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RESEARCH ARTICLE

Alpha-Fetoprotein Binds Toxins and Can Be Used to Treat Cancer

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"All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident."
Arthur Schopenhauer (1788-1860)

ABSTRACT

The inefficiency of the immune system to kill cancer cells leads to the disease. Like in pregnancy, oncofetal proteins counteract the immune attack. Alpha-fetoprotein is an immunosuppressive protein generated by the embryo and in insignificant amounts by adults. It delivers nutrients to embryo cells and to the monocytes that suppress both innate and adaptive immunity during pregnancy and cancer. The small subpopulation of suppressor monocytes and cancer cells absorb the alpha-fetoprotein-nutrient complex through the specific receptor that is mostly absent in normal adult cells. It comes out that suppressor monocytes are the main targets in cancer prophylactic or treatment, not cancer cells. By delivering toxins instead of nutrients alpha-fetoprotein kills suppressor monocytes canceling immune suppression, as well as killing cancer cells directly. It is the perfect synergy of the most powerful cancer immunotherapy with targeted chemotherapy. Alpha-fetoprotein chemical conjugates, as well as the complexes of this oncofetal protein with binding toxins, have shown promising results in cancer treatments. Oral porcine alpha-fetoprotein complexes with toxins can prevent and/or treat cancer, although their immunotherapy mechanism of action is undiscovered.

Introduction

For over a hundred years oncologists were inventing a 'magic bullet' that specifically kills cancer cells. Cancer cells, their DNA, specific enzymes, tumor suppressive proteins like p53, multiple drug resistance, epigenetics, etc., were targeted. The outcome is still not satisfactory. It is time to harness nature to beat cancer. Cancer cells appear permanently during our lifetime but it is the immune system that keeps the majority of us healthy. That is why cancer prevention and treatment should be focused on immunotherapies. Moreover, among the many types of immunotherapies priority should be given to the most powerful of them.

As with any defense system, the immune one has a subordination between the regulatory and executive elements. The innate immune system preceded the adaptive one, and monocytes evolved and became superior to lymphocytes in the hierarchy of immune cells. Myeloid-derived suppressor cells (MDSCs) are a small heterogeneous population of pathologically activated, mostly immature, myeloid cells that exert robust immunosuppressive functions. MDSC inhibits natural killer (NK) cells and macrophages of innate immunity as well as T and B lymphocytes' of adaptive immunity directly and indirectly through various mechanisms (Fig. 1).

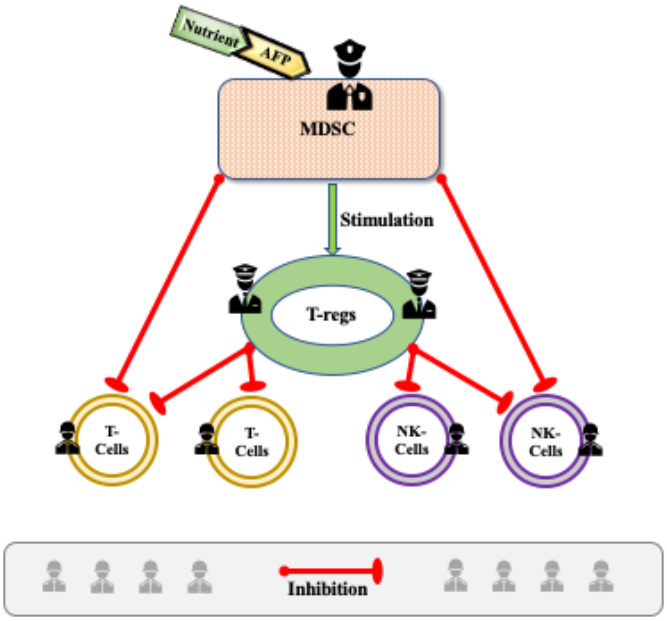


Figure 1. MDSC inhibits T and NK cells directly and indirectly through various mechanisms.

MDSCs originate from hematopoietic stem cells (HSCs) and then can leave the bone marrow and spread throughout the body becoming immune response calmers during pregnancy, cancer, regeneration, stress, autoimmune and infectious diseases, obesity, age, etc. [1, 2]. MDSCs are significant players in the profound immune suppression [3, 4]. They are the primary suppressors of the immune system's attack on cancer in the mechanism of the local immune suppression in cancer, where the contribution of MDSCs and regulatory T cells (Tregs) to the functional suppression of T- and NK cells and other immune cells cannot be overestimated. MDSCs-dependent Tregs are abundant in tumors where they dampen the immune response and protect cancer cells from killing by cytotoxic T lymphocytes, stimulate tumor vasculature, and promote tumor invasion [5]. MDSCs expand during oncogenesis and have been linked to accelerated disease progression and resistance to treatment in both preclinical tumor models and patients with cancer. The tumor microenvironment (TME) includes a wide variety of cell types and soluble factors capable of suppressing immune responses, but MDSCs accumulation in TME is a general and dominant process that prevents executive immune cells from erasing cancer [6]. Thus, the compromised function of NK cells in TME was consequent to their interaction with MDSCs [7]. MDSCs stand out as promising targets for the development of novel immunotherapeutic regimens with superior efficacy [8, 9]. So, not cancer cells but MDSC is the main target in cancer prevention and treatment. MDSCs depletion unleashes all of the subordinate lymphocytes that effectively erase

cancer cells. NK cells being activated rapidly kill any foreign cells without prior immunization or major histocompatibility complex (MHC) I restrictions, and kill cancer stem cells and metastases which are unmet needs in oncology.

New solutions to the urgent problems in cancer immunology - reduction in the number and the functional suppression of MDSCs in tumor-bearing hosts - have been proposed in clinics [10]. For example, MDSCs depletion with antibodies administered intravenously resulted in their reduction without affecting the number of neutrophils, monocytes, and other populations of myeloid and lymphoid cells. A transitory decrease in the elevated MDSC numbers was inversely correlated with the length of patients with advanced stages of cancer survival [11].

MDSC-targeted immunotherapy can be applied to many types of cancer, and it is not personalized. It is more powerful than other immunotherapies because it activates both the innate and adaptive immunity.

Immunology of pregnancy and cancer is similar [12]. A tumor is 'an embryo's evil mutant' and the mechanism of the mother's immune tolerance to a fetus during pregnancy is re-used in cancer. The single fertilized cell is able to grow into the fetus though it has half of the father's antigens. Even surrogate motherhood is possible without any relationship to the egg.

Oncofetal proteins activate in developing (fetal) as well as in growing cancer (onco) cells and induce maternal-fetal tolerance. Studying how oncofetal proteins drive placentation is essential to facilitate the process of providing better diagnostics for earlier screenings as well as treatment, ensuring the proper care for healthier babies and happier mothers [13]. On the other hand, early human placental development strongly resembles carcinogenesis in otherwise healthy tissues. It is critical to start thinking of oncofetal proteins in their roles as drivers of cancer cell proliferation, differentiation, invasion, survival, and especially, how cancer cells metastasize.

Oncofetal proteins: carcinoembryonic antigen (CEA) [14, 15], chorionic gonadotropin (hCG) [16-18], trophoblastic glycoprotein [19, 20], cancer antigen 125 (CA 125) [21, 22], CA19-9 [23, 24], prostate-specific antigen (PSA) [25, 26], and others are counteracting the immune attack.

Nevertheless, the impact of a dominant oncofetal serum protein α-fetoprotein (AFP) in embryo/fetus protection is outstanding. The 69 kDa single-chain glycoprotein appears in the early post-implantation. It belongs to the albuminoid gene family and is synthesized before albumin by the embryo yolk sac, fetal liver, and gastrointestinal tract. It is also synthesized by human hepatocellular carcinoma tumors. So, AFP has been utilized as a clinical pregnancy biomarker and fetal defects as well as for cancer growth [27, 28].

The AFP is considered an 'embryo albumin' because it serves as an albumin transport protein in adults. AFP's primary function is nutrients delivery to embryo cells as well as to the immune cells that suppress simultaneously both innate and adaptive immunity of the mother. Though the immunosuppressive AFP molecule directly limits the viability and functionality of human NK cells, monocytes, and dendritic cells (DCs) as reviewed in [29], the key mechanism of AFP-mediated immunosuppression in pregnancy, cancer, autoimmunity, etc., involves the binding of ligands which modulate immune reactions. AFP complexes with hormones, prostaglandins, polyunsaturated fatty acids (PUFAs), etc., impact the NK cells, monocytes and DC's phenotype, function, and metabolic pathways. These cells can absorb complexes by the neonatal Fc receptor (FcRn) which is a regulator of immunity [30, 31], and to which AFP has a higher than albumin binding affinity [32], or by a specific AFP receptor (AFPR) [27].

AFP is found in monomeric, dimeric, and multimeric forms and has multiple sites that bind different small molecules, such as zinc, copper, nickel, and heavy metals. Most molecules do not bind to the rat AFP (out of 125 molecules tested only 53 bind AFP) [33]. The serum carrier/transport function of mammalian AFPs was found to be more than 50 different biochemical known compounds with their individual dissociation constants [34].

Omega-3 PUFA docosahexaenoic acid (DHA) is the essential nutrient for embryo growth but is not synthesized by the mother who should take it with food during pregnancy. The rat AFP-PUFA affinity is more durable than those of albumin-PUFA by a factor of 54 [35]. Rat AFP binds 75% of DHA in the presence of x10 times excess albumin [36]. In pregnant women, albumin excess over AFP is much bigger (34,000 μg/ml and $<$ 0.2 μg/ml accordingly). Nevertheless, albumin cannot extract the PUFA from the AFP because of the almost-neutral pH in the blood.

With a half-life of 3-5 days, AFP needs only an hour to release a hydrophobic/amphiphilic ligand inside the cell compartment with an acidic pH [37]. Like other shuttle delivery proteins: hemoglobin, transferrin, IgG, and albumin [36]

AFP delivers 1-2 molecules, but numerous times. This way AFP transports dozens of DHAs and other molecules with MW <2 kDa through the placenta in a shuttle manner [38].

Similar to how oxygen binding changes the hemoglobin conformation, binding PUFAs changes the AFP conformation [39]. A circular dichroism experiment, or the change of the isoelectric point from 5.3 for free AFP to 4.7 for PUFAs bound AFP [27] indicates the difference in the AFP conformation. The conformation shift leads to the complex's higher binding affinity and sustainability [40, 41]. Metals can additionally stabilize AFP-ligand complex [42]. AFP has about 15 sites for zinc, however, zinc-binding does not lead to global changes in the AFP structure and stability [43].

Logically, not only an embryo, but other low differentiated cells (stem cells, HSCs, MDSCs, and cancer stem cells) deposited at the early embryo/fetal cells duplications rounds, should be able to secrete AFP and absorb AFP-nutrient complex back through the AFPR-mediated endocytosis. Embryonal and tumor tissues with a high level of proliferation use this mechanism. HSCs are not affected, as they are 'sleeping' in the bone marrow. T lymphocytes briefly express AFPR during blast-transformation [44]. Efficient routes of AFP uptake by multiple immune cells have been reported [45, 46]. Healthy, as well as malignant, mononuclear blood cells, and cancer cells use an autocrine AFP/AFPR nutrient delivery system [47-49].

AFP was unlikely to directly impact T cells, it may inhibit immune responses indirectly by activating regulatory suppressor cells [50]. AFP causes a selective down regulation of monocyte MHC class II molecules without altering other induced or noninduced monocyte markers or functions in monocytoid cell lines [51]. MDSCs are directly activated by AFP [52], and AFP influence MDSCs differentiation [53]. The unique 65-kDa AFPR discovered earlier [54] is widespread [55] but remains uncharacterized. AFPR was found on cells of the majority of cancers [27], which in some ways revert to the embryo state. Supposedly, when the cells transform into cancerous, they start to produce a certain type of glycan. This glycan attaches to AFPR silent in the membranes of normal cells enabling it to absorb the AFP/AFP-nutrient complexes [56].

The discovery of the AFPR on MDSCs was a breakthrough [57]. The AFPR is mostly absent in normal adult cells, except for MDSCs and cancer cells accumulated in TME. [125-I]-labeled AFP level can reach 6% of the inoculated amount per 1 g of tumor [58]. So, a lot of AFP-toxin conjugates were used as 'magic bullets' for cancer treatments [59]. AFP-toxins conjugates and non-covalent complexes kill MDSCs canceling immune suppression, as well as killing cancer cells directly [60].

Recombinant AFP-maytansine conjugate (molar ratio 1:5-7) lead to a statistically significant reduction in tumor volume in mice compared with control groups. All 10 mice in the AFP-maytansine arm survived (100%) with no obvious signs of bone marrow or other toxicity compared to no survivors in the control group [61], while 60 days of mice survival can be equal to 5 years of survival in humans.

The alternative to conjugate use of AFP in cancer treatment can be taught from embryo toxins. They can appear in the blood of pregnant women from food, drugs, or air, and damage embryo cells but are tolerated by the adult mother cells.

The toxin's binding affinity to AFP can correlate with embryo toxicity. Thus, unlike natural estrogens, AFP strongly binds the embryotoxic synthetic estrogen diethylstilbestrol (DES). A 3D computer model shows the DES docking in the AFP hydrophobic cavity [62]. AFP binds DES instead of DHA and crosses the placenta barrier. Currently, there are over 4194 known embryo toxins/teratogens, including 200 of the prescribed drugs [63]. A fluorescence-spectral method has shown AFP binding with warfarin and phenylbutazone [64]. Warfarin, Thalidomide, Accutane (isotretinoin), Lonidamine, and other drugs with a competitive albumins binding affinity to AFP can be combined with AFP to treat cancer.

The cancer drugs: cyclophosphamide, fluorouracil, doxorubicin, bleomycin, vincristine, and etoposide may be given safely to a woman in need during any trimester of pregnancy and do not hurt the child or the mother [65] that indicate their poor binding affinity to AFP. Testing the drugs for binding the AFP is easier than screening millions of potential cancer drug candidates. Computer modeling, taking into account the flexibility of the AFP [66], can help fit toxins into the AFP hydrophobic cavity.

AFP supports the mother's health to perform delivery successfully. The ability to support the immune system was used for the treatment of the autoimmune diseases. Purified AFP from abortive material was used as a registered drug [67] but was canceled later due to ethical reasons. Nevertheless, recombinant AFP has a bright future in immunopharmacology and clinical applications [27, 68, and 69]. The AFP is simple in design, biodegradable, no immunogenic, and has shown

an excellent safety profile at doses much higher than the AFP concentrations in serum during pregnancy [70, 71]. AFP has been proposed for example, as a novel therapeutic agent for inflammatory bowel disease [72].

The registered AFP and toxins/drugs data packages can shortcut clinical trials and accelerate the US FDA approvals.

Similar to embryo cells, a few cancers produce AFP which can bind the toxin in the blood and deliver it back to AFPR-positive cells. Daunomycin-DHA conjugate injected into mice with hepatoma cells producing AFP binds conjugate due to DHA, and the AFP-DHA-daunomycin complex inhibits tumor growth [73].

The amount of AFP in the blood is minuscule, and the majority of cancers do not produce AFP, and it should be delivered simultaneously or sequentially with the toxin. 75-300 μg (about 5 μg/kg!) of the registered drug AFP and amphotericin B in excess (1:60-100) were injected into cancer patients. Antifungal antibiotic amphotericin B is not a cancer drug. It was chosen as a compromise between its tolerability and binding affinity to AFP. The treatment was accompanied sometimes by immediate acute phase immune reaction that preceded cancer cells' death. AFP-amphotericin B infusions demonstrated response in 6 out of 8 cancer patients and an increase in quality of life [74].

The efficacy of the AFP complex can be raised with a potent toxin. Embryo toxins DES or dioxin are not recommended because they are mutagens and carcinogens. In the NCI 60 Cancer Cell Line screen, thapsigargin (TG) has a GI_{50} = 10^{-10} M, which compares favorably with agents such as paclitaxel (10^{-8} M) and doxorubicin (10^{-7} M) in this assay. 1 μM AFP-TG complex (1:2) has caused the death of 32% of MDSCs, compared to 5% in control *in vitro*; 0.15 mg/kg decreased MDSCs and tumor-associated macrophages numbers *in vivo* and lead to complete regression in 5 out of 6 of tumors in mice by day 7 of treatment with no further growth thereafter [71]. AFP-TG complex with 0.15 mg/kg doses has an advantage over 40-50 mg/kg of the AFP-maytansine conjugate at least in safety [61].

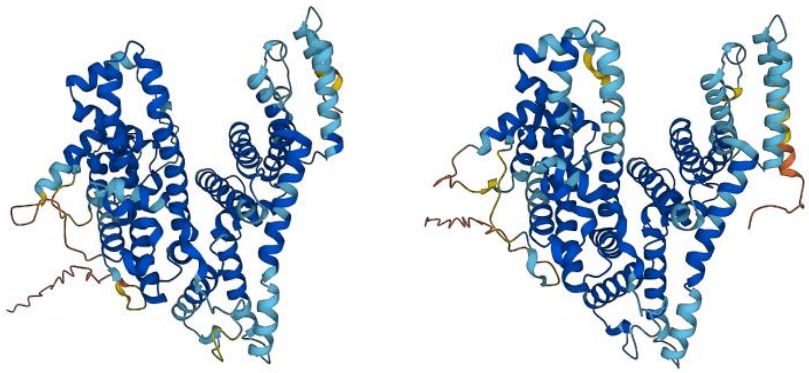
AFP noncovalent complexes with dioxin (1:2) [75], 1-S-1-acetoxychavicol acetate (1:1-5) [76], paclitaxel (1:4) [77], curcumin, genistein, and all-trans-retinoic acid [78], have shown anticancer effects in mice models. General efficacy and survival rate in the groups treated with AFP-toxin complexes are higher than in that treated with toxin alone.

Additional AFP-binding toxins in tolerable doses may be administered with AFP-potent toxin complexes (1:2) to elevate their anticancer effect. Utilizing its physiological delivery manner, AFP as an 'oncoshuttle' vehicle can bind and deliver more than the 1–5 drugs carried by AFP conjugates. This way, more toxins will be delivered with fewer AFP doses and fewer/no bystander effects [79].

"If one way be better than another, that you may be sure is nature's way."

— Aristotle

The dominant immunotherapy role in cancer treatments is supported by the experiments with the porcine AFP (pAFP)-toxin oral preparations. PAFP is close to but not identical to AFP with a high homology of the amino acid and 3D structure (Fig. 2), physicochemical, and similar immunologic properties.



A B

Figure 2. Human (A) and porcine AFPs (B) 3D structures [80].

PAFP has a pI = 4.6 and no micro heterogeneity [81]. Mono-type glycosylated pAFP is responsible for both immunosuppressive and nutrient delivery activity. This fact supports that it is the preferential ligand rather than the AFP molecule itself that exhibits the immunosuppressive activity in pigs and humans. PAFP is a better delivery protein than AFP as it has to deliver nutrients through six cell layers of porcine epitheliochorial placenta. AFP, by contrast, only has to cross three cell layers (trophoblast, embryonic connective tissue, and embryonic capillary endothelium) in the human hemochorial placenta. The AFP's ability to penetrate through polarized epithelial/endothelial cells, including those in the gastrointestinal tract, can be used for cancer treatments.

Oral porcine AFP with different toxins has unexpectedly demonstrated promising results in cancer treatments. The discovery that not only injectable but also oral pAFP-toxin complexes' have anti-tumor activity was a breakthrough [82]. Unlike the DES or dioxin, causing mutations in DNA, the toxins were selected from those targeted to mitochondrion, ER, or other cell organelles. On the one hand, that guarantees the inevitable apoptosis of the targeted cells, on the other - toxins 'encapsulation' by AFP and the treatment safety. Mice gavage with the pAFP complexes with atractyloside, thapsigargin, betulinic acid, rotenone, ajoene, isotretinoin, tocotrienol, and vitamin D3 have demonstrated good results in cancer treatments [79].

The suboptimal doses of the oral pAFP-atractyloside (1:2) complex were well-tolerated and produced major objective responses in 6 out of 12 metastatic colorectal cancer patients [83]. In the next trial, the ovarian cancer stage IV woman took twice-day capsules with the pAFP-atractyloside complex (3 mg/0.06 mg) suspended in oil and survived over 10 years [79]. Unlike during AFP-amphotericin B infusions, prolonged absorption of the capsules in the gut does not induce acute phase immune reactions.

In ancient Rome and Greece, women used an oral contraceptive called silphium - the lost ancient world's herbal birth control. Due to its value, the herb was depictured in a coin together with a crab which at that time was a cancer disease name (Fig. 3). Possibly silphium was used for cancer prevention/treatment too.

Figure 3. A coin of Magas of Cyrene c. 300–282/75 BC. Reverse: silphium and small crab symbols.

Wormwood (*Artemisia absinthium*) was also used in Roman times for birth control. Animal studies show it prevents *implantation of the early embryo* which indicates the activation of the mother's immune system. Supposedly, silphium and artemisinin can act through MDSCs similar to paclitaxel which in low non-cytotoxic concentrations significantly decreased the accumulation and immunosuppressive activities of MDSCs in primary skin tumors (an embryo's evil mutants) [84, 85].

Artemisinin, capsaicin, sinigrin, astaxanthin, resveratrol, quercetin, gossypol, and other moderate toxins from traditional medicines/spices have anti-cancer activity. It is possible, that they utilize host endogenous AFP, which level can be raised by the administration of AFP or pAFP. The pAFP-betulinic acid and pAFP-ajoene complexes demonstrated tumor-inhibiting activity and possibly can be permitted as supplements to prevent cancer/metastases at the early stages [86].

The mechanism of action of the oral pAFP-toxin complexes is not discovered yet. One of the hypotheses [79] is that they are absorbed like IgG-antigen and albumin-ligand complexes by the gut enterocytes through the FcRn [31, 32]. FcRn is

widely expressed by hematopoietic cells, including monocytes, neutrophils, intestinal macrophages, and DCs [87, 88]. The transcytosis of the complex through enterocytes can be similar to that one through placental syncytiotrophoblasts during pregnancy. Next, pAFP-toxin reaches the intestinal lymphatic system and kills FcRn-positive DCs and macrophages instead of AFPR-positive MDSCs, which are rare in the lymph nodes. AFP is known to induce DCs apoptosis [89], and AFP-toxin will do it for sure. The death of the regulatory immune cells is an alert for the immune system. Another assumption is apoptotic extracellular vesicles from the low differentiated cells mobilize HSCs in the bone marrow for *emergency erythropoiesis* to compensate for the loss of the regulatory immune cells. HSCs are able of enhancing the antitumor effect that is seen in several forms of immunotherapy. Redirecting differentiation of HSCs to immune cells promotes a survival benefit in tumor-bearing mice [90].

Conclusion

Oncofetal proteins counteract the immune attack during pregnancy and cancer. The AFP can deliver nutrients to the top immune regulatory monocytes and cancer cells. Monocytes/macrophages suppress both innate and adaptive immune responses and are the main targets in cancer treatment. Cancer cells, MDSCs and DCs absorb AFP-ligand through AFPR and/or FcRn. To cancel immune suppression in cancer, these cells can be killed by AFP-toxins preparations. AFP conjugates or complexes with toxins demonstrates a synergy of the MDSCs-targeted immunotherapy and the cancer cell-targeted chemotherapy that have shown promising results in animal models and in humans.

Alpha-fetoprotein binds toxins and can be used to treat cancer. It require low doses of AFP and toxins like embryo toxins/teratogens in pregnancy and promise to be effective and safe in cancer and metastasis prophylactics.

Oral AFP or pAFP-toxin complexes can treat cancer, though their immunotherapy mechanism of action is undiscovered.

Conflict of interest: The author has no conflicts of interest to declare.

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