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It is important to be aware that *BRCA1/2* carriers with CRPCa had a higher PSA level and ADT duration compared to non carriers. No differences were seen between carriers and non carriers in a Gleason score. Castro et al. reported that patients with prostate cancer who were positive for a *BRCA1/2* mutation had higher Gleason scores (≥ 8) and PSA level [6]. Our results also raise the question of whether adjuvant treatments may be beneficial for *BRCA* carriers. Because androgen receptor–maintained activity has been shown to increase chromosomal instability [7], an increased duration of ADT may possibly benefit patients with DNA repair defects.

The high prevalence of activating AKT/mTOR pathway alterations was found in prostate cancer [5, 8]. AKT was also involved in *BRCA1*-deficiency mediated tumorigenesis [9] and this fact was also confirmed in the study. AKT expression in prostate cancers was higher in *BRCA1/2* mutation carriers compared to non carriers.

Conclusion

Activation of AKT/m-TOR signaling pathway was found in prostate cancers. The activity of this pathway was decreased in CRPCa tissues. Germline *BRCA* mutations in CRPCa were revealed in up to 33% of patients. *BRCA1/2* carriers had an increased duration of ADT, a high level of PSA and elevated level of AKT expression compared to non-carriers.

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THE SIGNIFICANCE OF THE IMMUNE SYSTEM FOR THE EFFICIENT CANCER THERAPY

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During the last decade it was shown that immune effectors contribute to efficiency of cancer therapy. As a result, the functional state of the host immune system has been considered to have a major prognostic and predictive impact on an efficiency of cancer therapy. The aim of this study was to find out whether the united state of the immune system is associated with the efficiency of the neoadjuvant chemotherapy in breast cancer patients. One hundred six patients diagnosed with primary local breast cancer (I-III stages) were enrolled in the investigation. The cancer treatment obligatory included the appropriate courses of neoadjuvant chemotherapy. The response to neoadjuvant chemotherapy was used as criterion of the efficiency of the cytostatic therapy. The immune system state as an integral unit was estimated by NovoSpark Visualization approach. The favorable immune system states in BC patients were associated with effective NAC: 68 %

of patients from this group responded to cytostatic treatment in comparison with 45 % in women with unfavorable immune system states ($\chi 2=3,67$, $p=0,05$). Thus, the efficiency of the cytostatic therapy is associated with the immune system state in BC patients.

Keywords: breast cancer, chemotherapy efficiency, cytostatic therapy, immune system.

For a long time, anticancer therapies were believed to work either by killing cancer cells or by inducing a permanent arrest in their cell cycle [1]. But during the last decade, it is becoming clear that many of the available anticancer drugs mediate therapeutic effects by eliciting de novo or reactivating pre-existing tumor-specific immune responses suppressed by an established malignant tumor [2].

In clinical and experimental studies it was shown that immune effectors contribute to efficiency of cancer therapeutics [1, 2, 3]. As a result, the functional state of the host immune system has been considered to have a major prognostic and predictive impact on the fate of cancer patients treated with conventional or targeted chemotherapies [2, 3].

In general, cancer therapy efficiency was associated with functioning of cytotoxic mechanisms of immune system, such as activity of cytotoxic T-cells, NK and antibodies as mediators of antibody-dependent cell cytotoxicity [3]. But the outcome of chemotherapy can be influenced by the host immune system at multiple levels. This fact supports the idea that immune works in a coordinated fashion against numerous threats from the external or internal environment. Moreover, in our previous studies, we demonstrated that immune response to tumor can be presented as united strategy [4]. We have found that the immune system state can be split on at least two different ones. One state, which we called as favorable, was observed in BC patients without recurrence, and another state – unfavorable - was associated with relapses in BC patients within 3 year follow-up [4]. The aim of this study was to find out whether the united state of the immune system is associated with the efficiency of the neoadjuvant chemotherapy in breast cancer patients.

Material and Methods

One hundred six patients diagnosed with primary local breast cancer were enrolled in the investigation. The patient pathological stages ranged from I to III. Patients with diagnosed metastatic breast cancer (i.e. IV stage) were not enrolled. The cancer treatment obligatory included the appropriate courses of neoadjuvant chemotherapy, adjuvant chemo-, radio- and hormonal therapies according to the pathological stages and routine predictive criteria. The standard chemotherapies regimens predominantly used in the study are FAC, CAF. The response to neoadjuvant chemotherapy according to the WHO was used as criterion of the efficiency of the cytostatic therapy. The therapy was considered effective, when complete or partial responses were achieved in BC patients, and it was ineffective, when stable or progressive disease were observed after 2 cycles of the neoadjuvant treatment.

In order to test the immune system state, venous blood was obtained from the patients before cancer treatment. To characterize the immune system state as an integral unit, we estimated separate parameters of the innate and adaptive arms of immune system in the BC patients and then we integrated the obtained values into a single characteristic as a visual image using NovoSpark Visualization approach [4]. Presenting an immune system state in an integral visual image of NovoSpark Visualizer software for all 106 BC patients, we identified two groups of patients: with favorable (associated with disease-free survival) or unfavorable (associated with recurrences) of immune system state.

All the patients gave written informed consent to participate in this investigation. The study was approved by Ethics Committee of Tomsk Cancer Research Institute and performed according to the guidelines of Declaration of Helsinki.

Statistical analysis was performed using Statistica version 6.1 (StatSoft Inc). Differences between groups were evaluated using the $\chi 2$ test.

Results

In the group, including all BC patients, the half of them (53 women of 106) responded to NAC, but other half did not. The complete and particular responses were observed in four patients and forty nine patients, respectively. Forty two cases of stable diseases and eleven cases of progressive diseases consisted of the group of BC patients without therapeutic response.

Then we classified all BC patients depending on their immune system states with using our original approach. Only twenty two BC patients (21 %) had favorable immune system states prior cancer, but eighty four women (79 %) were characterized as having unfavorable immune system states. The favorable immune system states in BC patients were associated with effective NAC:

68 % of patients from this group responded to cytostatic treatment in comparison with 45 % in women with unfavorable immune system states (Table, $\chi 2=3,67$, $p=0,05$).

Conclusion

The efficiency of the cytostatic therapy is associated with the immune system state in BC patients. Responses to NAC were observed in 68% in patients with favorable immune system states in comparison with 45 % in women with unfavorable ones. Assessment of the immune system status is necessary to select an appropriate cytostatic therapy.

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OPTIMIZATION OF DENDRITIC CELL MATURATION PROTOCOL TO ASSESS THE SPECIFIC ACTIVITY OF ANTICANCER DNA-VACCINES IN EX VIVO SYSTEM

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Treatment of advanced stages of cancer using surgery, chemotherapy, radiation therapy or hormone therapy remains challenging and may not be effective, so it is important to design and improve new treatment methods. One of the promising methods is immune therapy, in particular, the development of anti-cancer vaccines, including genetically engineered DNA-vaccines that induce specific T-cell immune response against the tumor [1]. Lately, to estimate the immune response induced by T-cell vaccine, researchers use the method based on the ability of a vaccine to cause formation of effector T-cells in *ex vivo* system [2]. Generation of mature dendritic cells is a crucial step of that method. The goal of this study is improving the protocol of generation of mature dendritic cells (DC) to study immunogenicity of DNA-vaccines against **oncological diseases** *ex vivo* using PBMC from healthy donors [3]. There are various ways of generating mature dendritic cells, differing in the reagents used [4]. Unlike mature DC, immature DC are capable of endocytosis, therefore they can process and present antigen on their surface. So, immature DCs are used for antigen uptake. After loading with antigen, DCs are cultivated with maturing factors [1]. During the maturation, immature DC lose specific markers such as CD11c, CD83 and costimulatory signals CD86 and CD80 as well as start producing cytokines [5,6]. To obtain mature DC we tried several protocols. First, we obtained mononuclear cells that adhere to cultural plastic from human peripheral blood, then cultivated it with GM-CSF (50 ng/ml) and IL-4 (100 ng/ml) for two days [7]. After the cultivation immature DC were magnetically transfected with DNA-vaccines; maturation factors were added and cells were cultivated for two more days. We used three different maturation cytokine cocktails: 1) only TNFα (25 ng/ml) [6]; 2) TNFα (25 ng/ml), IFN-γ (3000 units/ml) and polyI:C (25 μg/ml); 3) TNFα (50 ng/ml), IFN-γ (3000 units/ml), polyI:C (25 μg/ml) and IL-1b (25 ng/ml) [8,9]. Using each cocktail, we obtained mature DC expressing costimulatory signals CD86, CD80, and markers CD11c and CD83. However, we obtained DC with more number of maturation markers when third maturation cocktail was used. The system *ex vivo* was approved for evaluation of the efficacy of DNA vaccines against melanoma pMEL-TCI-A0201 and pMEL-TCI in our laboratory [3]. DC obtained using the third cocktail, transfected with pMEL-TCI-A0201 and pMEL-TCI, provided more effective activation of specific cytotoxic T-lymphocytes.

Keywords: DNA-vaccine, dendritic cells, oncological diseases, cytokine cocktail.