrogens. AFP isolated from umbilical cord blood or amniotic fluid inhibits

dose-dependently estradiol production *in vitro*. In this case, progesterone production was insensitive to the action of AFP [2, 16].

It is also known that AFP is capable to have an impact on cell proliferation and differentiation. In particular, its effect on the intracellular signaling pathway with the participation of phosphatidylinositol 3-kinase (PI3K)/AKT was shown, and a direct effect on the production of insulin-like growth factor was established [18, 19]. Since AFP acts as a transport protein, it can deliver regulatory molecules to the cells having receptors for AFP, or act as one of the independent regulators of signal transduction pathways. Since receptors for AFP are found in dividing cells (including tumor cells, hematopoietic cells, immunity, etc.), so in fact, it is able to exercise informational control of proliferating cells and significantly affect the level of their functional activity.

Isolation and purification of AFP

Currently, there are several ways to isolate and purify AFP from various biological raw materials. Most often, material with a high AFP content is used for these purposes: serum of cord or abortive blood of the second trimester of pregnancy and amniotic fluid [2]. AFP is also obtained from fetal and tumor tissues [2]. The main problem that arises during the isolation of a highly homogeneous preparation of AFP is purification from serum albumin, since it is present in serum in high concentrations and has a number of properties similar to AFP (molecular weight, close isotopes, etc.). In addition, human AFP isolated by different methods and from various sources has significant microheterogeneity that revealed either by native electrophoresis or isofocusing, while one or two bands are present in electrophoresis under denaturing conditions [20]. Thus, isofocusing the protein isolated from cord blood serum at pH values from 4.5 to 5.2 using polyclonal and monoclonal antibodies, 9 AFP isoforms were found (Sittenfeld and Moreno, 1988). When isolating protein from hepatomas, the authors found even greater heterogeneity than when isolating AFP from normal tissues and fluids [20]. It is believed that the heterogeneity of this protein is associated with varying degrees of glycosylation and with the presence or absence of ligands, primarily fatty acids. Back in the 70s and 80s. it was shown (Parmelee et al., 1978; Kerckaert et al., 1975, 1979b; Nunez et al., 1976; Bayard and Kerckaert, 1977; McMahon et al., 1977; Nagai et al., 1982) that AFP delipidisalia leads to a change in its isotope, and unsaturation with fatty acids promotes the transition of the protein to another isoform. The possibility of linking the heterogeneity of AFP with the primary structure was not confirmed when constructing peptide maps of AFP from a normal source and hepatoma: electrophoresis of hydrolysis was identical for both proteins (Ruoslahti and Seppala, 1971). These data were also confirmed by the analysis of the primary structure of cDNA, mRNA and the amino acid sequence of AFP derived from them from carcinoma (Morinaga et al., 1983) and normal liver (Gibbs et al., 1987), as well as by comparing the N-terminal sequence of the protein from various sources. Thus, the problem of AFP heterogeneity during its production remains open.

The technology for isolation and purification of AFP is a complex, laborious and multistage process with a large amount of losses of the target material. The bulk of research in this direction was carried out in 1980–2000. At present, either recombinant technologies [21] or fragmentary synthesis of its segments with an average of 15–40 amino acids [22] are used to obtain AFP.

In general, the method for AFP obtaining from natural sources can be divided into several main stages [2, 20]:

- fractionation of source material — separation of ballast proteins and blood cells;
- inactivation of viruses (solvent/detergent type TRITON-X100 or TWEEN-80);
- stepwise isolation of AFP (on average 2–4 stages) using affinity/immune-affinity/reverse-phase chromatography on columns with a sorbent (Sepharose, Sephadex, agarose, metal chelates). In the case of immuno-affinity chromatography, antibodies to human AFP covalently immobilized in the matrix are used. The elution solutions vary depending on the type of antibodies;
- ultrafiltration of the eluate;
- checking the purity of the protein by electrophoresis or immunochemical method;
- stabilization of the resulting AFP solution by freeze drying with stabilizing additives.

Depending on the method of obtaining, the AFP yield from different authors averages 60–85%, but there are also indicators with more significant losses of AFP. For example, in [2] a practical example is described, in which 2.5 liters of serum containing 250 mg of AFP was obtained from 3 liters of abortive blood. After 12 stages of the production cycle, the dry matter yield was 86.4 mg AFP, i. e. about 35% of the initial protein content in abortive blood.

One of the most highly efficient schemes for the isolation and purification of AFP from

human cord blood was proposed by the authors of [20]. The scheme includes sequential chromatography on four columns (blue sepharose, two metal chelates, and one reverse phase), which contributes to the efficient isolation of high-purity protein and the yield of the target product is about 85% [20].

Most of the AFP isolation and purification schemes are described in detail in [2]. Thus, one of the most common is the immune-affinity method for AFP isolation using specific high-affinity polyclonal antibodies. It is optimal for isolation from a source with a high AFP content. In this case, the antiserum is pretreated with serum obtained from an adult organism (to get out it from antibodies to impurity proteins), after which it is mixed with the starting material to form a precipitate. The precipitate is dissolved in a low pH buffer. AFP and free antibodies are separated by gel filtration. Antibodies conjugated to various matrices (agarose, Sephadex or Sepharose) are also used. In this case, AFP is eluted with a solution with a low pH value or high concentrations of chaotropic agents (8 M urea).

A less common immuno-affinity method is an isolation scheme using monoclonal antibodies with low affinity and antibodies to impurity proteins [2, 20]. It is obvious that the use of low pH values and high concentrations of chaotropic agents for elution of the target product, in the case of using positive antibodies, can affect the nativeness of the protein. The disadvantage of this scheme is the strong interaction between the antibody and the target protein, which leads to a significant loss of the target product. When using low affinity monoclonal antibodies and negative antisera, the problems arise associated with high cost (monoclonal antibodies) and low sorbent capacity in both cases.

Other schemes for AFP purification have been developed, for example, using immobilized concanavalin A, with which AFP bound to the estradiol matrix binds specifically, separation using a two-phase system, various isolation schemes using high pressure chromatography or metal chelate sorbents [2].

All these methods make it possible to obtain a substance for the manufacture of an injectable form of the AFP drug. But at the same time, the general and main disadvantage of all the above schemes for obtaining AFP is the heterogeneity of the isolated protein, which implies additional purification steps, the removal of hydrophobic ligands, and regular immunochemical or electrophoretic control. In addition, there are problems associated with low capacity, low degree of purification and large losses of the target product up to 40%.

It is interesting that for all described schemes of AFP isolation from cord or abortive blood, it is fractionated with the removal of corpuscles and ballast proteins. However, as it was discussed above, a significant amount of AFP is bound to receptor complexes on cells of leukocyte origin, localized in the cytoplasm, or forms complexes with other proteins and ligands [3, 11]. From this point of view, it would be appropriate to introduce the stage of decellularization of the starting material, which, possibly, will significantly increase the final yield of the target product.

The most common methods of decellularization are destruction by hypotonic lysis, heating to high temperatures (+70–100 °C), destruction by ultrasound, as well as by exposure to low temperatures. It should be noted that when extracted at room or high temperature, the structure of proteins is disrupted and, as a consequence, a significant decrease or complete loss of their biological activity occurs. The use of low temperatures will completely avoid heating. Therefore, cryotechnologies are the most promising and prevailing in terms of a number of qualitative characteristics for these purposes [23–25]. The use of low temperatures in the preprocessing of biological raw materials contributes to a more complete destruction of cellular structures and the release of proteins into the extraction solution. At the same time, the degree of decellularization and destruction of cellular structures can be varied using a combination of fast or slow freezing and thawing modes, which is important due to the localization of AFP not only in the cytoplasm, but also in the Golgi complex, endoplasmic reticulum, and mitochondria [11]. It is known that the greatest destruction of cellular components occurs during slow cooling of biological material in the temperature range of water crystallization (especially at the eutectic point) and recrystallization [23]. Based on this, the study of the effectiveness of various modes of low-temperature destruction of cord or abortive blood for the isolation of AFP will be of high scientific and practical importance.

The purified AFP preparation, obtained from aborted human tissues, was registered (Russia, USA) and was used in clinics in the 80–90s mainly for the treatment of autoimmune diseases (Alfetin® 75 μg/ampoule; Profetal® 75 μg/ampoule), but was withdrawn in 2008 for ethical reasons. Advances in biotechnology over the past decade make it possible not only to

improve the methods of isolation and large-scale purification of natural AFP, but also to produce a recombinant protein [26]. Preconceptual study is underway as well to create artificially synthesized active peptide fragments of AFP, consisting of an average of 30–60 amino acid residues, and to study their properties and therapeutic efficacy [22, 27, 28].

At the same time, the clinical application of AFP, both natural and synthetic, is controversial [26]. On the one hand, natural AFP is glycosylated (3–5%), while synthetic AFP is not, which can affect the binding affinity/specificity of the drug or its reception. On the other hand, in the case of using synthetic AFP, medicinal substances can be preliminarily bound to stabilize the tertiary structure of the synthetic protein [29] and/or additional stability can be achieved due to the binding of metal ions at the stage of production [30, 31], which will eliminate the loss of pharmaceutical ligand, for example, by competition for binding to albumin. In addition to all that has been said, the potential risks of administering therapeutic doses of this oncofetal protein to adult patients are still actively discussed in the scientific literature [26, 31].

Clinical application

To date, there have been many studies of the therapeutic efficacy of an AFP-based drug in laboratory and clinical practice [7, 32–35]. However, as it was discussed above, there is a latent danger of using therapeutic doses of AFP in adults, which consists in the abundance of its reversible and transitional conformational states depending on environmental conditions (pH, temperature, osmolality, excessive ligand concentrations, oxidation and heat/glucose shock) [7]. Contrary to the earlier reports that AFP induces cancer cell apoptosis [36], it has also been shown that it can inhibit this process in several types of cancer cells, thereby promoting their proliferation, growth and progression [37–40]. In addition, AFP is able to dimerize with other proteins, such as nuclear receptors (eg, retinoic receptor), transcription factors and caspases, which, as a result, can also promote the growth of tumor cells [34, 37].

Nevertheless, the ability of AFP to bind and target drug delivery to target cells with receptors for it remains an attractive object for further research. The capture of the drug by AFP leads to a change in its conformation, which provides a high binding affinity, thus, it acts as a natural nanocontainer for the selected hydrophobic ligands [31]. AFP has 5 or more possible binding sites. Of these, the major hydrophobic binding pocket is of particular interest, since other sites cannot compete with albumin for ligand binding. Since albumin is present in the bloodstream in a huge excess, all non-covalently bound ligands on the surface of the AFP molecule will be lost [41].

Inside the cells with a low pH, AFP changes its conformation again and releases the transported substance. In a ligand-free conformation, AFP returns to the bloodstream in an intact form (AFP recirculation takes about 1 hour) and continues to transfer dozens of ligands in a shuttle manner [41].It was shown that AFP can bind and release the ligand several times with a change in pH [30, 42].

In an adult organism, AFP receptors are present extensively, primarily in AFP-positive myeloid suppressor cells and cancer cells. Other cell populations are practically not affected by the AFP-ligand complex, since they do not have receptors or there are too few of them, or the cells themselves are in an inactive state (for example, stem cells have a set of receptors for AFP, but are in an inactive state in the bone marrow) [43]. Thus, with the help of AFP, it is possible to exert a targeting effect on myeloid suppressors and cancer cells. In this regard, there are 3 main directions in the use of drugs based on AFP:

- immunotherapy (the first registered AFP drugs belong to the class of immunomodulators);
- therapy of autoimmune diseases;
- anticancer therapy.

Immunotherapy and therapy of autoimmune diseases. The ability of AFP with the help of transported ligands to activate or inhibit suppressor cells of myeloid origin makes it possible to control the intensity of immune responses in a targeted manner, which is used in the complex treatment of autoimmune diseases [31, 44, 45] and other immunopathological conditions caused by impaired synthesis of cytokines that regulate T-cell immunity (ulcerative colitis, autoimmune thyroiditis, etc.). In addition, as it was already discussed, AFP is capable of enhancing the action of alpha-interferon, a tumor necrosis factor, and modulating the activity of prostaglandins and leukotrienes, which are important in the development of the inflammation process [46, 47]. It has been shown that due to these properties, AFP is a participant in the acute phase of inflammation [48].

The use of synthetic AFP (MM-093) as monotherapy has been investigated in autoimmune diseases such as rheumatoid

arthritis, psoriasis and uveitis [49] and has been successful in phase I and II of clinical trials: the established safe doses can be used for several indications. In rheumatoid arthritis, however, MM-093 has not been shown to be effective in phase II of clinical trials. Perhaps this is due to the fact that AFP is not a drug in itself, and it should be used as a part of complex therapy, since the successful treatment of autoimmune diseases using AFP from natural sources was carried out in combination with other drugs (for example, in a complex with reduced doses of glucocorticoids) [2].

At the same time, the study of experimental autoimmune myasthenia in animal models provided clear evidence of the effectiveness of MM-093 monotherapy. It has been found that the use of MM-093 leads to a decrease in the complement cascade, which plays an important role in the onset of clinical symptoms of autoimmune myasthenia [50]. It is possible that AFP delivers local natural ligands to myeloid suppressors, which is sufficient for the positive results obtaining, or other mechanisms of action are involved. There were no safety concerns in this study.

Anticancer therapy. Another promising area of drugs application based on AFP is the delivery of cytostatics to tumor cells. Chemotherapy is one of the most commonly used methods of fighting cancer. The effectiveness of chemotherapy drugs is ensured by the fact that they are all especially effective in killing actively dividing and metabolizing cells. Unfortunately, in addition to tumor cells, the cells of the bone marrow, gastrointestinal tract, etc. normally have the same characteristics. As a result, the entire spectrum of side effects of chemotherapy is associated with the effect of the drug on normal actively dividing cells in the body. In view of this, one of the promising ways to increase the effectiveness of chemotherapy drugs is their selective delivery to tumor cells. The essence of the method is that the chemotherapy is attached to a vector molecule, which delivers it to a certain type of cells. Targeting of delivery is provided due to specific receptors characteristic only of target cells. AFP is one of the most promising proteins that can be used as vector molecules capable of targeting the delivery of chemotherapy drugs. It was shown that conjugates of natural AFP with cytostatics inhibited the growth of tumor cells *in vitro* and *in vivo* [51–54]. However, the use of natural AFP is limited by the abundance of its conformational states depending on the conditions of the microenvironment (which makes it difficult to predict the therapeutic effect), as well as technical and ethical aspects, since its only source is material of fetal-placental origin. For this reason, numerous studies are being carried out on the development and use of functionally complete recombinant AFP fragments. It is known that the receptor binding site is located in the C-terminal domain of the AFP molecule. Therefore, for the purpose of targeted delivery, it is optimal to use recombinant protein fragments based on the C-terminal domain [7, 55–57]. One of the main problems when using recombinant proteins is their production in a functional form. In most cases, when proteins are produced in large quantities in the bacterial system, they form in cells the so-called inclusion bodies, which consist of protein in an aggregated state [55, 58–60]. To obtain a functionally active protein, it is necessary to carry out the procedure of its renaturation. For hydrophobic proteins with a large number of disulfide bonds, such as the C-terminal domain of AFP, this problem is especially difficult. The development of an effective renaturation technique is the main difficulty in obtaining a functional form of a recombinant AFP fragment. This problem solution will make it possible to obtain a functional fragment of AFP in sufficient quantities to create its conjugates with cytostatics.

The creation of conjugates of AFP with cytostatics is a method aimed at the cancer cells themselves. But another approach to the use of AFP in the framework of anticancer therapy is also possible — depressing effect on myeloid suppressors. The point is that the accumulation of these cells is a key process of immunosuppression in various types of cancer [61]. Myeloid suppressors and T-regulatory cells are the main components of the immunosuppressive tumor microenvironment [62], providing tumor-mediated evasion from immune control [63]. Being normal cells in the body, myeloid suppressors enhance cancer growth and inhibit the activity of natural killer cells and specialized T cells that can destroy cancer cells. Thus, in order to overcome the tolerance to tumor cells on the part of the immune system and activate adaptive and innate immunity to cancer, myeloid suppressors must be depleted [31, 64].

It has been shown that such common chemotherapy drugs as doxorubicin, paclitaxel, gemcitabine, and 5-FU can deplete myeloid suppressors [65]. These drugs can be used not only as direct toxins against cancer cells, but also as immunomodulators that can selectively reduce myeloid suppressor-mediated immunosuppression and increase

the effectiveness of other therapies. Myeloid suppressors are more important target in anticancer therapy than cancer cells themselves, since killing one suppressor has more potent anticancer effect than killing a single cancer cell. For example, the effect of 5-FU immunotherapy within the framework of a complex treatment regimen prevailed over the effect of classical chemotherapy, as it is seen when comparing tumor growth suppression in mice [66]. Low-dose paclitaxel blocks myeloid suppressor-mediated immunosuppression *in vivo*, suppresses chronic inflammation in the tumor microenvironment, and can be used to increase the effectiveness of concomitant antitumor therapy [67]. Moreover, unlike 5-FU or doxorubicin, paclitaxel strongly binds to AFP, which ensures its targeted delivery to myeloid suppressors and enhances the antitumor effect of AFP [31]. Similar regimens for the combination of chemotherapy and immunotherapy are already used in the treatment of cancer [68, 69]. Chemotherapy leads to self-destruction of cancer cells (apoptosis, autophagy, etc.), while immunotherapy alters the tolerance of the patient's immune system to cancer cells. The combination of these two mechanisms in many cases can provide victory over cancer [70]. The use of non-covalent AFP-chemotherapy complexes combines immunotherapy and targeted chemotherapy. To date, a large number of drugs for the treatment of oncological diseases containing AFP or its fragments are at the stage of development, studies of the efficacy and safety of use [71–78].

Thus, summarizing the data presented, we can conclude that the study of the molecular mechanisms of AFP functioning and the therapeutic potential of drugs based on it is a promising area of modern fundamental science, pharmacy and medicine. It should be noted that despite the huge array of works devoted to the study of this protein, there are a number of unresolved issues that require further research. From a fundamental point of view, it is important to clarify the number of active AFP binding sites for interaction with various ligands, to assess the strength of this interaction and the stability of the complexes formed. It is extremely important to establish the biological activity of not only the native protein, but also its fragments, which may reveal new possibilities for its therapeutic use. Biotechnological solutions also require a solution to issues related to the production of AFP from natural raw materials, namely, the search for the most effective and gentle ways of its isolation, including the use of cryotechnologies, which together will avoid large losses of the target product during the production cycle and provide a high degree of its purification. Questions about the mechanisms of AFP reception also require further in-depth study. It is assumed that solving these problems will allow the creation of a registered AFP drug in the consecutive 5–10 years.

The review was prepared within the framework of the scientific work "Study of the regularities of the release of biologically active water-soluble substances from tissues of plant and animal origin when exposed to low temperatures" (State registration number 0106V002165, deadline 2020–2024), which is funded from the budget of the National Academy of Sciences of Ukraine.

REFERENCES

1. *Moldogazieva N. T., Terentiev A. A., Shait K. V.* Relationship between the structure and functions of alpha-fetoprotein: its conformational changes and biological activity. *Biomed. chem*. 2005, 51 (2), 127–151. (In Russian).
2. *Chereshnev V. A., Rodionov S. Yu., Cherkasov V. A., Malyutina N. N., Orlov O. A.* Alfa-fetoprotein. *Yekaterinburg: Uralskoe otdelenie RAN*. 2004, 376 p. (In Russian).
3. *Treshalina H. M., Smirnova G. B., Tsurkan S. A., Tcherkassova J. R., Lesnaya N. A.* The role of alpha-fetoprotein receptor in the delivery of targeted preparations in oncology. *Russian J. Oncol.* 2017, 22 (1), 4–14. https://doi.org/10.18821/1028-9984-2017-22-1-4-14 (In Russian).
4. *Nikolaeva L. B., Ushakova G. A.* The first pregnancy and first birth: a guide for doctors. *Moskva: GEOTAR-Media*. 2013, 264 p. (In Russian).
5. *FryerA. A., Jones P., Strange R., Hume R., Bell J. E.* Plasma protein levels in normal human fetuses: 13 to 41 weeks' gestation. *Br. J. Obstet. Gynaecol.* 1993, 100 (9), 850–855.
6. *Hsu J. J., Hseih T. T., Chiu T. H., Liou J. D., Soong Y. K.* Alpha-fetoprotein levels of paired samples between the amniotic fluid and maternal serum from 16 to 18 weeks' gestation in Chinese women. *J. Formos. Med. Assoc.* 1994, 93 (5), 374–378.
7. *Mizejewski G. J.* Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. *Exp. Biol. Med.* 2001, V. 226, P. 377–408.
8. *Naval J., Villacampa M. J., Goguel A. F., Uriel J.* Cell-type specific receptors for alpha-

fetoprotein in a mouse T-lymphoma cell line. *Proc. Natl. Acad. Sci. USA.* 1985, V. 82, P. 3301–3305.
9. *Suzuki T., Sasano H., Aoki H., Nagura H., Sasano N., Sano T., Saito M., Watanuki T., Kato H., Aizawa S.* Immunohistochemical comparison between anaplastic seminoma and typical seminoma. *Acta Pathol. Jpn.* 1993, 43 (12), 751–757.
10. *Moldogazieva N. T., Terentiev A. A.* Alpha-fetoprotein and growth factors. Structure-function relationships and analogies. *Usp. Biol. Chim.* 2006, No 46, P. 99–148. (In Russian).
11. *Bogdanov A. Yu., Bogdanova T. M., Ilin A. I.* Endocytic pathway of alpha-fetoprotein in mice bone marrow hematopoietic stem cells: molecular characterization and role in biological activity modification. *Cytology and Genetics*. 2014, 48 (1), 25–40. (In Russian).
12. *Shmagel K. V., Chereshnev V. A.* Alpha-fetoprotein: structure, function and role in embryogenesis. *Akusherstvo i ginekologiya.* 2002, No 5, P. 6–8. (In Russian).
13. *Clark G. F., Dell A., Morris H. R., Patankar M., Oehninger S., Sepp l M.* Viewing AIDS from a glycobiological perspective: potential linkages to the human fetoembryonic defence system hypothesis. *Mol. Hum. Reprod.* 1997, 3 (1), 5–13.
14. *Filella X., Molina R., Alcover J., Coca F., Zarco M. A., Ballesta A. M.* Influence of AFP, CEA and PSA on the in vitro production of cytokines. *Tumour Biol*. 2001, 22 (2), 67–71.
15. *Czokalo M., Wishnewski L.* Culture conditions modify the effects exerted by human fetal AFP on some lymphocyte functions *in vitro*. *Exp. Pathol.* 1981, 20 (4), 233–238.
16. *Keel B. A., Eddy K. B., Cho S., May J. V.* Human alpha-fetoprotein purified from amniotic proliferation *in vitro*. *Mol. Reprod. Dev.* 1991, 30 (2), 112–118.
17. *Leal J. A., May J. V., Keel B. A.* Human alpha-fetoprotein enhances epidermal growth factor proliferative activity upon porcine granulosa cells in monolayer culture. *Endocrinol*. 1990, 126 (1), 669–671.
18. *Li M., Li H., Li C., Wang S., Jiang W., Liu Z., Zhou S., Liu X., McNutt M. A., Li G.* 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 (3), 524–532. https://doi.org/10.1002/ijc.25373
19. *Zheng L., Gong W., Liang P., Huang X., You N., Han K. Q., Li Y. M., Li J.* Effects of AFP-activated PI3K/Akt signaling pathway on cell proliferation of liver cancer. *Tumour Biol*. 2014, 35 (5), 4095–4099. https://doi.org/10.1007/s13277-013-1535-z
20. *Tomashevsky A. I., Yuversky V. N.* A new scheme for the isolation and purification of human alpha-fetoprotein. *Bioorg. chem.* 1999, 25 (6), 412–417. (In Russian).
21. *Lin B., Peng G., Feng H., Li W., Dong X., Chen Y., Lu Y., Wang Q., Xie X., Zhu M., Li M.* Purification and characterization of abioactive alpha-fetoprotein produced by HEK-293 cells. *Protein Expr. Purif*. 2017, V. 136, P. 1–6. https://doi.org/10.1016/j.pep.2017.05.008
22. *Terentev A. A., Kazimirsky A. N., Lychkova A. E., Salmasi J. M.* Lymphocyte apoptosis enhancement by the synthetic peptide of human alpha-fetoprotein (afp 14-20) in experimental ulcer. *Exper. Clin. Gastroenterol.* 2014, 110 (10), 50–52. (In Russian).
23. *Fuller B. J., Lane N., Benson E. E.* Life in the frozen state. *London: CRC Press*. 2004, 672 p.
24. *Shabunin S. V., Vostrilova G. A., Shabanov I. E.* Screening of biologically active substances depending on the technological parameters of cryogenic fractionation of the placenta. *Problems of cryobiology*. 2005, 15 (3), 306–309. (In Ukrainian).
25. *Osetskiy A. I., Grischenko V. I., Snurnikov A. S., Shabanov I. Ye., Babijchuk G. A.* Cryosublimation fractionating of biological material. *Problems of cryobiology*. 2006, 16 (2), 230–240. (In Ukrainian).
26. *Mizejewski G. J.* Therapeutic use of human alpha-fetoprotein in clinical patients: Is a cancer risk involved? *Int. J. Cancer.* 2011, V. 128, P. 239–249. https://doi.org/10.1002/ijc.25292
27. *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, No 9, P. 603–10.
28. *Mizejewski G.* Mapping of structure-function peptide sites on the human alpha-fetoprotein amino acid sequence. *Atlas Genet. Cytogenet. Oncol. Haematol*. 2010, 14 (2), 169–216. https://doi.org/10.4267/2042/44695
29. *Uversky V. N., Narizhneva N. V., Ivanova T. V., Tomashevski A. Y.* Rigidity of human alpha-fetoprotein tertiary structure is under ligand control. *Biochem.* 1997, V. 36, P. 13638–13645.
30. *Dudich I. V., Semenkova L. N., Tatulov E., Korpela T., Dudich E.* Improved delivery of poorly water soluble drugs with alphafetoprotein stabilized with metal ions. *International Bureau PCT. WO2015075296 A1*. 05. 28. 2015.
31. *Pak V. N.* The use ofalpha-fetoproteinfor the treatment of autoimmune diseases and cancer. *Ther Deliv*. 2018, 9 (1), 37–46. https://doi.org/10.4155/tde-2017-0073
32. *Li M., Liu X., Zhou S., Li P., Li G.* Effects of alpha fetoprotein on escape of Bel 7402 cells from attack of lymphocytes. *BMC Cancer.* 2005, No 5, P. 96.
33. *Li M., Zhou S., Liu X., Li P.* Alpha-fetoprotein shield hepatocellular carcinoma cells from apoptosis induced by tumor necrosis factor-

related apoptosis-inducing ligand. *Cancer Lett*. 2007, V. 249, P. 227–234.
34. *Li M., Li H., Li C., Guo L., Zhou S.* 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, V. 285, P. 190–199.
35. *Zhang W., Liu J., Wu Y., Xiao F., Wang Y., Wang R., Yang H., Wang G., Yang J., Deng H., Li J., Wen Y., Wei Y.* Immunotherapy of hepatocellular carcinoma with a vaccine based on xenogeneic homologous alpha fetoprotein in mice. *Biochem. Biophys. Res. Commun*. 2008, 376 (1), 10–14. https://doi.org/10.1016/j.bbrc.2008.08.061
36. *Semenkova L. N., Dudich E. I., Dudich I. V.* Induction of apoptosis in human hepatoma cells by alpha-fetoprotein. *Tumour Biol.* 1997, V. 18, P. 261–273.
37. *Li M., Li H., Li C., Zhou S., Guo L., Jiang W.* 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, V. 124, P. 2845–2854. https://doi.org/10.1002/ijc.24272
38. *Laderoute M. P., Pilarski L. M.* The inhibition of apoptosis by alpha-fetoprotein (AFP) and the role of AFP receptors in anti-cellular senescence. *Anticancer Res*. 1994, No 14, P. 2429–2438.
39. *Wang X., Wand Xu B.* Stimulation of tumor-cell growth by alpha-fetoprotein. *Int. J. Cancer*. 1998, V. 75, P. 596–599.
40. *Wang X. W., Xie H.* Alpha-fetoprotein enhances the proliferation of human hepatoma cells in vitro. *Life Sci*. 1999, V. 64, P. 17–23.
41. *Pak V. N.* α-fetoprotein-binding toxins and teratogens against cancer. *Ther Deliv*. 2019, No 1, P. 1–3. https://doi.org/10.4155/tde-2018-0068
42. *Hirano K., Watanabe Y., Adachi T., Ito Y., Sugiura M.* Drug binding properties of human alpha-fetoprotein. *Biochem. J*. 1985, V. 231, P. 189–191.
43. *Belyaev N. N., Abdolla N., Perfilyeva Y. V., Ostapchuk Y. O., Krasnoshtanov V. K., Kali A., Tleulieva R.* Daunorubicin conjugated with AFP selectively eliminates MDSCs and inhibits experimental tumor growth. *Cancer Immunol. Immunother*. 2018, 67 (1), 101–111. https://doi.org/10.1007/s00262-017-2067-y
44. *Gabrilovich D. I.* Myeloid-derived suppressor cells. *Cancer Immunol. Res.* 2017, 5 (1), 3–8. https://doi.org/10.1158/2326-6066.CIR-16-0297
45. *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 (6), 603–610.
46. *Aussel C., Fehlmann M.* Alpha-fetoprotein stimulates leukotriene synthesis in P388D1 macrophages. *Cell. Immunol*. 1986, V. 101, P. 415–424.
47. *Nakanishi M., Rosenberg D. W.* Multifaceted roles of PGE2in inflammation and cancer. *Semin. Immunopathol*. 2013, 35 (2), 123–137. https://doi.org/10.1007/s00281-012-0342-8
48. *Mizejewski G. J.* Alpha-fetoprotein (AFP) and inflammation: Is AFP an acute and/or chronic phase reactant? *J. Hematol. Thrombo. Dis*. 2015, 3 (1), 191–199. https://doi.org/10.4172/2329-8790.1000191
49. *Murgita R. A.* Recombinant human alpha-fetoprotein as an immunosuppressive agent. *Pat. USA. US7423024*. 09. 09. 2008.
50. *Kusner L. L., Sengupta M., Aguilo-Seara G., Sherman I.* Preclinical pilot study of alpha-fetoprotein on moderation of MG weakness. *Abstracts of the 13th International Conference on Myasthenia Gravis and Related Disorders. NY: The New York Academy of Sciences*. 2017.
51. *Mizejewski G. J.* Biological role of alpha-fetoprotein in cancer: prospects for anticancer therapy. *Expert. Rev. Anticancer Ther*. 2002, 2 (6), 709–735. https://doi.org/10.1586/14737140.2.6.709
52. *Chereshnev V. A., Rodionov S. Iu., Vasil'ev N. V., Orlov O. A., Cherkasov V. A.* Alpha-fetoprotein immunotherapy as a stage of combined treatment of cancer patients. *Vopr. Onkol*. 2005, 51 (1), 86–92.
53. *Posypanova G., Severin S.* Alpha-fetoprotein and recombinant alpha-fetoprotein fragments as drug delivery tools. In: Alpha-fetoprotein: functions and clinical applications. Lakhi N., Moretti M. (Eds). *Nova Science Publishes, NY, USA*. 2016, P. 277–300.
54. *Pak V. N.* The use of alpha-fetoprotein for the delivery of cytotoxic payloads to cancer cells. *Ther. Deliv*. 2014, 5 (8), 885–892. https://doi.org/10.4155/tde.14.59
55. *Sharapova O. A.* Recombinant fragments of human alpha-fetoprotein for creating targeted drug delivery (Ph. D. dissertation). *Available from the Scientific Electronic Library of theses and abstracts*. 2012.
56. *Bereznikova A. B., Posypanova G. A., Makarov V. A., Antipova O. V., Severin E. S.* AFP octapeptide is a promising peptide vector for targeted delivery of cytostatics to tumor cells. *Vopr. biol. med. farm. chemistry*. 2012, No 5, P. 15–21. (In Russian).
57. *Sharapova O.A., Pozdnyakova N.V., Severin S.E., Laurinavichyute D. K., Yurkova M. S., Andronova S. M., Fedorov A. N., Severin E. S., Posypanova G. A.* Isolation and characterization of the recombinant human α-fetoprotein fragment corresponding to the c-terminal structural domain. *Bioorganic chem*. 2010, 36 (6), 696–703. (In Russian).
58. *Garc a-Fruit s E., Ar s A., Villaverde A.* Localization of Functional Polypeptides in

Bacterial Inclusion Bodies. *Appl. Environ. Microbiol*. 2007, 73 (1), 289–294. https://doi.org/10.1128/AEM.01952-06
59. *Gil-Garcia* M., *Navarro S., Ventura S.* Coiled-coil inspired functional inclusion bodies. *Microb. Cell Fact*. 2020, 19 (1), 117. https://doi.org/10.1186/s12934-020-01375-4
60. *J ger V. D., Lamm R., K sters K., l* c *G., Oldiges M., Jaeger K. E., B chs J., Krauss U.* Catalytically-active inclusion bodies for biotechnology-general concepts, optimization, and application. *Appl. Microbiol. Biotechnol*. 2020, 104 (17), 7313–7329. https://doi.org/10.1007/s00253-020-10760-3
61. *Colligan S. H., Tzetzo S. L., Abrams S. I.* Myeloid-driven mechanisms as barriers to antitumor CD8+T cell activity. *Mol. Immunol*. 2020, V. 118, P. 165–173. https://doi.org/10.1016/j.molimm.2019.12.012
62. *Weiss J. M., Subleski J. J., Back T., Chen X., Watkins S. K., Yagita H., Sayers T. J., Murphy W. J., Wiltrout R. H.* Regulatory T cells and myeloid-derived suppressor cells in the tumor microenvironment undergo Fas-dependent cell death during IL-2/αCD40 therapy. *J. Immunol*. 2014, 192 (12), 5821–5829. https://doi.org/10.4049/jimmunol.1400404
63. *Atretkhany K. N., Drutskaya S.* Myeloid-derived suppressor cells and proinflammatory cytokines as targets for cancer therapy. *Biochemistry (Mosc)*. 2016, 81 (11), 1274–1283. https://doi.org/10.1134/S0006297916110055
64. *Belyaev N. N.* Myeloid-derived suppressor cells (MDSC) as a main tumor induced negative regulators of cancer immunity and possible ways for their elimination. *KazNU Bulletin Biol. Series*. 2014, 1 (60), 79–83.
65. *Alizadeh D., Trad M., Hanke N. T., Larmonier C. B., Janikashvili N., Bonnotte B., Katsanis E., Larmonier N.* Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. *Cancer Res*. 2014, 74 (1), 104–118. https://doi.org/10.1158/0008-5472.CAN-13-1545
66. *Vincent J., Mignot G., Chalmin F., Ladoire, S., Bruchard M., Chevriaux A., Martin F., Apetoh L., R b C., Ghiringhelli F.* 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res*. 2010, 70 (8), 3052–3061. https://doi.org/10.1158/0008-5472.CAN-09-3690
67. *Sevko A., Michels T., Vrohlings M., Umansky L., Beckhove P., Kato M., Shurin G. V., Shurin M. R., Umansky V.* Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. *J. Immunol*. 2013, 190 (5), 2464–2671.
68. *Bracci L., Schiavoni G., Sistigu A., Belardelli F.* Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ*. 2014, 21 (1), 15–25. https://doi.org/10.1038/cdd.2013.67
69. *Wang Z., Liu Y., Zhang Y., Shang Y., Gao Q.* MDSC-decreasing chemotherapy increases the efficacy of cytokine-induced killer cell immunotherapy in metastatic renal cell carcinoma and pancreatic cancer. *Oncotarge*. 2016, 7 (4), 4760–4769. https://doi.org/10.18632/oncotarget.6734
70. *Pak V. N.* Magic bullet and immunotherapy against metastasis. *J. Cancer Prev. Curr. Res*. 2016, 6 (3), 00206. https://doi.org/10.15406/jcpcr.2016.06.00206
71. *Qin H., Lerman B., Sakamaki I., Wei G., Cha S. C., Rao S. S., Qian J., Hailemichael Y., Nurieva R., Dwyer K. C., Roth J., Yi Q., Overwijk W. W., Kwak L. W.* Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice. *Nat. Med*. 2014, 20 (6), 676–681. https://doi.org/ 10.1038/nm.3560
72. *Arshad N. M., In L. L., Soh T. L., Azmi M. N., Ibrahim H., Awang K., Dudich E., Tatulov E., Nagoor N. H.* Recombinant human alphafetoprotein synergistically potentiates the anti-cancer effects of 1'-S-1'-acetoxychavicol acetate when used as a complex against human tumours harbouring AFP-receptors. *Oncotarget*. 2015, 6 (18), 16151–16167. https://doi.org/10.18632/oncotarget.3951
73. *Dominguez G. A., Condamine T., Mony S., Hashimoto A., Wang F., Liu Q., Forero A., Bendell J., Witt R., Hockstein N., Kumar P., Gabrilovich D. I.* Selective targeting of myeloid-derived suppressor cells in cancer patients using DS-8273a, an agonistic TRAIL-R2 antibody. *Clin. Cancer Res*. 2016, 23 (12), 2942–2950. https://doi.org/10.1158/1078-0432.CCR-16-1784
74. *Wilkerson A., Kim J., Huang A. Y., Zhang M.* Nanoparticle systems modulating myeloid-derived suppressor cells for cancer immunotherapy. *Curr. Top. Med. Chem*. 2017, 17 (16), 1843–1857. https://doi.org/10.2174/1568026617666161122121412
75. *Wang H. F., Ning F., Liu Z. C., Wu L., Li Z. Q., Qi Y. F., Zhang G., Wang H. S., Cai S. H., Du J.* Histone deacetylase inhibitors deplete myeloid-derived suppressor cells induced by 4T1 mammary tumorsin vivoand *in vitro*. *Cancer Immunol. Immunother*. 2017, 66 (3), 355–366. https://doi.org/10.1007/s00262-016-1935-1
76. *Pak V. N.* Selective targeting of myeloid-derived suppressor cells in cancer patients through AFP-binding receptors. *Future Sci. OA*. 2018, 5 (1), FSO321. https://doi.org/10.4155/fsoa-2018-0029

77. *Belyaev N. N., Abdolla N., Perfilyeva Y. V., Ostapchuk Y. O., Krasnoshtanov V. K., Kali A., Tleulieva R.* Daunorubicin conjugated withalpha-fetoproteinselectively eliminates myeloid-derived suppressor cells (MDSCs) and inhibits experimental tumor growth. *Cancer Immunol. Immunother*. 2018, 67 (1), 101–111. https://doi.org/10.1007/s00262-017-2067-y

78. *Mollaev M., Gorokhovets N., Nikolskaya E., Faustova M., Zabolotsky A., Zhunina O., Sokol M., Zamulaeva I., Severin E., Yabbarov N*. Type of pH sensitive linker reveals different time-dependent intracellular localization, *in vitro* and *in vivo* efficiency inalpha-fetoproteinreceptor targeted doxorubicin conjugate. *Int. J. Pharm*. 2019, V. 559, P. 138–146. https://doi.org/10.1016/j.ijpharm.2018.12.073

СУЧАСНЕ УЯВЛЕННЯ ПРО СТРУКТУРНО-ФУНКЦІОНАЛЬНІ ВЛАСТИВОСТІ АЛЬФА-ФЕТОПРОТЕЇНУ ТА МОЖЛИВОСТІ ЙОГО КЛІНІЧНОГО ЗАСТОСУВАННЯ

О. К. Гулевський
Ю. С. Ахатова

Інститут проблем кріобіолоогії
та кріомедицини НАН України, Харків

E-mail: Julija_Veselovskaja@meta.ua

Подано дані зарубіжної та вітчизняної літератури останніх 40 років стосовно особливостей молекулярної структури, функцій, отримання і застосування одного з найбільш досліджуваних та необхідних протеїнів — альфа-фетопротеїну людини (АФП). Виконано короткий огляд фундаментальних досліджень із застосуванням сучасних методів, включаючи різні види електрофорезу, хроматографії, електронної мікроскопії та імуноаналізу, що дало змогу охарактеризувати основні фізико-хімічні властивості та локалізацію вільних і зв'язаних форм АФП, а також структуру, різновиди і топографію його рецепторів. Наведено дані про механізми синтезу АФП, його конформаційні особливості, сайти зв'язування та внутрішньоклітинний метаболізм. Викладено уявлення про фізіологічні функції й механізми транспортування АФП в організмі. Подано дані щодо отримання АФП з натуральної сировини, а також рекомбінантним і синтетичним шляхом. Узагальнено також інформацію про наявні на сьогодні можливості клінічного застосування АФП і перспективи використання у протипухлинній терапії для таргетного доставлення хіміопрепаратів з урахуванням останніх досягнень в цій галузі.

Ключові слова: альфа-фетопротеїн, джерела отримання альфа-фетопротеїну, застосування альфа-фетопротеїну, таргетне доставлення хіміопрепаратів.

СОВРЕМЕННОЕ ПРЕДСТАВЛЕНИЕ О СТРУКТУРНО-ФУНКЦИОНАЛЬНЫХ СВОЙСТВАХ АЛЬФА-ФЕТОПРОТЕИНА И ВОЗМОЖНОСТЯХ ЕГО КЛИНИЧЕСКОГО ПРИМЕНЕНИЯ

А. К. Гулевский
Ю. С. Ахатова

Институт проблем криобиологии
и криомедицины НАН Украины, Харьков

E-mail: Julija_Veselovskaja@meta.ua

Изложены данные зарубежной и отечественной литературы последних 40 лет об особенностях молекулярной структуры, функциях, получении и применении одного из наиболее изучаемых и востребованных протеинов — альфа-фетопротеина человека (АФП). Выполнен краткий обзор фундаментальных исследований с применением современных методов, включая различные виды электрофореза, хроматографии, электронной микроскопии и иммуноанализа, что позволило охарактеризовать основные физико-химические свойства и локализацию свободных и связанных форм АФП, а также структуру, разновидности и топографию его рецепторов. Приведены данные о механизмах синтеза АФП, его конформационных особенностях, сайтах связывания и внутриклеточном метаболизме. Изложены представления о физиологических функциях и механизмах транспортировки АФП в организме. Представлены данные о получении АФП из натурального сырья, рекомбинантным и синтетическим путем. Обобщена также информация об имеющихся на сегодняшний день возможностях его клинического применения и перспективах использования в рамках противоопухолевой терапии для таргентной доставки химиопрепаратов с акцентом на описание последних достижений в этой области.

Ключевые слова: альфа-фетопротеин, источники получения альфа-фетопротеина, применение альфа-фетопротеина, таргетная доставка химиопрепаратов.