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مجله المثنى الطبيه

Breast cancer: role of proinflammatory cytokines in the clinical presentation

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Abstract

Breast cancer is among the most commonly diagnosed cancer types in women worldwide and is the second leading cause of cancer-related disease in the USA. Recent data have suggested that many cytokines may be essential roles in the pathogenesis of breast cancer. The goal of this paper is to examine the major cytokines involved in breast cancer immunotherapy and discuss their basic biology and clinical presentation. IL-1, IL-6, IL-10, and TNF-α in patients with breast cancer (n = 63) and healthy individuals as control (n = 68). We found that there were higher levels of IL-6, IL-10 and TNF-α in patients with breast cancer compared to healthy controls ($P<0.05$) and highly correlated to NF-κB. We conclude that the significance of selected cytokines is potential clinical markers of breast cancer in humans.

Keywords: Breast Cancer; Cytokine; Clinical markers; Immunotherapy

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Introduction

Breast cancer is among the most commonly diagnosed cancer types in women worldwide and is the second leading cause of cancer-related disease in the USA [1]. Improved diagnostic methods and medical therapies are necessary for early detection and treatment and an improved prognosis of breast cancer. It is thus vital to both examine and evaluate the role of the various existing proteins as biomarkers in carcinogenesis and to assess the contribution of these proteins in anti-cancer activity, for consideration in therapeutic strategies. The association between inflammation and cancer is well established, and deregulated expression of multiple inflammatory cytokines in malignant breast disease has been recognized [2].

Cytokines are signaling peptides, proteins, or glycoproteins that are secreted by many cell types, including immune, epithelial, endothelial, and smooth muscle cells [3]. They either enhance or inhibit inflammation in response to pathogens, "non-self" molecules, and toxins. Cytokines are molecular messengers that allow the cells of the immune system to communicate with one another to generate a coordinated, robust, but self-limited response to a target antigen. The growing interest over the past two decades in harnessing the immune system to eradicate cancer has been accompanied by heightened efforts to characterize cytokines and exploit their vast signaling networks to develop cancer treatments [4].

Recent years have seen a number of cytokines, including GM-CSF, IL-7, IL-12, IL-15, IL-18 and IL-21, enter clinical trials for patients with advanced cancer. There is ongoing pre-clinical work supporting the neutralization of suppressive cytokines, such as IL-10 and TGF-β in promoting anti-tumor immunity [5]. In addition, advances in adoptive cell therapy have relied on the use of cytokines to create an *in vitro,* highly controlled environment for the optimal development of anti-tumor T cells [6, 7, 8].

Furthermore, Cytokines are excellent candidate factors for the orchestration of promotion of breast cancer development in humans, various cytokines may promote formation of blood vessels within the breast tumor microenvironment, can independently and/or synergistically influence the activity of immune cells, the intensity of inflammatory processes, and the invasiveness of breast cells, therefore contributing to metastatic spread [9]. We hypothesized that patients suffering from breast cancer would have higher levels of certain cytokines that could potentially serve as pre-clinical markers for breast cancer.

Methods

The study has been approved by the Ethics Committee of Institute of Cytology and Preventive Oncology. The approval number of this project is MS-6375658 and all patients provided written informed consent prior to participation, The Institutional Review Board of University approved collection and analysis of all samples.

The final diagnosis of 63 patients with breast cancer was based on biopsy specimen analysis. In order to establish disease staging, all patients underwent ultrasonography, computed tomography, as well as chest x-ray examinations. 45 patients qualified for surgical removal of the breast tumor (Stage I or II according to the Tumor-Node-Metastasis-TNM classification), 14 patients presented with inoperable, locally advanced disease, and 4 patients had distal metastases (3 to the liver and 1

to brain). At the time of the study, none of the patients had received any cytotoxic agents or drugs within the last 6-months before the study. All patients were recruited from individuals hospitalized in the Department of Cell Research and Immunology, Institute of Cytology and Preventive Oncology. The general characteristics of the individuals enrolled in the study, together with a statistical comparison of these features between the examined groups, are presented in Peripheral blood samples 10 mL were collected from all included individuals. Samples were processed immediately according to standard laboratory protocols, and plasma was separated, frozen, and stored at −80°C until further assessment.

Reagents

Hoechst 33258, Sytox green, Trizol reagents were purchased from Invitrogen (Carlsbad, CA, USA). BD OptEIA™ ELISA set were from BD Biosciences (San Diego, California, USA). Antibodies and suppliers were as follows: rabbit polyclonal anti elastase (IgG, Calbiochem, San Diego, California, USA), chicken anti rabbit Alexa fluor 488 (IgG, Molecular probes, Eugene, Oregon, USA), goat polyclonal anti human IL-1β (IgG, Santa Cruz Biotechnology, Santa Cruz, California, USA), rabbit monoclonal anti human TNF-α (D5G9, IgG, Cell Signaling Technology, Denver, MA, USA), mouse monoclonal anti human IL-8 (2A2, IgG1, BD Bioscience Pharmingen, San Diego, California, USA), normal rabbit IgG, normal goat IgG and normal mouse IgG (Santa Cruz Biotechnology, Santa Cruz, California, USA). Percoll was from Amersham Biosciences Corp. (Uppsala, Sweden). Genomic DNA purification kit, 2× PCR Master Mix, 2× Maxima SYBR Green RT-PCR Master Mix were purchased from Fermentas Life Sciences (Vilnius, Lithuania). Recombinant TNF and recombinant IL-8 was purchased from R&D system (Minneapolis, MN, USA). Primers were purchased from Eurofins Genomics India Pvt Ltd (Bangalore, India). 4-Aminobenzoic acid hydrazide (ABAH), Dextran T-500, Dichlorofluorescein diacetate (DCF-DA), Diphenyleneiodonium chloride (DPI), Gelatin from cold water fish skin, Paraformaldehyde, Poly-L-Lysine, Phorbol 12-myristate 13-acetate (PMA), Recombinant IL-1β, RPMI 1640 (Phenol red free) and Tween-20 were purchased from Sigma Aldrich (St. Louis, MO, USA) [10, 11, 12].

Cytokine Estimation

Levels of IL-1, IL-6, IL-8, IL-10, and TNF-α in plasma were determined by selective ELISA kit as per the manufacturer's protocol (OptEIA; BD Biosciences, San Diego, CA, USA) [13, 14].

Cytokines Expression Analysis

Total cellular RNA from PMNs was extracted by Trizol reagent. 5 μg of total RNA was digested with RNase free DNase and reverse transcribed into cDNA using RevertAid™ H minus First Strand

cDNA synthesis kit using oligo (dT) primers as per manufacturer's instruction. cDNA were amplified with PCR (Light Cycler 480 System, Roche Diagnostics). Real-time RT-PCR was performed with a Maxima SYBR Green RT-PCR Kit on Roche light cycler. Reactions were 25 μl volumes including 12.5 μl of 2× Maxima SYBR Green RT-PCR Master Mix, 1 μl of cDNA template, and 0.2 μmol/l primers that were designed to amplify a part of each gene. The three-step PCR protocol applied consisted of 35 cycles of 95°C for 15 seconds; 57°C for 30 seconds for NF-kB. After PCR, a melting curve analysis consisting of 1 cycle: 95°C for 0 second, 70°C for 10 second, 95°C for 0second, Cooling 1 cycle: 40°C for 3 minute was performed to demonstrate the specificity of the PCR product as a single peak. [15].

Statistics

Data are presented as the mean ± standard deviation and were analyzed using SPSS software, version 11.0 (SPSS, Inc., Chicago, IL, USA). A t-test and analysis of variance were used to compare differences between the groups. P<0.05 was considered to indicate a statistically significant difference. To evaluate the effects of continuous variables on breast cancer staging and levels of cytokines, multivariate regression analyses were performed using a stepwise selection method.

Results

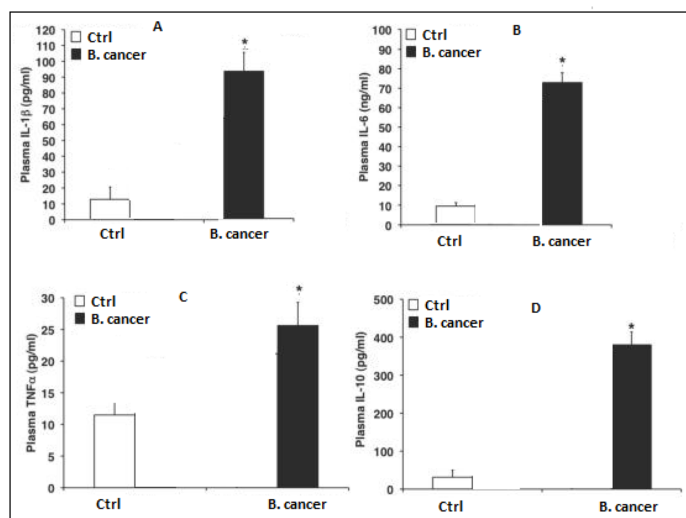

Figure 1.

Plasma levels of IL1-β (A), IL-6 (B), TNF-α (C), IL-10 (D).

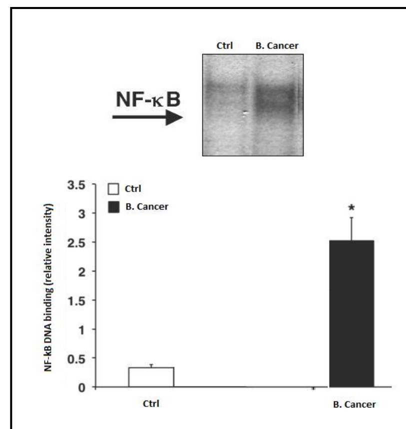

Figure 2.

DNA binding activity of NF-κB in control and breast cancer samples

To examine whether breast cancer is associated with expression of proinflammatory cytokines, we analyzed cytokines levels in the plasma by commercial ELISA kits. Plasma cytokines (TNF-α, IL-1β, IL-6 and IL-10) levels were also markedly elevated in patients with breast cancer in comparison to control (Fig.1). Furthermore, when we compared these cytokines levels between patients with early diagnosed, locally advanced and metastatic disease, we found statistically significant differences more with TNF-α concentrations (data not presented).

To investigate the mechanisms underlying the enhanced inflammatory response in the breast cancer we evaluated NF-κB activity. breast DNA binding of NF-κB was significantly increased in breast cancer in comparison to control (Fig. 2).

Discussion

The generation of potent, specific, and durable anti-tumor immunity requires a variety of cytokines that regulate important functions related to the balance between tumor rejection by antigen-specific effectors cells and suppressive mechanisms that allow tumors to escape immunologic detection [16, 17, 18]. The cytokines are critical for tumor immunosurveillance and have demonstrated therapeutic anti-tumor activity in murine models and in the clinical treatment of several human cancers. Single-agent IFN-α and high-dose IL-2 have been approved in the treatment of melanoma and renal cell carcinoma [19].

For many years, researchers have been highlighting the fact that biochemical and molecular crosstalk between immune and cancer cells is crucial for systemic progression of malignancies [20]. However, very few of these observations have been confirmed in an actual clinical setting. Therefore, we decided to comprehensively analyze a wide panel of cytokines in patients with breast

cancer. We used these data to verify potential associations of cytokines to evaluate the potential clinical diagnostic value of cytokine levels [21, 22].

Several studies have demonstrated a positive correlation between ectopic cytokines like IL-8 expression and the invasive potential of breast cancer cells. In breast cancer cell lines, invasion is directly proportional to IL-8 expression, and overexpression or treatment with recombinant IL-8 promotes invasion [23, 24].

Other members of the IL-2-related cytokine family are under intense investigation for additional anti-tumor applications based on encouraging murine tumor models [25, 26]. In addition, several innovative strategies have been developed that utilize cytokines to promote effective anti-tumor immunity, including bifunctional molecules such as antibody-cytokine fusions, expression of cytokines in recombinant viral vectors, or irradiated whole tumor cells as vaccines, by PEGylation to enhance the kinetics, and for ex vivo manipulation of cells, such as dendritic cells and adoptively transferred T cells [27, 28].

We found that, among all analyzed cytokines, only IL-6, IL-8, IL-10 levels significantly differed between patients with breast cancer and other individuals. From a molecular standpoint, elevations in the levels of these ILs may strongly promote cancer progression in patients with breast malignancies, as these cytokines are involved in the activation of multiple upstream signaling pathways that influence the activity of numerous transcription factors, modulate the cellular proteome at both the genetic and translational level [29].

A variety of innovative strategies for delivery of therapeutic cytokines have been promising in treatment of malignancy. These include cytokine-antibody fusion molecules (immunocytokines), recombinant viral vectors to deliver cytokine genes, transgenic expression of cytokines in whole tumor cells, and chemical conjugation to polyethylene glycol (PEGylation) to improve the kinetics of the cytokine [30].

Several researchers are constantly trying to discover novel substances that could be routinely used in clinical practice as markers of breast cancer [31]. Thus far, these attempts have met with variable success. In our study, we found that systemic levels of IL-6, IL-8, IL-10, and TNFα show potential as diagnostic markers for the detection of breast cancer. Unfortunately, even though our preliminary results are very promising, at this stage, these markers do not seem suitable for independent decision making because of a relatively small sample size. Our study contributes to a comprehensive understanding cytokines of prognostic biomarkers during clinical presentation, and could be useful for developing new strategies for targeted therapies in patients with breast cancer.

Competing interests

The authors declare that there is no conflict of interest.

Author's Contributions

SR contributed to the conception of the idea, literature search and drafting the manuscript. AS and SR contributed to the interpretation of findings, critical evaluation and editing of the manuscript. Both authors critically reviewed and accepted the final version of the manuscript.

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