BreastCare

Cellular Immune Responses and Immune Escape Mechanisms in Breast Cancer: Determinants of Immunotherapy

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Summary

More recently, immunotherapy has emerged as a novel potentially effective therapeutic option also for solid malignancies such as breast cancer (BC). Relevant approaches, however, are determined by the 2 main elements of cancer immunoediting – the elimination of nascent transformed cells by immunosurveillance on the one hand and tumor immune escape on the other hand. Correspondingly, we here review the role of the various cellular immune players within the host-protective system and dissect the mechanisms of immune evasion leading to tumor progression. If the immune balance of disseminated BC cell dormancy (equilibrium phase) is lost, distant metastatic relapse may occur. The relevant cellular antitumor responses and translational immunotherapeutic options will also be discussed in terms of clinical benefit and future directions in BC management.

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Introduction

An intact immune system plays a dual role in cancer: it can prevent/control as well as shape/promote cancer by a process called 'cancer immunoediting' [1]. On the one hand, in a host-protective elimination phase named 'immunosurveillance', the immune system can recognize and suppress tumor growth by destroying nas-

cent transformed cells or inhibiting their outgrowth. This antitumor immunity is substantiated by the main cellular effectors of the innate and the adaptive immune system, namely natural killer cells, natural killer T cells, and T cells (TCs), as well as increased pro-immune humoral factors (e.g., interferons) in the tumor microenvironment. On the other hand, in the tumor-promoting phase referred to as 'immune escape', the immune system can further tumor progression either by selecting cancer cells that are more capable of surviving the host's immunocompetence or by modifying the tumor microenvironment in such a way that tumor outgrowth is facilitated [2]. In between the above phases is the equilibrium where cancerous cells are kept under control but are not eliminated by the immune system. This balance of antitumor and tumor-promoting factors may maintain the tumor in a functionally inactive state of dormancy over a period of many years [3].

The processes mentioned above also make up the rationale for the development of immunotherapeutic options in breast cancer (BC) [3–5], as characteristically in this tumor entity, already at very early stages, cancer cells are able to disseminate hematogenously from the primary tumor site, and distant metastases often occur only after many years of latency [6]. In this context, one predominant organ associated with the dissemination and survival of BC tumor cells is, besides others such as locoregional lymph nodes, the bone marrow (BM). Of note, the detection of disseminated BM tumor cells correlates with an increased rate of secondary osseous and visceral metastases and with a worse overall survival [7–11].

Consequently, in addition to surgical resection of the primary tumor and locoregional irradiation, curative BC therapy aims at eliminating disseminated micrometastatic tumor cells. In this context, besides cytostatic and/or hormonal therapies, new supportive treatment options like immunotherapy are increasingly gaining oncological interest. Hence, we also review aspects of BC immunoediting processes with respect to potential immunotherapeutic approaches.

Cellular Immune Responses

Tumor Site

In human BCs, like in other malignancies, the presence of primary tumor-infiltrating lymphocytes (TILs), especially of the Th1 and cytotoxic variety, is correlated with the absence of metastatic invasion and improved clinical outcome in terms of overall survival rates [12]. This holds particularly true for BCs with aggressive features such as high histologic grade or estrogen receptor-α negativity [13, 14].

Intriguingly, in triple-negative invasive BC patients receiving neoadjuvant chemotherapy and subsequent surgical treatment, the immunohistochemical or mainly the hematoxylin and eosin staining analysis of primary tumor needle biopsy specimens revealed that the pathologic complete response rates of tumors showing a high TIL score were significantly higher than those of tumors with a low TIL count [15, 16]. These results suggested that the pretreatment host immune response may enhance the ability of anthracycline/taxane-based neoadjuvant chemotherapy to eliminate cancer cells [17]. This hypothesis was further corroborated by large studies which demonstrated triple-negative as well as HER2-overexpressing BC phenotypes with high levels of intratumoral cytotoxic TCs to have heightened sensitivity to anthracycline-based chemotherapy, as assessed by the immediate response to neoadjuvant therapy and long-term disease-free survival rates [18–20]. Further, in HER2-positive BC patients, high levels of TILs were also found to be associated with improved therapeutic responses to the monoclonal antibody trastuzumab [21].

In summary, the data available suggest that particularly in aggressive subtypes such as triple-negative and HER2-positive BC the immune response plays a pivotal part in tumor chemosensitivity and clinical outcome.

Bone Marrow Site

TCs play a central role in cell-mediated tumor immunity. Conventionally, the precursors of TCs are produced in the BM but subsequently leave the BM and mature in the thymus. Mature cells then emigrate from the thymus into the circulation. Repeatedly, circulating naive TCs leave the blood to enter peripheral lymphoid organs where they may encounter their specific tumor antigen and become activated as effector (cytotoxic or helper) TCs.

Relating to BC immunity and the generation of tumor-specific effector and memory TCs, blood/BM interactions proceed in 3 steps [22, 23]:

i) Naive TCs as well as tumor cells are recruited from the circulation to the BM ('homing') via constitutively expressed adhesion molecules [24, 25];

ii) BM-resident antigen-presenting cells, particularly dendritic cells, can scan, process, and cross-present BC-associated antigens to prime TCs [22, 26]; and

iii) activated TCs proliferate and may become effector and memory cells that either recirculate or remain in the BM compartment.

The coexistence of BM-resident tumor-specific TCs and disseminated BC cells maintains the quiescent state, i.e., the immune balance, which is referred to as tumor dormancy, while a loss of TC function can lead to tumor metastasis even after years of latency [27–30].

Furthermore, especially in the case of BC, in a significant number of patients during the course of disease, tumor-specific TC responses could be proven to have been induced and maintained in the form of BM memory TCs (TMCs) – a subset of BC-specific TCs that persist long-term [31].

TMCs are an ideal source for the generation of therapeutic effector TCs expressing the CD8 glycoprotein at their surfaces. This is due to the fact that secondary CD8+ TC responses take place more quickly and more effectively than primary responses [32–34]. Correspondingly, in a trial of advanced metastasized BC patients with tumor-reactive TMCs in the BM, no tumor-specific TCs were detected in the peripheral blood at the beginning. After therapy with reactivated autologous BC-reactive BM TMCs, however, about 44% of patients showed tumor-specific TCs in the peripheral blood [35, 36].

Tumor Immune Escape

Mechanisms

In the studies cited above [35, 36], which will be discussed in more detail in the subsequent chapters, roughly half of the BC patients treated did not respond to the immunotherapy employed. In these patients, counterregulation mechanisms may have taken action leading to post-therapy tumor immune escape along the following lines:

i) The immune recognition of tumor cells can be circumvented because of loss of tumor antigen expression that may occur in at least 3 ways [2]: through development of tumor cells lacking expression of potent rejection antigens; by means of downregulation of major histocompatibility complex (MHC) class I proteins that present these antigens to tumor-specific TCs; or via loss of tumor cells' antigen processing capacity that is mandatory to develop the antigenic peptide epitopes and load them onto the MHC class I molecules.

ii) At the tumor cell level, resistance to cytotoxic lysis by immune cells may be brought about by enhanced expression of anti-apoptotic effector molecules such as FLIP and BCL-XL [37] or by persistent activation of pro-oncogenic transcription factors like STAT3 [2]. Alternatively, tumor cells can evade immune-mediated killing through expression of mutated inactive forms of death receptors [37]. Concertedly, these mechanisms may promote tumor progression.

iii) Tumor immune escape may develop due to local immunosuppression in the tumor microenvironment. Such an immunosuppressive state can be established either by tumor cells producing immunosuppressive cytokines (e.g., vascular endothelial growth factor and transforming growth factor-ß (TGF-ß)) or by recruitment of specific immunosuppressive leukocyte populations (e.g., regulatory TCs (Tregs) and myeloid-derived suppressor cells) [2, 37, 38].

Clearly, the rate of BC progression is determined by the balance between the above immune-inhibitory factors of tumor immune escape and the host-protective capacity of the immune system as well as the immune-stimulatory conditions of immunotherapeutic approaches.

Potential Anti-Escape Strategies

In BC patients with failed immune response to cellular immunotherapeutic approaches, a significantly higher proportion of Tregs was found in the BM [35]. Tregs are a subpopulation of TCs which generally suppress (hence also called suppressor TCs) or downregulate activities of effector TCs thus modulating immune reactions, maintaining tolerance to self-antigens, and abrogating autoimmune disease. Consequently, in future immunotherapy studies, patients might benefit from ex vivo Treg depletion prior to TC stimulation.

Additional strategies potentially capable of reducing the immunosuppressive effects of Tregs are currently investigated in murine models or first clinical studies and comprise i) denileukin diftitox, a fusion protein of interleukin 2 and diphtheria toxin targeting Treg cells [39–41], ii) direct antibody blockade of the immunosuppressive moieties of Tregs [37, 38], and iii) pharmacological agents such as cyclooxygenase-2 inhibitors as well as the antineoplastics temozolomide (tumor DNA methylator), fludarabine (purine analog), and cyclophosphamide (CTX) [42, 43]. Strikingly, CTX, a well-known chemotherapeutic compound, when being applied solely at low doses in a metronomic regimen could be demonstrated to induce a selective profound reduction in circulating Tregs through enhanced apoptosis and decreased proliferation of this cell type [44, 45]. Simultaneously, spontaneous antitumor TC responses were found restored [42, 45].

Correspondingly, in a recent study of metastasized BC patients, Ge et al. [46] reported metronomic CTX treatment over 3 months to cause a transient reduction in circulating Tregs by more than 40% associated with a strong and lasting increase in breast tumor-specific TCs, which significantly correlated with disease stabilization and overall survival. Consequently, low-dose CTX might be successfully integrated into a future concept of chemoimmunotherapy.

Cellular Immunotherapy

Possible Accesses

Cellular immunotherapy approaches are based on 2 different principles [47]:

i) The body's own immune system can be actively and specifically stimulated through confrontation of immune cells with autologous or allogeneic tumor antigens in situ. This leads to a primary activation of naive effector cells or to a secondary reactivation of memory cells which were formed during an earlier confrontation of the immune system with the specific antigen. This procedure corresponds to an active immunization, i.e., a vaccination.

ii) Autologous or allogeneic immune cells (tumor-specific TMCs from BM [35, 36] or peripheral blood [48], TILs [49], or engi-
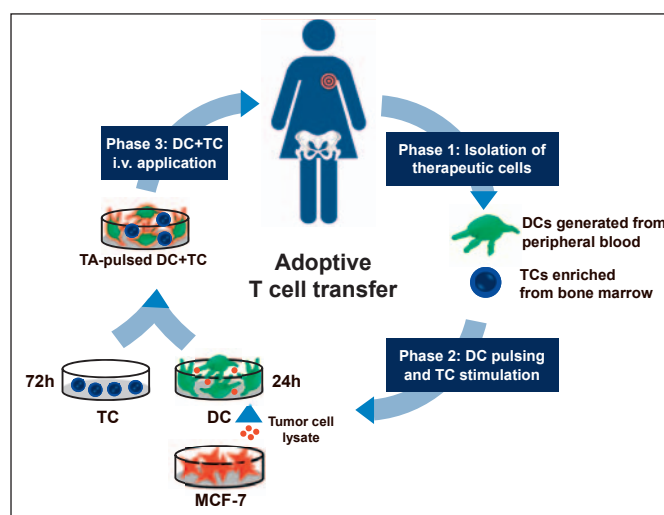
Fig. 1. Adoptive immunotherapy (ADI) of metastasized breast cancer patients. Process of ADI treatment preparation (PB = Peripheral blood; BM = bone marrow; DC = dendritic cell; TC = T cell; MCF-7 = human breast cancer cell line MCF-7; TA = tumor antigen). Adapted from [36, 57].

neered TCs [50]) with a specific affinity for tumor-associated antigens can get activated ex vivo and subsequently applied directly into the human organism as cellular immunotherapy. This is equivalent to a passive immunization, i.e., an adoptive immunotherapy (ADI).

Vaccination

Vaccine-based therapies do firstly require the identification of tumor-specific antigens (expressed on malignancies only) and tumor-associated antigens (expressed on all types of cells but overexpressed on cancer cells). In breast cancer, relevant antigens that could be targeted by vaccination include MUC-1, MAGE-A3, and NY-ESO-1 which are characteristically expressed on estrogen receptor-negative and/or triple-negative tumor subvariants [51, 52]. In vaccination strategies, liposome-based and synthetic peptide vaccines are employed. Additionally, the administration of in vitro activated dendritic cells can be involved [53]. In BC patients, attempts along the above lines are currently being evaluated in clinical trials which are comprehensively expounded elsewhere [54].

Adoptive Immunotherapy

Results from animal experiments [55–57] were the rationale for the establishment of a phase I trial where patients with advanced metastasized BC were treated with reactivated autologous tumor-specific BM TMCs [35, 36]. In 16 BC patients with tumor-reactive BM TMCs, another BM aspiration was performed which provided tumor-specific TMCs for the following flow of ADI treatment:

i) TMCs were activated by antigen-pulsed dendritic cells with antigens originating from lysates of a microbiologically tested MCF-7 cell line. After incubation of the antigen-presenting cells with TMCs, the cell suspension (2×10^6 to 5.7×10^7 TCs) was intravenously applied under antibiotic prophylaxis (fig. 1). This

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immune cell transfer was well tolerated except for influenza-like symptoms in 2 patients.

ii) As early as 1 week after intravenous ADI, tumor antigen-reactive TMCs could be detected in the peripheral blood of about 50% of the patients (ADI responders). The responding patients had received the highest total number of TMCs and harbored the lowest tumor burden. Of note, significantly higher levels of tumor-specific TMCs had been observed in the BM of patients with subsequently positive ADI response compared to non-responding patients.

iii) In none of the treated patients with overt bone metastases tumor-reactive TCs were detectable in the peripheral blood after ADI. This massively reduced immune responsiveness may be related, at least in part, to the increased release of immunosuppressive TGF-ß from the bone during the osseous metastatic process [36, 58–61].

iv) Finally and most importantly, ADI responders had a significantly longer median survival than non-responders (58.6 vs. 13.6 months; p = 0.009) with 3 out of 16 patients still being alive at last follow-up and more than 7 years after ADI [36].

In summary, results hint at a relationship between immune response and cancer prognosis and suggest that preferably BC patients without bone metastases but with immunologic response to adoptive TC transfer might benefit from this treatment option.

Perspectives

The central aim of forthcoming studies will be the control of tumor escape mechanisms as described in the preceding chapters.

Firstly, approaches to deplete or reduce Tregs in their capacity to suppress the immune system might substantially improve spontaneous or immunotherapy-related tumor defense. One strategy in this context may be a combined chemoimmunotherapy of metronomic CTX with an adoptive TC transfer (ADI). This might include a selection of defined tumor antigens with immune adjuvants preventing the re-activation of type-2 TC responses during stimulation. Additionally, new approaches to polyclonal TC expansion deserve consideration attempting to replace autologous dendritic cells by artificial antigen-presenting cells with improved TC-stimulatory properties [62].

Secondly, although still in the experimental phase, recent initiatives aim at grafting patients' primary TCs with a second TC receptor known to recognize a defined tumor antigen [47]. There is hope that, in the long run, such engineered tumor-specific TCs (CAR-T cells) may be successfully used for adoptive immunotherapy purposes [63, 64].

Thirdly, other current attempts focus on adoptive TC therapy with TILs [65–68]. In this context, tumor-reactive TCs are harvested from tumor-infiltrated lymph nodes or tumor tissue. In metastatic melanoma patients, a transfer of ex vivo activated and expanded autologous TILs after a preceding lympho-depleting chemotherapy was demonstrated to induce tumor regression in about 50% of treated patients [67, 68]. Therefore, a pivotal scien-
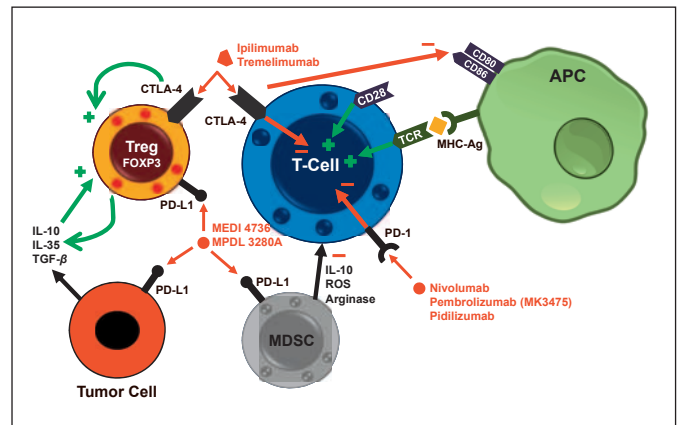
Fig. 2. Checkpoint inhibitors (CTLA-4 = Cytotoxic T-lymphocyte-associated antigen 4; PD-(L)1 = programmed cell death protein (ligand) 1; APC = antigen-presenting cell; MDSC = myeloid-derived suppressor cell; ROS = reactive oxygen species; TCR = T cell receptor; MEDI 4736/MPDL 3280A = anti-PD-L1 monoclonal antibodies). Adapted from [54].

tific focus is on the improvement of the tumor antigen recognition capacity and, hereby, the therapeutic efficacy of TILs or genetically engineered TCs [68–78].

Finally, immune checkpoints are currently the focus of clinical research (fig. 2). The expression of 'cytotoxic T-lymphocyte-associated antigen 4' (CTLA-4) on the plasma cell membrane of TCs induces a downregulation of their activity and thereby leads to immunosuppression. This effect is mediated by B7 expression on antigen-presenting cells as for example dendritic cells. CTLA-4 is in competition with stimulating CD28 for binding to B7. Therefore, the expression of one or the other results in TC suppression (CTLA-4) or stimulation (CD28).

The 'programmed cell death protein-1' (PD-1) is also expressed on the plasma cell membrane of TCs. Activated by its ligands PD-L1 and PD-L2, the generation of Tregs is induced and the activity of immune cells is downregulated [79]. PD-L1 is expressed by about 30% of BC cells and results in relevant immunosuppression [80].

Several immune checkpoint inhibitors are now under clinical investigation. Nivolumab, pidilizumab, and pembrolizumab are anti-PD-1 antibodies. In metastatic BC, a study with pembrolizumab has just been presented [81]. In only 18.5% of the patients included a clinical response was detected. Nevertheless, this response was long-lasting. Nivolumab showed a clinical benefit in patients with malignant melanoma where cases expressing PD-L1 on the tumor cells were correlated with best responses [82]. In those patients, even a prolonged overall survival was observed. BC-related data is promising although systematic studies are still missing.

As described above, blockade of CTLA-4 by antibodies such as ipilimumab or tremelimumab enhances the immune reaction against tumor cells. In malignant melanoma patients, ipilimumab resulted in favorable clinical responses. Consequently, this antibody is now being tested in other solid carcinomas, with BC-related data still missing. Nevertheless, a combination of checkpoint inhibitors might be a promising therapeutic option. In patients with malig-

nant melanoma, for instance, ipilimumab together with pembrolizumab was tested against a solitary ipilimumab therapy. This trial showed a relevant benefit in the cohort of patients with combined therapy thus justifying further studies [83]. Modulating the immune system, however, is not free of clinical side effects. Suppressing immunosuppressive effects may be related e.g. to several autoimmune reactions (thyroiditis, colitis, pancreatitis etc.). Therefore, thorough clinical observation within trials is mandatory.

All in all, in contrast to other neoplasms such as melanoma, BC treatment with immune checkpoint-abrogating agents has only recently become a field of interest. Hence, most clinical trials based on immune checkpoint blockade are pilot or phase I/II studies [54].

Conclusion

In BC, the presence of TILs has been shown to correlate with a favorable long-term prognosis primarily of high-grade/highly proliferative lesions. TILs were also associated with a favorable response to neoadjuvant and adjuvant anthracycline-based chemotherapies. These findings support the addition of immunotherapeutic strategies to conventional treatment concepts.

In late-stage metastasized BC patients, the adoptive transfer of BM TCs (ADI) can induce the presence of tumor antigen-reactive TCs in the peripheral blood. This positive immunologic response appears to depend significantly on the number of transferred specific memory TCs and on the absence of BM metastases. Immune responders show a significantly prolonged overall survival.

Eventually, future strategies to potentially overcome tumor immune escape should comprise Treg depletion from BM preparations before their ex vivo activation. Additionally, a functional inhibition of the immunocompromising capacity of Tregs or a functional TC stimulation may be achieved either by directly targeted antibodies (CTLA-4, PD-1, PD-L1, IL-2 receptor) or by pharmacologic agents such as cyclooxygenase-2 inhibitors or CTX. Besides a checkpoint inhibition, immunomodulating metronomic low-dose CTX plus ADI appears to be a promising choice of combinatorial chemoimmunotherapy. Finally, current developments relating to artificial antigen-presenting cells, engineered tumor-specific TCs, and TILs should be followed up.

Disclosure Statement

No conflicts of interest were disclosed.

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