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Myeloid-derived suppressor cells (MDSC) as a main tumor induced negative regulators of cancer immunity and possible ways for their elimination

Extremely increased immunosuppressive activity of myeloid derived suppressor cells (MDSC), a heterogeneous population of early myeloid precursors, is one of the main reasons for failure of the immune system, which includes natural killer cells, macrophages, dendritic cells and cytotoxic T lymphocytes, to inhibit tumor growth on the late stage of its development. MDSC suppress antitumor immunity and as a result significantly decrease the positive effect of anticancer immunotherapy. In this regard development of new approaches to effective elimination of MDSC from organisms that bear a growing tumor is an urgent problem of modern cancer immunology. Utilization of alpha-fetoprotein (AFP) as a vector molecule composed of cytotoxic conjugate, which is specifically recognized by MDSC receptors, for the purpose of their elimination and hereby activation of antitumor immunity is proposed.
Keywords: cancer immunoediting, myeloid derived suppressor cells (MDSC), antitumor immunity, alpha-fetoprotein.

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Миелоидты супрессорлық жасушалары (МСЖ) ісіке қарсы иммунитеттің негізгі ісікиндуцирлік кері әсерлі реттегіші және олардың элиминациялану жолының мүмкіндігі

Бастапқы миелоиды негізін салушы гетерогенді популяция миелоиды супрессорлық жасушалардың (МСЖ) иммуносупрессорлық белсендігінің ерекше жоғарлауы ісіктің соңғы кезеңінде дамуын басатын табиғи киллер жасушалар, макрофактар, дендриттік жасушалар және цитотоксикалық Т-лимфоциттерді қамтитын, иммундық жүйенің жетіспеушілігің басты себептернің бірі. МСЖ ісікке қарсы иммунитетті супрессиялап, нәтижесінде қатерлі ісіктің иммунотерапиясының өң тимділігін едәуір төмендетеді. Осыған байланысты ісігі бар организімдерден МСЖ тимді элиминациялаудың жаңа жолдын жетілдіру, осы заманғы онкоиммунологияның өзекті тапсырмасы болып табылады. МСЖ-ды элиминациялау мақсатымен, оның арнайы рецепторын танитын, цитотоксикалық коньюгатты бар векторлық молекула ретінде альфа-фетопротеин (АФП) ді, ісікке қарсы иммунитетті активтендіру үшін қолдану ұсынылады.
Түйін сөздер: қатерлі ісіктің иммуноредактирленуі, миелоидтық супрессорлық жасушалар, ісікке қарсы иммунитет, альфа-фетопротеин

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Миелоидные супрессорные клетки (МСК), как основные опухолеиндуцированные негативные регуляторы противоопухолевого иммунитета, и возможные пути их элиминации

Чрезвычайно повышенная иммуносупрессорная активность миелоидных супрессорных (МСК) клеток, гетерогенной популяции ранних миелоидных предшественников, является одной из основных причин недостаточности иммунной системы, включающей натуральные киллерные клетки, макрофаги, дендритные клетки и цитотоксические Т-лимфоциты, ингибировать рост опухоли на поздней стадии ее развития. МСК супрессируют противоопухолевый иммунитет и, как результат, значительно снижается положительный эффект иммунотерпаии рака. В этой связи разработка новых подходов к эффективной элиминации МСК из опухоленесущего организма является актуальной задачей современной онкоиммунологии. Предлагается использовать альфа-фетопротеин (АФП) в качестве векторной молекулы в составе цитотоксического конъюгата, специфически распознаваемой рецепторами МСК, с целью их элиминации и, в конечном итоге, активации противоопухолевого иммунитета.
Ключевые слова*:* иммунноредактирование рака, миелоидные супрессорные клетки, противоопухолевый иммунитет, альфа-фетопротеин.

After 50 years existence of M.Bernet's hypothesis of immunological surveillance, cancer immunologists arrived at a conclusion that on the late stage of cancer development the immune system does not attack tumor cells but promotes their growth. In federated form novel view on the problem of relationships between tumor and immunity has been expressed in hypothesis of cancer immunoediting [1].

According to the hypothesis development of interaction between malignant tumor and immune system passes through three essential phases: elimination, equilibrium and escape. Elimination represents realization of immunological surveillance, when innate and adaptive immune systems, working together, recognize growing nascent transformed cells and destroy them without noticeable clinical manifestation. If a few resistant transformed cells survive the elimination phase proceeds to equilibrium phase, in which adaptive immune system prevents tumor growth, but simultaneously edits tumor, making it less immunogenic. Tumor goes into a dormant state and the equilibrium phase may occur over a period of many years. In the phase of escape cancer cells that have acquired the ability to circumvent immune recognition and destruction begin to rapidly proliferate, forming a visible tumor. Two related reasons are behind this event: loss of immunogenicity due to immunoediting and changes in the immune system in response to immunosuppressive activity of growing tumor and its microenvironment.

Exactly the immunosuppressive tumor microenvironment are represented by tumor-associated fibroblasts [2], suppressive endothelial cells [3], tumor-associated macrophages [4], tolerogenic dendritic cells [5], FoxP3+ Treg cells [6] and MDSC [7] provides functional anergy of cytolytic NK cells, CD8+ cytotoxic T cells and conversion of conventional CD4+ T helper cells into suppressive Treg cells. As a result uncontrolled tumor growth arises.

Among the cells involved in suppression of antitumor immunity, MDSC have a particular place because they obligatory accompany the development of malignant tumor both in experiments [8] and in clinics [9]. Historically, these cells have been appeared in the field of cancer immunology in the late 80's of last century under the name of "natural suppressor cells» (NS) [10] and have been described as myeloid progenitors lacking in linear differentiation markers, which showed suppressive activity against antitumor immunity.

Since 1998 a new stage in the study of myeloid suppressor cells started with publication of the work by V.Bronte and colleagues [11], which described the cell population inhibiting suppressive activity of effector CD8+ T cells with the properties of NS cells. This cells expressed granulocyte/monocyte markers, such as CD11b (Mac-1) and Lyt-6G (Gr-1). Over the next decade numerous studies on cells with such phenotype, named as "myeloid derived suppressor cells" (MDSC) [12], have emerged. To date several detailed reviews on the problem of MDSC have been published [8-9, 13]. Modern ideas about MDSC and their role in suppression of anti-tumor immunity are briefly described below.

MDSC in various murine tumor models usually express integrin CD11b and linear differentiation antigen "granulocyte receptor 1» (Gr-1), which are markers of granulocytic and monocytic lines respectively. There are two subpopulations of MDSC according to the presence of some additional markers and shape of nucleus: monocytic (M-MDSC) and granulocytic (G-MDSC). A number of data show that phenotype of these cells in tumor-bearing mice is not identical to immature myeloid cells of intact animals. After tumor eradication or destruction of myeloid cells with monoclonal antibodies to CD11b immune system restores and acquires lost antitumor activity.

Phenotype of human MDSC is not completely clear. These cells in human do not express linear markers of mature cells (CD3, CD19, CD56, CD14, HLA-DR), but express myeloid markers CD31, CD33, CD11b, and CD15. MDSC population with CD14+ HLA-DR-/low phenotype has been found in prostate cancer and melanoma. Obviously, different types of cancer can induce various MDSC subpopulations.

Apparently MDSC accumulation is a general and dominant process in immunosuppression observed in bone marrow, spleen, blood, liver of tumor-bearing animals and human blood at different types of cancer. Myeloid cells acquire supressive capacity under the influence of tumor factors, such as GM-CSF, M-CSF, IL-3, IL-10, TGFβ, VEGF, IL-6, IL-1β, and SCF. Production of these factors by tumor is always associated with a poor prognosis of disease.

Tumor-associated cytokines and chemokines can also control the differentiation of neutrophils into MDSC and enhance MDSC homing into tumor. MDSC express a number of chemokine and cytokine receptors, including CCR2, CXCR4, CXCR2, CD117 and VEGFR1. Among them CCR2 is considered as a marker of MDSC. MDSC have been shown to migrate into tumor in response to cellular damage, infection or

inflammatory mediators that belong to the family of S100 proteins binding calcium. These proteins mediate MDSC accumulation in the locus of tumor microenvironment by blocking differentiation of myeloid precursors into mature dendritic cells and hemoattraction of MDSC into tumor (NF-κB-dependent signaling pathway).

The main property of MDSC, isolated from tumors of animals, is the suppression of T and NK cell activation. The mechanism of this suppression varies depending on the subpopulation of MDSC.

Suppression of T cells as a considerable function of MDSC is associated with the production of peroxynitrite (ONOO-), which is generated by arginase (ARG1) and inducible NO-synthase (iNOS). iNOS induced by TNF□ and IFN□ converts L-arginine into L-citrolin following by production of NO, which in turn lead to formation of peroxynitrite. iNOS inhibits T-cell activation and proliferation by blocking of Jak 3 and STAT 5 activation, inhibits expression of MHC II genes and induces apoptosis.

ARG1 converts L-arginine into L-ornithine, a substance needed for formation of polyamines (putrescin, spermidine, spermine), involved in cell transformation and tumor cell proliferation. ARG1 induction in MDSC by Th2-type cytokines (IL-4 and IL-13) leads to disruption of CD3 expression, a component of T cell receptor. In addition, ARG1 in MDSC increases production of superoxide anion, which after interaction with NO forms piroxinitrit and hydrogen peroxides that cause suppression of T cell functions in cancer.

iNOS can be induced in myeloid cells by VEGF, GM-CSF and IL-6. Two mechanisms of participation of NO in suppressive activity of MDSC have been suggested. The first one is connected with synergic interaction of ARG1 and production of superoxide and NO with participation of iNOS. The second one is likely dependent only on iNOS and NO production, which directly inhibits CTL activity induced both by antigens and cytokines.

In addition to NO transforming growth factor beta (TGF□) is involved in suppressive activity of MDSC. Production of this well-known cytokine is usually induced by IL-13 which is secreted, presumably, by NKT cells, which, in turn, receive a signal for activation from antigen-presenting cells through a tumor antigen (glycolipid) associated with CD1b molecule. TGF□ directly inhibits antitumor activity of CTL.

In addition to their ability to directly suppress immune effector cells, MDSC act as tolerogenic antigen-presenting cells and induce activation of CD4+CD25+Foxp3+ Treg cells that represent another family of tumor-associated suppressor cells. This induction of Treg cells requires ARG1 production by MDSC and expression of CD80. Antigen-specific immunosuppression induced by G-MDSC requires participation of IFN□ but immunosuppression induced by M-MDSC depends on participation of NO. Tumor associated MDSC also inhibit other cells involved in the antitumor response such as dendritic cells and macrophages.

MDSC as one of the major factors of antitumor immunity suppression can limit positive effect of cancer immunotherapy. Thereby development of new approaches that would effectively eliminate *in vivo* the number and/or function of MDSC in tumor-bearing hosts are an urgent problem of cancer immunology [14].

These approaches include: (1) blocking production of factors secreted by tumor and its microenvironment, that attract and activate MDSC (for instance inhibition of GM-CSF production by vitamin D3); (2) elimination of MDSC with various cytotoxic agents (gemsitabin, 5-fluorouracil, docetaxel, doxorubicin, cyclophosphamide), (3) stimulation of MDSC maturation into mature dendritic cells, macrophages and granulocytes (all-trans-retinoic acid), (4) monitoring of immunosuppressive function of MDSC (nitroaspirin, inhibitors of phosphodiesterase 5), (5) monitoring of accumulation and suppressive function of MDSC (sunitinib, anti-CD117 (c-Kit)). However, none of these agents has selective effect on MDSC. Moreover, many of them testing *in vivo* on tumor-bearing mice showed antitumor effect only in combination with immunotherapy (immunization with tumor antigens, cell therapy with dendritic cells). Therefore a search for more specific tools that would allow eliminating MDSC without damaging effect on other cells of antitumor immunity is necessary.

We suggest using alfa-fetoprotein (AFP) as a vector molecule carrying cytostatic agent that can be specifically recognized by receptors to AFP on MDSC surface. Such approach has not been used by anybody regarding MDSC in the world. AFP is an oncofetal 68–72 kDa protein, found in high concentration in blood of patients with malignant tumor of liver, gonads and in embryonic carcinoma [15]. It is also produced during pregnancy where it plays an important role as an immunosuppressive molecule that blocks rejection of fetus by maternal immune system [16].

Immunosuppressive activity of AFP has been studied for more than 30 years [17]. It has been established that effects of AFP are mediated through binding with numerous cell receptors that have been recently reviewed by G.J. Mizejewski [18]. AFP has been shown to internalize into cytoplasm of tumor cells, as well as fetal and placental cells by clatrin-dependent endocytosis. Currently about 30 AFP-binding proteins have been identified, which belong to the family of scavenger receptors, including several classes of integral transmembrane proteins with a molecular mass of 18-250 kDa. Obviously, these proteins are required for the transport function of AFP, which, like serum albumin, binds and transports multiple ligands into cells, including bilirubin, fatty acids, retinoids, steroids, heavy metals, flavonoids, phytoestrogens, dioxins, and various organic drugs.

In our laboratory it has been shown that AFP is a specific inductor of NS cells that are identical to MDSC [19-22]. Three populations of these cells expressed 13 AFP-binding proteins with molecular mass of 18-305 kDa but only 4 of them bound serum albumin. 7 specific AFP-binding membrane and cytoplasmic proteins with molecular masses of 42, 50, 65, 67, 74, 147 and 182 kDa had high constant of association (10^{10}-10^{11} M^{-1}).

Thus MDSC are an attractive cell target for pharmacological impact on anticancer immunity at cancer treatment, and the use of cytotoxic AFP conjugates is a new promising approach to realization of this urgent problem.

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