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Dynamic changes in the tumor microenvironment under the effect of estradiol as a diagnostic tool and target for targeted cancer therapy

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ABSTRACT

Activation of the estrogen receptor-α (ER-α) signaling pathway is a significant factor in the initiation of carcinogenesis in various types of tumors due to the genomic and non-genomic effects of estradiol in cancer cells. However, data on the expression of ER-α and aromatase on stromal and immune cells in the tumor microenvironment (TME) point to an additional mechanism by which estrogens increase tumor malignancy. There is growing evidence that TME can affect tumor immunity by increasing the immune response or reducing immunoreactivity.

The important role of estrogen and the estrogen receptor signaling pathway in the response of the tumor microenvironment in cancer of various localizations, not only classical hormone-dependent cancers, has been proven. However, the clinical effectiveness of blocking the effect of estrogen on tumor growth has been primarily shown in cancer of the female reproductive system. At the same time, data on the significant role of TME in the development of endocrinotherapy resistance in breast cancer treatment are of great interest.

Despite the possibilities of standard therapy, a more in-depth study on the role of various TME components in cancer evolution, creation of a micrometastatic niche, as well as in the response to therapy may result in development of new strategies for cancer treatment. It is also necessary to study the possibilities of overcoming the immunosuppressive effect of the estrogen receptor signaling pathway on TME in order to increase the survival rates in patients with hormone-dependent cancers, particularly, breast cancer.

Keywords: estrogen receptor expression, tumor microenvironment, review, tumor-associated fibroblasts, T-lymphocytes, tumor-associated macrophages

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Динамические изменения опухолевого микроокружения под влиянием эстрадиола как диагностический критерий и мишень лекарственной терапии рака

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РЕЗЮМЕ

Активация сигнального пути эстрогенового рецептора-альфа (ЭР-α) является значимым фактором в инициации канцерогенеза при различных типах опухолей ввиду геномных и негеномных эффектов эстрадиола в опухолевых клетках. Тем не менее данные об экспрессии ЭР-α и ароматазы на стромальных и иммунных клетках в микроокружении опухоли (МО) говорят о дополнительном механизме, с помощью которого эстрогены повышают злокачественность опухоли. Появляется все больше доказательств того, что МО способно влиять на опухолевый иммунитет, повышая иммунный ответ или снижая иммунореактивность.

Доказано немаловажное значение роли эстрогена и ЭР-сигнального пути в реакции микроокружения опухоли при раке различных локализаций, не только классических гормонально-зависимых опухолей. Однако клиническая эффективность блокирования влияния эстрогена на рост опухолевых клеток доказана в основном при раке женской репродуктивной системы. При этом весьма интересны данные о значимой роли микроокружения опухоли в развитии резистентности к эндокринотерапии рака молочной железы.

Несмотря на возможности стандартной терапии, более углубленное изучение роли различных компонентов МО в эволюции опухоли, создании ниши микрометастазов, а также в ответе на терапию может привести к появлению новых стратегий лечения рака. Также необходимо изучить возможности преодоления иммуносупрессивного влияния сигнального пути рецептора эстрогена на МО с целью увеличения показателей выживаемости больных гормонально-зависимыми опухолями, в частности раком молочной железы.

Ключевые слова: экспрессия рецептора эстрогена, микроокружение опухоли, опухоль-ассоциированные фибробласты, Т-лимфоциты, опухоль-ассоциированные макрофаги

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INTRODUCTION

Estrogen receptor-α (ER-α) is a transcription factor regulating cell proliferation, migration, and survival. In the mammary gland, ER-α plays a key role in tumor growth, being activated by 17β-estradiol (E2). The estrogen receptor signaling pathway is based on dimerization of ER located in the nucleus in response to steroid hormone binding. Dimerized nuclear ERs bind to estrogen-sensitive elements (ESE) in the promoter regions of target genes, regulating their transcription [1].

In classical hormone-dependent tumors, ER expression as a prognostic and predictive marker is currently evaluated only on tumor cells. Therefore, the efficacy of blocking the ER-dependent signaling mechanism is analyzed from the standpoint of reducing the proliferative activity of malignant cells. So, endocrine therapy is the most effective treatment method for ER-positive breast cancer (BC). However, its effectiveness is limited by primary and acquired tumor resistance. Undoubtedly, resistance may be associated with ER expression loss, which is observed in 15–20% of patients with disease progression. However, ER mutations and amplifications are considered to be rare in the primary tumor and are detected only in 0.5% and 2.6% of luminal breast cancers, respectively. However, in metastatic foci, ER mutations in the ligand-binding domain of the receptor were noted in about 20% of cases [2].

It was found that endocrine therapy resistance can also provoke dynamic changes in the tumor microenvironment (TME). A lot of data have been accumulated on the significant role of TME components in treatment response, resistance, and tumor progression. Interactions between cancer-associated fibroblasts (CAFs), adipocytes, immune cells, endothelial cells, pericytes, extracellular matrix (ECM), and soluble factors lead to tumor evolution and disease progression. Therefore, the role of TME in cancer progression, as well as in a response of the tumor stroma to drug therapy in various tumors is intensively studied [3–6].

Resistance mechanisms independent of tumor cells have been studied in murine models of breast cancer. It was found that estradiol promotes the growth of tumor cells without ER-α expression due to the activation of stromal estrogen receptors [7].

TUMOR STROMAL COMPONENTS AS DIRECT PARTICIPANTS OF THE ER-SIGNALING MECHANISM MODULATION

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are the most numerous cells in the tumor stroma. They act as a paracrine source of chemokines, soluble factors for cell activation of signaling pathways involved in cancer cell survival, invasiveness, and metastasis [8]. In breast cancer, ER expression was detected on CAFs, and in response to estradiol, they secrete growth and angiogenesis factors, as well as immunoregulatory and proinvasive soluble factors. It was found that soluble factors from CAF conditioned medium induce tamoxifen resistance in murine models of ER-positive cancers. This may be due to CAF secretion of growth factors, proteases, and the b1 integrin signaling pathway activation in response to endocrine therapy [9].

Despite similar ER expression level on normal fibroblasts and CAF, E2-sensitive genes and LRH-1 (liver receptor homolog-1) are hyperactive in CAF [8]. *LRH-1* is a target gene for E2 and a transcriptional regulator of the aromatase gene (*CYP19A1*) [10]. Aromatase is co-expressed on breast cancer cells with LRH-1, which indicates the paracrine mechanism of E2 synthesis and the role of TME in estrogen-mediated carcinogenesis in breast cancer. Endometrial CAFs also express ER and can promote tumor proliferation when co-cultured with human endometrial cancer cells. They induce *in vitro* tumor cell proliferation partly by activating PI3K and MAPK signaling pathways, which are regulated by an ER-dependent signal in breast cancer and lung cancer [1].

When studying human CAF co-cultured with MCF-7 breast cancer cell lines, two CAF subpopulations were identified based on different CD146 expression in breast cancers. CAFs without CD146 expression inhibit the ER expression on MCF-7 cells, reduce their sensitivity to estrogen, and increase tamoxifen resistance. The presence of CD146+ CAF stimulated ER expression and supported estrogen-dependent proliferation and sensitivity to tamoxifen. Conditioned medium of

CD146+ CAF restored sensitivity to tamoxifen in breast cancer cells which were resistant to it. Gene expression profile of breast cancer patients with CD146− CAF correlated with a decrease in the frequency of a clinical response and a worse disease prognosis [11].

M.M. Morgan et al. showed an increase in ER signaling activation in the presence of 17β-estradiol, while co-culturing MCF-7 cells with human breast fibroblasts in a 3-dimensional model. The addition of fibroblasts increased proliferation rate and caused estrogen-induced hyperplasia, which was explained by the inhibition of apoptosis during co-culture [12]. According to other data, dermal fibroblasts and bone marrow mesenchymal stem cells also affected ER-signaling pathway regulation in MCF-7 and T47D cells [10, 13]. In the first case, the authors showed that CAF induce resistance to tamoxifen by increased mitochondrial activity in breast cancer cells. In the second case, the paracrine stromal signaling mechanism led to a decrease in the activity of the ER signaling pathway in MCF-7 and T47D cells. The transfer of exosomes from stromal cells to breast cancer cells is the mechanism of resistance to endocrine therapy. The transfer of microvesicles containing OncomiR-221 microRNA from CAF to a breast cancer cell induces expansion of cancer stem cells (CSCs) with an increased self-renewal ability and resistance to endocrine therapy. It was previously shown that tamoxifen led to an increase in the number of CSCs in breast cancer in murine and human models of breast cancer and in the patient's primary tumor.

The question of different origin of the luminal and basal-like types of breast cancer remains important. A high degree of plasticity between these types of tumors is assumed. The transformation of luminal or basal-like cancers into each other under the influence of the microenvironment was demonstrated *in vitro*, which indicates the relationship with the progenitor cell [14]. PDGF receptors and their ligands are important factors that modulate the molecular type of tumor and the response to antiestrogenic therapy. It was established that platelet-derived growth factor (PDGF-CC) is an independent prognostic marker of poor survival in breast cancer.

Using xenografts of triple-negative breast cancer of MDA-MB-231 cell lines orthotopically inoculated into an immunodeficient mouse, an increase in ER-α expression was shown with a decrease in activity or inhibition with a response to tamoxifen. CAFs of all analyzed tumors expressed PDGFR-α and PDGFR-β, which indicates the paracrine type of PDGF-CC signaling mechanism from the epithelium to the stroma. In response to the activation of the PDGF-CC signaling cascade, CAFs secrete HGF, IGFBP3, and STC1 molecules that induce the formation of the luminal breast cancer phenotype [15, 16]. Thus, CAFs act as determinants of the molecular subtype of breast cancer and represent a promising target for therapy that modulates the tumor epithelial component [15].

Myeloid-derived stromal cells

Myeloid-derived stromal cells (MDSCs) are an important component of TME, affecting immune tolerance and promoting tumor development [17]. ER-α expression was also detected on tumor MDSCs, as well as in the bone marrow and peripheral blood in ovarian cancer. In a murine model of E2-dependent ovarian cancer, ovariectomy led to an increase in overall survival. At the same time, E2 caused tumor progression and reduced the effect of ovariectomy. This effect was observed only with normal immune status. However, the immunodeficient mouse did not benefit from ovariectomy due to the absence of T cell infiltration of the tumor.

Thus, it was found that the antitumor effect eliminating the influence of E2 is realized by modulating acquired immunity. When E2 was prescribed, T helper cells and cytotoxic T lymphocytes decreased, but the number of MDSCs in the spleen and tumor niche increased. At the same time, the immunosuppressive activity of granulocytic MDSCs was increased. In a murine model of ovarian cancer, exposure to E2 in the peritoneal cavity increased activation of signal transducer and activator of transcription 3 (STAT3), which is a signaling mechanism that regulates myeloid cell differentiation and development, by transcriptional regulation of JAK2 and SRC. Similar data were obtained in murine models of lung cancer and breast cancer. Under E2 stimulation, tumor growth stopped when MDSCs were affected by antibodies to Gr1 [18].

Extracellular matrix

Extracellular matrix (ECM) is an important component of the tumor tissue, which plays an essential role in tumor progression and treatment resistance in various cancers, including breast cancer. It was established that the tumor is a non-healing wound, since the host microenvironment receives signals for tissue repair by formation of fibrous tissue. Breast cancer progression is accompanied by stromal changes and the appearance of stiffness. The basement membrane of a normal mammary gland clearly separates the epithelial component from the stroma. Laminin, type IV collagen, fibronectin, and entactin are the main components of the basement membrane which are produced by the epithelium, endothelium, and stromal cells. Interstitial ECM consists of collagen fibrils, fibronectin, glycoproteins, and proteoglycans [19].

The biochemical characteristics of ECM make it possible to modulate the cellular response to various soluble factors, such as hormones, polypeptide growth factors, and chemokines. Malignant breast tissue becomes stiff, which is due to a change in the biochemical properties of ECM. ECM remodeling involves continuous synthesis of matrix proteins, their connection, interaction, and cleavage by proteases. This leads to an increase in ECM stiffness, which is a consequence of increased collagen deposition following lysyl oxidase (LOX) expression and parallel orientation of collagen fibers [1]. The reorganization of collagen into thin, linearly oriented fibers correlates with tumor progression and the clinical outcome, and increased ECM stiffness may also be the cause of certain cancer types [20].

M.P. Jansen et al. showed an association of the ECM gene cluster with ER-positive breast cancer progression in patients taking tamoxifen. The authors analyzed 112 ER-positive locally advanced tumors in patients with breast cancer and identified types of responses to tamoxifen therapy. Differences in the expression of 91 genes between tumors sensitive and resistant to tamoxifen were noted. Overexpression of the ECM genes *TIMP3, FN1* (fibronectin 1), *LOX, COL1A1* (collagen type 1 alpha 1 chain), *SPARC,* and *TNC* (tenascin C) was associated with progression of the disease in all cases [21].

Another study focused on these 6 genes and examined 1,286 tumor samples. The level of mRNA expression was associated with disease progression. The results showed that high expression of FN1, LOX, and SPARC was associated with low metastasis-free survival rates in patients who received adjuvant systemic therapy [22]. Studies have shown that fibronectin is associated with breast cancer progression. In breast cancer cell cultures, fibronectin led to endocrine therapy resistance via binding to beta-1 integrin. It was found that upon contact with fibronectin, ER-α activity does not decrease after 1 hour of exposure to estradiol. Estradiol induces endocytosis in breast cancer cells, and the ER-α of the cell membrane in the form of endosomes is directed to the nucleus. Pharmacological or biological endocytosis inhibition led to ER transcriptional activity inhibition.

Therefore, in the presence of fibronectin, ER-α undergoes endocytosis and is brought back to the cell surface by beta-1 integrin. So, ER-α is not co-localized with the lysosomal component, so it is obvious that the interaction of beta-1 integrin with fibronectin determines the fate of ER and the response to tamoxifen. Thus, ECM directly regulates the effect of the estrogen signaling mechanism on breast cancer cells [23].

INFLUENCE OF THE ER SIGNALING MECHANISM ON THE INFLAMMATORY RESPONSE AND TUMOR IMMUNE MICROENVIRONMENT

Numerous studies have confirmed the significant role of chronic inflammation in tumor progression. TME produces cytokines that activate protumorigenic proliferation pathways, leading to immune evasion and metastasis. The proinflammatory cytokine interleukin (IL)-6 increases growth and invasiveness of ER-positive breast cancer. Local CAFs act as paracrine sources of IL-6, activating STAT3 and ER-positive tumor cell proliferation *in vitro* and *in vivo* [24].

Tumor necrosis factor (TNF) regulates the expression of genes associated with the metastatic phenotype of ER-positive breast cancer and increases aromatase expression in cultured human adipose-derived stromal cells. An association between aromatase transcription and TNF and IL-6

cytokines in breast cancer was also found. A similar correlation was observed between aromatase and cyclooxygenase-2 (COX-2). COX-2 affects prostaglandin E2 (PGE2) synthesis, which causes an increase in aromatase transcription by elevating the concentration of cyclic adenosine monophosphate (cAMP) in breast cancer [25].

Significant correlations were found between the expression of ER, TNF, and NF-kB in breast cancer. The NF-kB signaling pathway is involved in the initiation of tumor growth and inflammation. Activation of NF-kB is observed in some types of cancer and is associated with the profile of IL-6 and TNF cytokines. Binding of DNA to NF-kB and activator protein-1 is associated with resistance to antiestrogens in ER-positive breast cancer cell lines and tissue samples [26]. Moreover, exposure to E2 in a murine model of tobacco-induced lung cancer increased the inflammatory response through increased activation of the NF-kB signaling pathway and expression of VEGF and IL-17A. A combination of aromatase inhibitor and NSAIDs prevented lung carcinogenesis in mice, reducing the activity of STAT3 and MAPK signaling pathways, the level of circulating IL-6, and expression of IL-17A. Thus, a relationship between the E2 signaling pathway and regulators of tumorigenic inflammation is obvious. This opens up promising strategies for targeted cancer therapy through additional E2 signal inhibition [27].

Involution of breast tissue after pregnancy and obesity are serious risk factors associated with inflammatory TME and breast carcinogenesis. After pregnancy and subsequent involution of the mammary glands after lactation suppression, the risk of developing cancer increases within 10 years. A high risk of breast cancer and a poor prognosis of this cancer type are associated with inflammatory mediators involved in the involution process. Nevertheless, ER expression status is not discussed at this point. Some studies have found low incidence of ER-positive tumors in this cancer type. Other data indicate a decrease in the estrogen and progesterone receptors due to a high estrogen level [28].

Postmenopausal period and obesity are associated with an increased risk of developing ER-positive breast cancer, endometrial cancer, and tamoxifen resistance. As a result, the risk of relapse increases during endocrine therapy [29]. This can be explained by the fact that a characteristic feature of inflammation is recruitment of macrophages to the adipose tissue. Adipocytes and macrophages trigger activation of the proinflammatory transcription factor NF-kB. The degree of macrophage infiltration is also associated with the development of resistance to tamoxifen. Research data suggest that tumor-associated macrophages (TAMs) protect cancer cells from an antitumor immune response [30].

Tumor-associated macrophages

Macrophages isolated from humans and mice are able to suppress the T cell response *in vitro*, and removal of macrophages leads to an increase in the number of CD8+ T cells in a breast cancer model when exposed to chemotherapy. It was shown that circulating M2-like monocytes were elevated in this population in comparison with healthy volunteers and patients with benign lesions. Another study revealed an association between CD204 expression on TAM and clinical and pathohistological characteristics in patients with invasive breast cancer [1, 31]. In a study involving 108 patients with luminal breast cancer subtypes, high CD204 expression was associated with a decrease in relapse-free survival and long-term event-free survival [32].

The immune response involving macrophages is tissue-specific and depends on local TME polarization by various cytokines. Polarized M1 macrophages produce proinflammatory cytokines, including interferon (IFN), IL-12, and TNF to trigger the tumor immune response and antigen presentation. M2-macrophages produce type 2 cytokines – IL-4, IL-5, IL-6, and IL-10, which promote tumor growth and cause immune evasion. TAMs are represented by the M2 phenotype, being a promising target for drug therapy. TAM infiltration is observed in a large number of various cancer types and is associated with a poor prognosis [1, 33]. In ER-positive cancer, premenopausal patients show an increase in the number of TAMs compared with postmenopausal women. While TAM infiltration was associated with a poor prognosis in ER-positive and ER-negative breast cancer, an increase in TAMs with their proliferation is more often detected in hormone receptor-negative tumors [34]. However, M1 polarization was not observed in these studies, as opposed to M2 polarization. In addition, the immunohistochemical analysis of

breast cancer samples showed aromatase expression in TAM, indicating local production of E2 in TME and increased proliferation of cancer cells in ER-positive tumors.

There is evidence that E2 is able to induce M2 polarization and TAM infiltration. In a murine model of ER-positive cancer, E2 increased infiltration of TAMs with the M1 phenotype, and in the control group, infiltration of CAFs with the M1 phenotype was shown. E2 also increased secretion of VEGF by M2-polarized TAMs, VEGFR expression, and the content of macrophages in murine lungs during tobacco-associated carcinogenesis [35–37].

A study of the tumor growth in high-grade serous ovarian carcinoma induced by E in a murine model found that E2 not only enhanced tumor growth, but also increased M2 CAF infiltration compared with an untreated ovariectomized mouse [33]. It is known that endometrial M2-polarized TAMs affect ER activation through epigenetic regulation and IL-17A secretion, increasing E2-associated proliferation of endometrial cancer cells [38].

Thus, a potential positive feedback between the ER-signaling pathway and M2 CAF infiltration has been identified in certain cancer types. These connections may become a therapeutic target. Recent studies on lung cancer xerographs confirmed an effect of the phytoestrogen resveratrol similar to that of selective estrogen receptor modulators (SERMs). Resveratrol reduces tumor growth by inhibiting M2 TAM polarization and decreases STAT3 signaling pathway activation [39].

Tumor-infiltrating CD4+/CD8+ T lymphocytes and natural killer cells

The composition of TME lymphocytes varies significantly depending on the type of cancer. At the same time, they promote tumor progression or activate antitumor immunity, depending on the primary tumor. The CD4+ T cell polarization is one of the evading immune mechanisms. Th1-mediated cellular response is associated with tumor suppression and hyperactivation of INF and IL-12. The Th2-associated cellular response is based on IL-4 production and implements a protumorigenic effect. Studies have noted that increased E2 induces Th2-type response and increases IL-4 production. An increase in the infiltration of ER-negative breast cancer by immune Th1 cells, B cells, and cytotoxic T lymphocytes was revealed in comparison with ER-positive tumors.

Thus, a negative relationship was noted between the ER-α signaling pathway and immune infiltration. An increased number of tumor-infiltrating lymphocytes, in particular CD8+ T cells, significantly increases overall survival of patients with ER-negative tumors [40]. Analysis of gene expression in ER-positive cancers showed that treatment with the aromatase inhibitor letrozole increased tumor infiltration with B cells and T helper cells both at early and late stages of treatment [41].

Granule exocytosis is one of the ways to initiate apoptosis by cytotoxic T lymphocytes and natural killer (NK) cells to fight pathogens and tumor cells [25]. CD8+ cytotoxic T cells play an important role as effectors in acquired immunity. Cells expressing a foreign antigen in association with the main histocompatibility complex (MCH I) are recognized by cytotoxic T lymphocytes through a specific interaction between the T cell receptor and the presented antigen. This interaction causes the activated T cells to release the proteins perforin and granzyme B, resulting in lysis of the cell membrane and cell death. These mechanisms may affect malignant cells due to atypical antigen presentation.

X. Jiang et al. co-cultured ER-positive hepatocellular carcinoma cells with E2, and the expression of granzyme B inhibitor, proteinase-9 inhibitor (PI-9), increased. This mechanism protected cells against NK-related and cytotoxic apoptosis [42]. E2-induced expression of PI-9 was also detected in the culture of ER-positive MCF7 cells, which decreased the influence of NK-cells. Elimination of PI-9 blocking reduced the protective effect of E2 from NK-mediated apoptosis. Thus, E2 enhanced immunosuppression through inhibition of NK and cell death mediated by cytotoxic T lymphocytes [43].

Analysis of 12,439 tumor samples, of which 8,775 were ER-positive, showed that intratumoral CD8+ T cells were associated with a 27% decrease in the risk of death from breast cancer [44]. The analysis of PD-L1 expression revealed that 20% of ER-positive tumors show positive marker expression in comparison with 58% of patients with triple-negative breast cancer phenotype [45, 46].

Regulatory T lymphocytes

Activation of T cells and their differentiation are mandatory in acquired immunity. Regulatory (T-reg) T cells expressing FoxP3 are involved in suppressing the antitumor immune response by secreting immunosuppressive cytokines and inhibiting T cell expansion [47]. Physiological doses of E2 increased the amount of CD4+CD25+ T-regs and Foxp3-expression in many tissues of immunodeficient mice after ovariectomy. At the same time, ER-positive CD4+CD25-negative cells acquired the ability to express CD25 after exposure to E2. The CD4+CD25+ T cells transformed by estradiol acquired the immunosuppressive T regulatory lymphocyte phenotype and inhibited proliferation of T cells *in vitro*. It was also found that Foxp3 expression by murine estradiol-stimulated T-regs was critically important for their functioning. And an increase in the number of FoxP3+ T-regs in patients was a predictor of a poor prognosis in various types of cancer [25, 48].

The results of a meta-analysis indicate that FoxP3+ T-reg infiltration significantly correlates not only with poor overall survival in ER-positive breast cancer, but also with higher survival rates in ER-negative breast cancer patients [49]. When prescribing letrozole to patients with ER-positive breast cancer, a significant decrease in FoxP3+ T-regs was shown after therapy [1]. E2 also stimulates *in vitro* expression of the programmed death ligand (PD-L1) on ER-positive endometrial cells and breast cancer cells through PI3K signaling pathway activation. The interaction between cells with PD-L1 expression and PD-1positive T cells inhibits recruitment of cytotoxic T cells, which leads to immune evasion. Data on E2 hyperactivation of both PD-L1 and PD-1 suggest a critical impact of the E2 signaling pathway on the PD-1 / PD-L1 signaling mechanism [50, 51].

Targeting the ER-signaling pathway in tumor microenvironment as a way to increase tumor immunoreactivity

Despite the fact that immunotherapy is an effective treatment strategy for cancer, often the immunosuppressive microenvironment reduces its possibilities. Immune checkpoint inhibitors (CTLA4) and PD-1 / PD-L1 are currently the most discussed drugs. However, the response rate to treatment remains about 20–35% with varying response duration depending on the stage, tumor type, and PD-L1 expression [25, 52]. In addition, during treatment, resistance to therapy and disease progression may occur [31, 32]. It was found that impaired DNA repair mechanisms and an increase in somatic mutation load and neoantigen presentation correlate with tumor heterogeneity and better clinical outcomes [1, 53]. Mechanisms leading to immune evasion include impaired ability to antigen presentation and decreased neoantigen presentation by MHC-1 [54, 55].

It should be noted that the described mechanisms can serve as potential predictors of a response to treatment with immune checkpoint inhibitors and a target for increasing the effectiveness of breast cancer therapy. Despite the data on the clinical effectiveness of anti-PD-1 / PD-L1 therapy for metastatic triple-negative breast cancer, it was found that the objective response frequency does not exceed 12%, and the clinical response duration of more than 24 weeks is observed in 20% of cases [56, 57]. The immune-suppressive TME may be the biological explanation for such disappointing results.

When analyzing 61 ER-positive cancer samples of primary breast cancer, C.A. Egelston et al. found that tumor-infiltrating CD8+T lymphocytes have a weakened ability to produce effector cytokines and degranulation capacity, despite PD-1 expression. In addition, the ability of CD8+ T lymphocytes treated with CD3:CD19 bispecific antibodies to influence breast cancer cells as effectively as peripheral blood mononuclear cells was shown [58].

In studies on cell lines, ER-α acts as a negative transcription regulator of the *PD-L1* gene. Moreover, TCGA data in the breast cancer sample analysis showed that the PD-L1 mRNA level in ER-positive tumors was significantly lower than in ER-negative tumors [59]. There are also data indicating a positive effect of blocking the ER-signaling mechanism on the increase in TME immunoreactivity. For example, in lung cancer, the antiestrogen fulvestrant increases tumor sensitivity to immune-mediated lysis [60]. Fulvestrant is an ideal candidate for combined use with anti-PD-1 / PD-L1 agents due to its proven safety and a lack of cross-toxicity. This strategy may improve the immediate and long-term results of cancer immunotherapy [61].

CONCLUSION

Literature data indicate the important role of estradiol and activation of the ER-signaling mechanism in TME, which provokes immunosuppression and tumor progression. These features have been identified in various cancers and are not limited to tumors of the female reproductive system. Nevertheless, in clinical practice, the study of estrogen and antiestrogenic effects on TME is of the greatest value in classical hormone-dependent tumors.

The results of studies showing that antiestrogen therapy has the potential for a reversible effect on immunosuppressive TME due to a pronounced response in hormone-dependent tumors are promising. However, a significant role of TME in resistance to endocrine therapy, particularly in luminal breast cancer, was revealed. These data will further lead to expansion of the panel of predictive and prognostic molecular markers of malignant diseases with mandatory identification of potential drug therapy targets in TME.

The relationship between the tumor immune response and the ER-signaling mechanism of TME also opens up prospects for improving the effectiveness of immune checkpoint inhibitors and overcoming resistance to hormones. There is a need to standardize the method for detecting the expression of ER and aromatase in TME, taking into account the possibilities of activating the immune response through combined use of antiestrogens and immune checkpoint inhibitors. In addition, studies should focus on gender differences and demographic data, including menopausal status and body mass index with information on the degree of obesity, to clarify the degree of E2 involvement in tumor immunity.

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