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Immune checkpoint inhibitors in breast cancer treatment

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Abstract

Breast cancer is a major cause of cancer-related death among women worldwide. Currently breast cancer is one of the major cancer types for which new immune-based cancer treatments are in development. Triple-negative breast cancer (TNBC), is characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PR), and HER2/ neu. TNBCs are generally high-grade, aggressive tumors with a high rate of distant metastasis and limited treatment options. Novel therapeutic strategies are needed to improve the management of patients with TNBC. A promising avenue of clinical research in breast cancer is the use of immune checkpoints. The aim of the study was to evaluate expression levels of selected immune checkpoints- PD-1 (programmed cell death protein 1), and PD-L1 (programmed cell death 1 ligand 1) in breast cancer patients. Immunogenicity appears to be gaining importance in triple negative and HER2-positive molecular subtypes of breast cancer, and the results in this study provide a basis for further investigation into the role of immune checkpoints in breast cancer.

Aim of study

To understand the role of the immune system in defending the body against cancer cells, and to analyze the effectiveness of immune therapy in breast cancer patients and to see if it should be used as a main type of cancer treatment.

The aim of the study was to evaluate expression levels of PD-1 and PD-L1 immune checkpoints in formalin- fixed, paraffinembedded breast cancer samples and determine their associations with clinicopathological features

Introduction

Unlike other solid tumor types, such as melanoma with elevated mutational load, thus activating an antitumor immune response, breast cancer has not traditionally been thought to be immunogenic. But recent studies on tumor-infiltrating lymphocytes, immune milieu, checkpoints and significant heterogeneity within breast cancer subtypes, have cast new light on the role of immune system in breast cancer. [3]

In recent years, immunotherapy has emerged as a possible fourth leader after surgery, chemo and radiotherapy, targeting cancer not by its anatomic location or tendency to divide, but by the inherent mechanisms the immune system uses to distinguish between healthy and pathologic tissue. The immune system is amazingly complex. It can recognize and remember millions of different enemies, and it can produce secretions and cells to match up with and wipe out each one of them. Scientists have discovered that the immune system is capable of destroying tumor cells, and that the body defends itself against cancer in much the same way that it defends itself against infection. Harnessing the immune system to recognize and destroy tumor cells has been the central goal of anti-cancer immunotherapy. There are more than one type of immunotherapy: Monoclonal antibodies (MABs), Checkpoint Inhibitors, Cytokines, Vaccines to treat cancer and CAR T-cell therapy.[2]

The type of immunotherapy focused on in this report is, immune Checkpoint Inhibitors.

Checkpoint inhibitors are a type of immunotherapy. They block proteins that stop the immune system from attacking the cancer cells. Cancer drugs do not always fit easily into a certain type of treatment. This is because some drugs work in more than one way and belong to more than one group. Checkpoint inhibitors are also described as a type of monoclonal antibody or targeted treatment.[2]

The immune system protects the body from disease, killing bacteria and viruses. One main type of immune cell that does this is called a T cell. T cells have proteins on them that turn on an immune response and other proteins that turn it off. These are called checkpoints. Some checkpoint proteins help tell T cells to become active, for example when an infection is present. But if T cells are active for too long, or react to things they shouldn't, they can start to destroy healthy cells and tissues. So other checkpoints help tell T cells to switch off. [2]

Some cancer cells make high levels of proteins. These can switch off T cells, when they should really be attacking the cancer cells. So the cancer cells are pushing a stop button on the immune system. And the T cells can no longer recognize and kill cancer cells. [2]

Drugs that block checkpoint proteins are called checkpoint inhibitors. They stop the proteins on the cancer cells from pushing the stop button. This turns the immune system back on and the T cells are able to find and attack the cancer cells. These drugs block different checkpoint proteins including: CTLA-4 (cytotoxic T lymphocyte associated protein 4), PD-1 (programmed cell death protein 1), PD-L1 (programmed death ligand 1), CTLA-4 and PD-1 are found on T cells. PD-L1 are on cancer cells. [2]

These inhibitors treat melanoma, non-small-cell lung cancer, kidney cancer, bladder cancer, head and neck cancers, stomach cancer, colorectal cancer, and Hodgkin's lymphoma.[2]

Method

Inclusion criteria:

50 patients with operable breast cancer, stages I-II, suitable for breast conservative and negative surgery sentinel node biopsy, and operated on in the Department of Surgical Oncology. Archival formalin- fixed, paraffin-embedded tumor samples were used for PD-1 and PD-L1 assessment. All samples were taken with written informed consent. Ethics committee

approval was obtained from the Institutional Review Board of the Medical University of Lodz. [3]

Exclusion criteria:

Breast cancer patients with concomitant or previous autoimmune diseases were ruled out, other immune aberrations or a medical history of any malignancy. Pregnant or lactating women were also excluded from the studies.[3]

PD-1 and PD-L1 assessment:

Total RNA isolation.

Total RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue using the Roche High Pure miRNA Isolation Kit according to the manufacturer's instruction. In brief, FFPE slices were processed in a 2 ml Eppendorf tubes, deparaffinized with 100% xylene, followed by wash with 100% ethanol and dried at 55°C for 10 min. The dry tissue was resuspended in Paraffin Tissue Lysis Buffer (included in the kit) and digested with proteinase K at 55°C overnight. Subsequent steps of RNA purification on column were performed following the manufacturer's recommendations. The yield and quality (the ratio of absorptions at 260/280 nm) of RNA product were measured using PicoDrop spectrophotometer (Picodrop Limited, UK). The purified total RNA was immediately used for cDNA synthesis or stored at −80°C until use. [3]

cDNA generation.

Generation of cDNA was performed with High Capacity cDNA Reverse Transcription Kits (Applied Biosystems) according to protocol of the manufacturer. 1 μg of total RNA was used as starting material, to which was

added 2x RT master mix containing 2 µl of 10x RT Buffer, 0.8 µl of 25x dNTP Mix (100 mM), 2 µl of 10x RT Random Primers, 1 µl MultiScribe™Reverse Transcriptase and 1 µl RNase Inhibitor per each 20 µL reaction. Reverse transcription was performed under conditions optimized for use this kit (25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min). The samples were kept frozen at −20 C. [3]

Results

Measurement of mRNA expression was done using standard TaqMan® Gene Expression Assays (Applied Biosystems): programmed cell death 1, and its ligand PD-L1, CD274 molecule (CD274, Hs01125301_m1) and beta actin as the endogenous control. TaqMan PCR assays were performed in 10µl reactions included 50 ng cDNA, 5 µl KAPA PROBE FAST qPCR Kit Master Mix ABI Prism (Kapa Biosystems) and 0,5 µl appropriate TaqMan Gene Expression Assay. All reactions were run in duplicate on a 7900HT Fast Real-Time PCR System (Applied Biosystems) in 96 well PCR plates. The following thermal cycling specifications were performed: 20s at 95°C and 40 cycles each for 30s at 95°C and 30s at 60°C. Data was analyzed using SDS 2.4 software (Applied Biosystems). [3]

Discussion

The immunogenicity of breast cancer is an emerging area of research, and highlighted at the ASCO meeting (American Society of Clinical Oncology) in May/ June 2015, St. Gallen Early Breast Cancer Conference in March 2015 and San Antonio Breast Cancer Symposium in December 2014. Loi et al. indicated that breast cancer itself does not have mutational capacity leading to immunogenic responses seen in other tumor types, although it is generally higher in the triple-negative and HER2-positive groups, as compared with the luminal breast cancers. This is in concordance with the research demonstrating higher PD-1 and PD-L1 expression in aggressive phenotypes such as triple negative and HER2-positive as compared with luminal A and luminal B HER2-negative breast cancers. We also showed

that elevated PD-L1 expression was more noticeable in high grade tumors. Bertucci et al. demonstrated that PD-L1 expression was higher in aggressive inflammatory breast cancer (IBC) than in non-IBC. In IBC, PD-L1 overexpression was associated with estrogen receptor-negative status, basal and ERBB2-enriched aggressive subtypes. Moreover, these authors indicated that PD-L1 overexpression was associated with better pathological response to chemotherapy and may be a biomarker for patient selection for immunogenic chemotherapy .

In the research only patients with early stage breast cancer were enrolled, so the study was unable to assess the pathologic response to chemotherapy in the neoadjuvant setting. Sabatier et al. revealed that high PD-L1 expression was associated with poor-prognosis features (high grade, ER-negative, PRnegative, ERBB2-positive status, high proliferation, basal and ERBB2-enriched subtypes). These authors concluded that reactivation of dormant tumor-infiltrating lymphocytes by PDL1-inhibitors could represent promising strategy in PDL1-upregulated basal breast cancer. These findings were also confirmed by Soliman et al. who showed that basal/ triple negative breast cancer cell lines expressed the highest levels of PD-L1. Disis et al. further substantiated the immunogenicity of triple negative breast cancer, demonstrating an amplification of the adaptive immune response through B-cell pathways with antibody secretion binding to tumor antigens.

In the current study immune biomarker expression in archival paraffin-embedded tumor samples have been examined , but in the previous research described elsewhere a striking difference ($p<0.0001$) between immune checkpoint PD-1 expression in CD8+ T cells in blood samples of breast cancer patients and healthy controls was found, with significantly lower levels in the latter group. Moreover, in the previous study, there was a negative correlation between PD-1 expression and progesterone receptor (PR) status in blood of breast cancer patients ($p=0.024$). The lack of PR, associated with the poor prognosis triple negative subtype, correlated with higher PD-1 expression, and thus augmented inhibition of the immune system. This is consistent with the current study in which higher PD-1 and PD-L1 expression in aggressive breast cancer subtypes e.g. triple negative

and HER2-positive were found, in contrast to subtypes with better prognosis such as luminal A and luminal BHER2-negative. In view of the small sample size these results should be considered preliminary and require further elucidation.

However, they provide a basis for further investigation into the role of immune checkpoints in breast cancer, especially in aggressive phenotypes, and potentially also justification for more personalized therapies in breast cancer patients on immunological grounds, to enhance the effects of the other already well-established multimodality treatment- chemotherapy, and anti-HER2 therapy. Nanda et al. presented the first report of clinical activity of an immune checkpoint inhibitor-pembrolizumab, in triple negative breast cancer in a group of pre-treated patients with recurrent/ metastatic disease in a phase Ib study . Pembrolizumab is a highly selective, humanized IgG4/kappa isotype mAb created to stop PD-1 interaction with its ligands PD-L1 and PD-L2, thereby stimulating the immune system to eliminate cancer. Emens et al. demonstrated a 19% objective response rate to the PD-L1 inhibitor MPDL3280A (9.5% of complete and 9.5% of partial response) with ongoing responses in 75% of pretreated patients with metastatic triple negative breast cancer. Loi et al. added that agents targeting the PD-1/PD-L1 pathways look promising in the more aggressive subtypes of breast cancer. Even if the response is limited to a small proportion of patients, it seems to be durable. Therefore, performing “immune profiling” of the tumor, including immune gene signatures, evaluation of TILs and immune checkpoints assessment in tumor, its microenvironment, as well as immune profiling of the peripheral blood, will help us understand the differences between responders and non-responders. The role of immunotherapy in breast cancer patients should become clearer with time. Hence, further investigation in larger studies is urgently required. The International Breast Cancer Study Group IBCSG 45-13 is currently conducting a phase Ib/II trial of anti-PD-1 monoclonal ANtibody in AdvanCed. Trastuzumab-resistant, HER2-positive breast cAncer, PD-L1-positive (PANACEA) , to evaluate the efficacy of MK-3475 and trastuzumab in patients with HER2-positive metastatic breast cancer [25]. During the 14th St. Gallen International Breast

Cancer Conference Primary Therapy of Early Breast Cancer, March, 2015, Curigliano suggested including TILs and immune checkpoints in pathology reports, especially in the more immunogenic breast cancer phenotypes such as triple negative and HER2-positive. [3]

Conclusion

In conclusion, breast cancer has not been considered to be immunogenic. However, immunogenicity appears to be gaining importance in triple negative and HER2-positive molecular subtypes of breast cancer, and the results in this study provide a basis for further investigation into the role of immune checkpoints in breast cancer. [3]

References

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