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Review Article

Cancer Vaccine

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Abstract

Prevention is the most effective strategy for combating cancer, making it a priority over reactive treatments. The majority of cancer vaccines primarily stimulate T and B cells within the adaptive immune system. Nevertheless, like embryos, tumors originate from a single cell and often evade rejection by the innate immune response. This immune evasion is akin to mechanisms seen in pregnancy, where immune-suppressive monocytes play a crucial role in establishing tolerance. Oncofetal alpha-fetoprotein (AFP) facilitates nutrient delivery during pregnancy and in tumor growth via its receptor (AFPR). AFPR is also present in myeloid-derived suppressor cells (MDSCs), which are elevated in the tumor microenvironment. AFP and AFP-binding nutrients can skew the immune response toward tolerance. Conversely, depleting AFPR-positive monocytes can unleash natural killer (NK) cells, CD8+ T cells, and M1 macrophages—essential components for targeting cancer cells—thereby improving the lymphocyte-to-monocyte ratio (LMR), a recognized prognostic marker. Additionally, AFP-toxin conjugates represent a promising targeted chemotherapy approach and hold potential as vaccines for treating both primary and metastatic cancers.

Keywords: Cancer vaccine; Immunotherapy; MDSC; AFPR; PUFA; AFP-toxin conjugate; CSC; Targeted chemotherapy; LMR; Breast cancer

Introduction

The innate immune system serves as the first line of defense against cancer, while adaptive immunity, involving T and B cells, plays a critical role in later stages of tumor progression. Within the Tumor Microenvironment (TME), key innate immune cells such as myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, macrophages, dendritic cells (DCs), and neutrophils are key players [1]. These cells can either inhibit or promote tumor growth through the secretion of various cytokines and chemokines, significantly influencing tumor development. Targeting innate immune pathways offers the potential to reshape the TME, suppress tumor growth, and advance cancer immunotherapy [2].

MDSCs are particularly important as regulators of immune responses in both pregnancy and cancer [3]. A tumor can be conceptualized as a "mutant embryo" [4] suggesting that the immune mechanisms that prevent pregnancy could inform the design of effective cancer vaccines. Depleting MDSCs can activate both innate and adaptive immunity, improving the Lymphocyte-to-Monocyte Ratio (LMR), which is associated with favorable cancer outcomes [5].

Oncofetal Alpha-Fetoprotein (AFP) plays a dual role by delivering nutrients to AFP receptor (AFPR)-positive cells, including both embryonic and MDSC populations, thereby suppressing maternal immune responses during pregnancy and promoting tumor immune evasion [6]. AFP-toxin conjugates have the potential to deplete MDSCs while mobilizing the immune system to target cancer cells. Given that many cancer cells express AFPR [7], these conjugates can function as targeted immuno- and chemotherapies, offering effective treatment with minimal toxicity [8,9]. Furthermore, AFP-toxin conjugates may serve as vaccines to prevent the onset of early-stage cancers and spread of metastases.

Discussion

Given that one in two people in developed countries will be diagnosed with cancer in their lifetime, prioritizing cancer prevention is essential. An ideal vaccine would not only generate a specific response from the adaptive immune system but also address multiple tumor types by reversing innate immune suppression and activating adaptive immunity.

MDSCs are crucial in pregnancy and cancer

MDSCs play a crucial role in successful pregnancy by regulating maternal-fetal tolerance, facilitating implantation, and supporting fetal survival [10,11]. While MDSCs suppress the immune response to prevent embryo rejection, their depletion can enhance the cytotoxicity of decidual NK cells, potentially leading to embryo rejection [12]. The mechanisms of immune tolerance generated by MDSCs in both pregnancy and can-

cer are strikingly similar, suggesting that targeting these cells could help prevent cancer progression and metastasis.

MDSCs arise from hematopoietic stem cells and can differentiate into monocytic (M-MDSCs) and granulocytic (G-MDSCs or PMN-MDSCs) subtypes. These cells are highly conserved across mammals, maintaining similar regulatory programs and functions. Under normal physiological conditions, MDSCs account for less than 1% of peripheral blood mononuclear cells (PBMCs). However, they become prominent in conditions such as chronic inflammation, cancer, autoimmune diseases (AD), allergies, and infections, where normal myelopoiesis is disrupted, leading to increased MDSC production. Generated in the bone marrow, MDSCs represent a transitional stage in the differentiation of suppressive immune cells [13].

MDSCs contribute to cancer progression and metastasis through various mechanisms: they inhibit T-cell proliferation, impair NK cell function, induce regulatory T cell (Treg) formation, polarize macrophages to the M2 phenotype, create a tolerogenic environment, and promote angiogenesis [14-16]. MDSCs are a major obstacle to cancer immunotherapies and targeting them therapeutically could improve efficacy of immunotherapy [17].

As immature monocytes, MDSCs exhibit low antigen expression, complicating efforts to develop therapies that specifically target these cells without affecting others [18]. Two types of AFPRs have been described on human monocytes [19], with Dr. Belyaev notably discovering AFPRs on M-MDSCs [20]. He proposed that MDSCs are primary tumor-induced negative regulators of cancer immunity and explored potential methods for their elimination using AFP [21]. Research has shown that AFP can directly activate MDSCs [22] and influence their differentiation [23]. Furthermore, AFP-daunorubicin conjugate has been demonstrated to reduce MDSC numbers from over 1% to less than 1% [20].

Cancer stem cells

Remarkably, even though a mother and embryo are only partially related—or not at all in surrogate pregnancies—the maternal immune system tolerates the "alien" embryo for months, similar to how cancer cells can also evade immune detection and grow for years. This striking similarity underscores the parallels in immune responses between pregnancy and cancer [24].

Both embryos and cancer cells originate from a single cell, and like many tissues, tumors consist of functional cell populations, including stem cells, transit-amplifying cells, and mature cells. Cancer Stem Cells (CSCs) represent a subset of cancer cells with characteristics akin to normal stem cells, capable of generating all cell types within a tumor. CSCs are often responsible for metastasis and can suppress the immune response through various mechanisms, frequently involving myeloid cells [25,26]. Approximately 73% of current CSC surface markers are shared with embryonic or adult stem cells, and these markers are rarely expressed in normal tissue [27].

Targeting CSCs could potentially lead to complete cancer eradication [28]. Certain agents, for instance, polyene macrolides like nystatin and amphotericin B, have been shown to effectively target CSCs [29]. Thapsigargin (TG) has also been shown to selectively target highly resistant CSCs [30]. The effectiveness of these agents can be further enhanced through targeted delivery.

Moreover, CSCs may exploit the autocrine AFP-mediated nutrient supply typically utilized by embryonic cells. Notably, AFP has been shown to increase the cytotoxicity of agents such as dioxin by factors of 200 to 1400 [31], highlighting AFP potential role in cancer therapy.

AFP delivers ligands through AFPR-mediated endocytosis

AFP is produced and secreted during fetal development by liver hepatocytes, the visceral endoderm of the yolk sac, and, to a lesser extent, by the embryonic intestine and kidneys. AFP is used as both a pregnancy and tumor marker [32]. Often referred to as "embryo albumin," it performs a similar transport function during pregnancy and virtually disappears after birth. Placental cells can both secrete and absorb AFP via its receptor, AFPR [33]. Secreted AFP crosses the placenta to extract nutrients from the mother's bloodstream and then delivers them to the embryo. While AFP concentrations in maternal blood are typically below 200 ng/ml, albumin levels are much higher ranging from 35 to 50 mg/ml. Nevertheless, AFP surpasses albumin in its ability to bind certain nutrients, such as polyunsaturated fatty acids (PUFAs) [34]. AFP possesses a binding cavity capable of accommodating 1-2 molecules of PUFAs, as well as other compounds such as diethylstilbestrol [35] and dioxin [31]. Laboratory studies have identified saturated fatty acids, particularly palmitic acid (C16:0) and stearic acid (C18:0), as the primary fatty acids bound to AFP [36]. The transport function of AFP has been extensively documented in the literature [37-40].

AFP-mediated immunosuppression

The primary mechanism of AFP-mediated immunosuppression involves AFP-mediated transport of lipid ligands, which modulate cellular metabolism and are converted into signaling molecules [6]. This immuno-metabolic effect is particularly pronounced when AFP delivers PUFAs [41]. For instance, omega-3 docosahexaenoic acid (DHA) bound to AFP promotes anti-inflammatory effects, while omega-6 arachidonic acid contributes to pro-inflammatory responses. Tumor-derived AFP (tAFP) directly activates NK cells, leading to their subsequent apoptosis, and induces immune and metabolic dysfunction in monocyte-derived DCs [42,43].

Several published studies support the use of recombinant AFP for AD treatments [44,45]. Furthermore, the safety of such approach is strongly supported by the fact that AFP levels can be elevated in healthy individuals with no negative consequences [46].

The immune suppressive activity of MDSCs is stimulated when they endocytose AFP with its payload [47]. These cells are considered critical targets for treatment of cancers and metastasis [48]. Depleting MDSCs can unleash NK cells to target CSCs and metastatic cells effectively [49].

MDSCs in breast cancer

Breast cancer is the leading cause of cancer-related death among women worldwide. The application of cancer vaccines in breast cancer as monotherapy could not induce satisfying anti-tumor immunity [50]. There is a notable correlation between MDSCs levels and various factors such as inflammation, immune suppression, malnutrition, and poor prognosis in breast cancer patients. Specifically, patients with MDSC levels exceeding 1% of total PBMCs exhibit significantly shorter overall survival compared to those with MDSC levels below 1% [51] **(Figure 1)**.

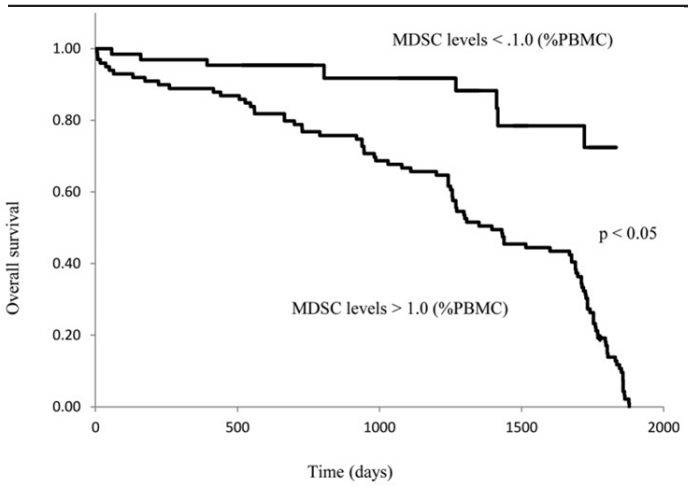

Figure 1: MDSC levels in patients with stage IV breast cancer and their overall survival. MDSC, myeloid-derived suppressor cell; PBMC, peripheral blood mononuclear cells (Adapted from [51]).

In murine models of triple-negative breast cancer, MDSCs have been shown to support tumor growth. For example, transplanting MDSCs from donor mice can effectively counteract the protective effects of calorie restriction against primary tumor growth, although this does not impact lung metastasis in the recipient animals [52]. MDSCs play a significant role in tumor progression in breast cancer and are emerging as an important therapeutic target [53]. Additionally, breast cancer cells express AFPRs, which can be targeted using AFP-toxin conjugates [54]. This suggests that AFP-toxin conjugates hold promise as a potential breast cancer vaccine.

AFP-toxin conjugates

Amphotericin B (4.2-7.0 mg) has been administered in combination with 0.075-0.15 mg AFP once every 3 days for one month and had produced a response in 6 out of 8 terminal patients with different cancers [55]. With a half-life of 4-5 days, AFP functions as a shuttle, delivering numerous amphotericin B molecules (half-life of 48 hours) to the AFPR-positive cells. Other drugs, such as paclitaxel [56] and warfarin [57], also bind to AFP and could potentially serve as targeted cancer therapeutics using AFP as a shuttle.

Covalent AFP-toxin conjugates are currently being explored as potential cancer therapies [37-40]. The AFP-maytansine conjugate, known as ACT-903, represents the most advanced development in this area, featuring a molar ratio of 1:5.96 [8, 9]. This conjugate offers several advantages over other MDSC-depleting strategies, as it not only targets MDSCs but also has dual targeting capabilities for a wide range of cancers that express AFPR. Covalent conjugates like ACT-903 are stable in the bloodstream, ensuring improved safety and prolonged efficacy. ACT-903 marks a significant advancement in cancer treatment strategies, as it simultaneously enhances the immune response by eliminating MDSCs in the TME, improves the LMR, and directly targets cancer cells [58]. This innovative approach functions not only as a cancer therapeutic but also as a potential cancer vaccine.

MDSCs are "what they eat"

MDSCs are sensitive to various agents [59, 60] and possess a double-edged nature. They can support a healthy state in contexts such as pregnancy, tissue regeneration, obesity, aging, and organ transplantation, but they can also impair immune responses in cancer and various bacterial and viral infections [61]. While MDSCs have protective roles in AD, allergies, and organ transplantation, they play a crucial negative role in the progression of many cancers [62].

Certain cancer cells and PMBCs are known to secrete AFP and reabsorb it with its payload through AFPR-mediated endocytosis, facilitating tumor growth [63-65]. When AFP with its PUFA payload is taken by MDSCs, it results in promotion of the expansion of these cells [66]. AFP acts as a growth factor beneficial to the fetus by promoting cell growth and proliferation; however, it has the same effect on the growth and progression of disease in cancer patients [67]. This dual role has led to AFP being described as a double-edged sword [68].

Active ingredients used in traditional medicine can bind to AFP and modulate the metabolism and activity of MDSCs, suggesting that AFP bound to the appropriate ligands could be utilized in cancer treatments. For example, Arshad et al. showed that binding 1'-S-1'-acetoxychavicol acetate to AFP significantly potentiates its anti-cancer activity [69]. Thapsigargin is another plant toxin being developed as an anti-cancer drug [70]. In vivo studies demonstrated that a dose of 0.15 mg/kg of the AFP-TG complex rapidly reduced MDSCs but not tumor-associated macrophages (TAMs), leading to a complete tumor regression in 5 out of 6 mice by day 7 of treatment [71]. Embryo toxins and teratogens that have high binding affinity to AFP can potentially be used for cancer therapy [72, 73]. E.g., Mifepristone, typically used to induce an abortion, regulates macrophage-mediated NK cells function in the decidua [74]. It is also being investigated for its potential as a breast cancer treatment [75]. There is growing interest in using mifepristone in combination with AFP as a universal cancer vaccine, with the aim of benefiting all cancer patients, not just women [76].

Oral cancer vaccine

It is reasonable to speculate that oral administration of AFP can be effective in modulating the immune system. The main advantage of such approach is that oral administration of AFP does not require high purity, and a complex of AFP with its ligand can be administered with a high margin of safety. Furthermore, it has been shown that gastrointestinal tract can facilitate the absorption of IgG-antigen complexes, albumin-ligand complexes [77], and likely AFP-ligand complexes. This would allow the AFP-toxin complex to pass through the gut and reach the intestinal lymph nodes, where it can deplete monocytes that endocytose AFP. Depleting these AFPR-positive monocytes would improve the LMR and enhance immune function both in the gut and systemically.

Studies with porcine AFP (pAFP) isolated from fetal pig serum, which shares amino acid and three-dimensional structural similarities with human AFP [78] shown that it can bind 2.6 moles of DHA and arachidonic acid (AA) (20:4, n-6) per mole of protein [79]. It has the capability to cross the epitheliochorial placenta [80] and may also traverse enterocyte line in the gut.

Oral administration of pAFP combined with AFP-binding toxins has been evaluated in xenograft models. In these studies, tumor-bearing mice were administered pAFP complexes (1:2) containing agents such as atractyloside (ATR), TG, rotenone, betulinic acid, ajoene, tocotrienol, cholecalciferol, and paclitaxel by gavage. Tumor inhibition was observed, along with extended survival in the mice [4, 81].

In clinical study, pAFP-ATR capsules (containing 0.3 mg of pAFP and 0.006 mg of ATR) administered twice daily for two months resulted in a 50% response rate among 12 metastatic colorectal cancer patients [82]. Notably, two patients with small metastases achieved complete responses, suggesting that this approach may function as a cancer vaccine. Anecdotally, a woman with stage IV ovarian cancer took capsules with 6 mg of pAFP and 0.12 mg of ATR daily for two months as a monotherapy and survived for over 10 years [4]. These results underscore the potential of AFP conjugates as both therapeutics and vaccines, though further research is necessary to fully explore their efficacy, particularly as oral formulations.

Conclusion

Activating innate immunity—the body's first line of defense—is crucial for preventing cancer progression and the development of metastasis. Monocytes play a significant role in the immune response, with MDSCs being the key players. In cancer patients, these AFPR-positive cells endocytose AFP and its associated nutrients, leading to immune suppression. In contrast, AFP-toxin conjugates—both covalent and non-covalent—have shown the potential to deplete MDSCs, improve the LMR, reverse immune suppression, and enhance the immune system's ability to combat various diseases, including cancer.

By combining MDSC targeting with the direct targeting of cancer cells, AFP-toxin conjugates hold promise as a curative strategy for many patients. An AFP-toxin conjugate vaccine could represent a paradigm shift in cancer therapy.

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