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Original Research Article

Interleukin-10 Gene Expression in Breast Carcinoma

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Abstract

Introduction: Breast cancer is regarded as the most common cancer among women, and about 6.6% of cases are diagnosed among women 40 years old or younger. Inflammatory cells can regulate the tumor microenvironment and are clearly implicated in tumor development by facilitating proliferation, migration, and survival. Several cytokines, including interferon-a, interleukin (IL)-2, IL-6, IL-8, IL-10, and tumor necrosis factor-a, have essential and coordinated functions in breast carcinogenesis. ***Materials and Methods:*** This is a prospective study and observational study conducted in the Department of Pathology, Dr. V.R.K. Women's Medical College Teaching Hospital & Research Centre over a period of 1 year with a sample of 40 patients. Two to five grams of tumour tissue and another sample from the normal peri-tumoural breast tissue were taken from the resected breast immediately after surgery. Specimen were fixed in 10 % buffered formalin, processed and embedded in paraffin. Paraffin section was stained with hematoxylin and eosin staining for histopathology and grading of the tumour. Immunohistochemistry was done for detection of cytokine IL 10 following a standardized protocol described earlier. ***Results:*** The mean patient age was 50 years (range 30-70years). Even though either side of the Breast can be affected in Ca. Breast, for the reasons not known there is slight preponderance to left side breast. Most of the gross tumours were of size 4-5 cms (n=17 i.e, 42.5%) followed by 5- 6 cm (n=10 i.e, 25%). Majority of the patients are categorized as grade-II i.e. 47.5% of cases and next is grade-I tumours i.e. 30.0% cases. All grade 3 tumours expressed strong expression of IL-10 indicating that probably IL-10 expression is more in high grade tumours. ***Conclusion:*** IL-10 may serve as a useful biomarker with potential prognostic value as there is statistically significant association of high IL-10 mRNA levels and the breast tumour tissue when compared with peri-tumoral tissue. Evidence from various studies suggests that IL-10 within tumour tissue has an important role in initiation and progression of breast carcinoma. This is also supported by high serum IL-10 levels noticed in such patients, although the mechanisms involved in the process are not exactly known.

Keywords: Breast Carcinoma, Interleukin-10, Bloom Richardson Scoring System.

INTRODUCTION

Breast cancer is regarded as the most common cancer among women, and about 6.6% of cases are diagnosed among women 40 years old or younger [1]. Breast cancer accounts for 40% of all types of cancers diagnosed in women and are the third leading cause among all cancer deaths in Western countries, although the death rate has decreased in most developed countries with the help of improved treatments and earlier diagnosis [2].

Over the last few years, several mechanisms have been postulated regarding the etiology and progression of breast cancer [3]. It has been shown that chronic inflammatory responses play essential roles in development of all kinds of cancers. Inflammatory cells can regulate the tumor microenvironment and are clearly implicated in tumor development by facilitating proliferation, migration, and survival [4]. Several cytokines, including interferon-a, interleukin (IL)-2, IL-6, IL-8, IL-10, and tumor necrosis factor-a, have essential and coordinated functions in breast carcinogenesis [5]. As a multifunctional anti-inflammatory cytokine, IL-10 represses the inflammatory response to tumor microenvironments. It is usually secreted by immune cells, such as monocytes, T cells, macrophages (if stimulated appropriately), certain subsets of dendritic cells, and B cells [6].

Inflammation plays a significant role in BC development and is an important part of the BC microenvironment. Interleukin-10 (IL-10) is an important anti-inflammatory and immunomodulatory cytokine in the human immune response. IL-10 is

located on chromosome 1 (1q31-1q32), composed of five exons and four introns. [7] Single nucleotide polymorphism (SNP) is the most common genetic variation. In the SNP database, three promoter SNPs of IL-10, rs1800896 (-1082A/G), rs1800871 (-819T/C), and rs1800872 (-592A/C) were extensively investigated in many diseases. Because they might affect IL-10 gene transcription and translation, resulting in abnormal cell proliferation and cancer development. [8]

The possible mechanism is that IL-10 is activated by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways through its receptor IL-10 R1 which binds to STAT3. Then STAT3 is translocated into the nucleus, where it binds to STAT-binding elements in the promoters of proliferation-related genes. [9] It has been reported that IL-10 gene polymorphism plays an important role in the occurrence and development of cancers such as BC, gastric cancer, lung cancer. And some studies reported the high IL-10 expression levels in the BC paraffin section and its expression is correlated with worse outcomes in patients with malignant tumors. [10]

MATERIALS AND METHODS

This is a prospective study and observational study conducted in the Department of Pathology, Dr. V.R.K. Women's Medical College Teaching Hospital & Research Centre over a period of 1 year with a sample of 40 patients.

Inclusion Criteria:

Properly labelled mastectomy specimens
Fresh unfixed tissue samples
Tumour tissue and adjacent normal tissue for analysis
Complete clinicopathological data

Exclusion criteria

Patients with in-situ cancers and patients with tumour size less than 1 cm were excluded.

Sample Collection and Immunohistochemistry

Two to five grams of tumour tissue and another sample from the normal peri-tumoural breast tissue were taken from the resected breast immediately after surgery. Specimen were fixed in 10 % buffered formalin, processed and embedded in paraffin. Paraffin section was stained with hematoxylin and eosin staining for histopathology and grading of the tumour. Immunohistochemistry was done for detection of cytokine IL 10, ER, PR and HER2 following a standardized protocol described earlier [16]. A set of 5 µm-thick paraffin sections were cut. Formalin fixed tissues were deparaffinized in xylene and rehydrated gradually by dipping in 100 %, 95 % and 70 % alcohol and distilled water.

Antigen retrieval was done by microwave treatment in citrate buffer (10 mM, PH-6.0). It was followed by serum blocking, application of diluted (1:100) primary antibody IL-10 and incubation overnight at 4 °C. The secondary antibody application and staining was done following a standard protocol using Vectastain universal kit and Diaminobenzidine (DAB) substrate kit. The sections were then counterstained with hematoxylin, followed by dehydration and finally mounted in DPX and evaluated under microscope. Immunoreactivity in the tissue was judged independently by two experts who were blinded to the clinical data. Negative controls were included in each slide run with omission of primary and secondary antibodies. Interleukin10 expression in tumour tissue was regarded as positive when 10 % or more tumor cells were positive.

RESULTS

The mean patient age was 50 years (range 30-70years). Majority of cases were seen in 4th to 6th decade**.**

Table 1: Distribution of Age

Age Group (Years)	Number	Percent
30-40	03	7.5%
41-50	14	35%
51-60	17	42.5%
61-70	06	15%
Total	40	100%

The age group distribution of the subjects in the present sample is depicted in Table 1. Accordingly, the commonly affected age group 51-60 years (42.5%) followed closely by 41-50yrs (35%). The mean age of the sample is 49 years, the maximum and minimum age being 61 years and 37 years respectively.

Table 2: Distribution of Lesions According to Side Affected

Laterality of Tumour	Number	Percent
Right Breast	17	42.5%
Left Breast	23	57.5%
Total Cases	40	100%

Even though either side of the Breast can be affected in Ca. Breast, for the reasons not known there is slight preponderance to left side breast in table 2.

Table 3: Size Distribution of Tumour in our Study Sample

Size	Number	Percent
2 X 3 Cm	3	7.5%
3 X 4 Cm	5	12.5%
4 X 5 Cm	17	42.5%
5 X 6 Cm	10	25%
6 X 7 Cm	5	12.5%
Total	40	100%

Most of the gross tumours were of size 4-5 cms (n=17 i.e, 42.5%) followed by 5- 6 cm (n=10 i.e, 25%)

Table 4: Distribution of Cases According to Histological Grade by Bloom Richardson Scoring System

Histological Grade	Number of Cases	Percent
Grade-I	12	30.0%
Grade-II	19	47.5%
Grade-III	09	22.5%
Total Cases	40	100%

Majority of the patients are categorized as grade-II i.e. 47.5% of cases and next is grade-I tumours i.e. 30.0% cases in table 4.

Table 5: Distribution of Lymph Node Metastasis in Study Subjects

Lymph Node Spread	Number of Cases	Percent
Reactive Lymph Node	16	40%
Metastatic Lymph Node	24	60%

Table 6: Mean RQ Values for IL-10

	Neoplastic tissue	Surrounding peri-tumoural tissue
No of cases showing strong expression	**33 (82.5%)**	**04 (10%)**
No of cases showing weak expression	**07 (17.5%)**	**36 (90%)**

All grade 3 tumours expressed strong expression of IL-10 indicating that probably IL-10 expression is more in high grade tumours.

DISCUSSION

Breast cancer is the most common cancer among women, comprising about one fourth of all female cancers worldwide [11]. It is well-recognized that the functional status of immune system has direct bearing on breast cancer. But, the exact biological mechanism involved in breast cancer pathophysiology is still not clearly understood [12]. Numerous reports suggest that modulation of the innate and adaptive response though B-cells, T-cells, macrophages, dendritic cells (DC), natural killer (NK) cells and other mediator by the immune system is critical in initiation and progression of breast cancer. Role immune system and inflammation in breast cancer has been extensively reviewed elsewhere [13].

Cancer immunotherapy employs a variety of options, including enhancement of stimulatory signals required for T-cell activation, genetically engineered cells to secrete cytokines to enhance the intensity of anti-tumor immune response, and direct exogenous therapeutic use of cytokines. Immunoregulatory cytokines are an important component of biological milieu associated with breast cancer [14]. Several cytokines including Interferon (IFN)-α, β and γ; Interleukin-2 (IL-2), IL-6, IL-10, Tumor Necrosis Factor-α (TNF-α) are known to play important role in coordinated manner in breast carcinogenesis [15].

IL-10, initially known as cytokine synthesis inhibitory factor (CSIF), is primarily a potent antiinflammatory cytokine that inhibits gene expression and T-cell/macrophage cytokine synthesis and inhibits their antigen-presenting capacity [16]. It suppresses production of IL-1α, IL-1β, TNF-α, IL-6, IL-8, IL-12, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein-1α (MIP-1α), Regulated upon activation, normal T-cell expressed, and secreted (RANTES), leukemia inhibiting factor, and IL-10 itself [17]. IL-10 also inhibits IFN-γ synthesis by activated Th-cells and peripheral blood

mononuclear cells (PBMC) and induces mast cell proliferation [18].

IL-10 is also a strong stimulator of B-cell differentiation for immunoglobulin secretion [19]. IL-10 inhibits nuclear factorκB (NF-κB) translocation considered as a mechanism for inhibiting immediate-early pro-inflammatory response [20]. IL-10 is located in chromosome 1 and mature human IL-10 consists of 160 amino acids with molecular weight of approximately 18 kDa in monomeric form. The homodimeric protein with single transmembrane domain subunits binds to class II cytokine receptor [21]. Human IL-10 contains four exons which show 73% amino acid homology with murine IL-10. IL-10 structure, its receptor along with their role in physiological functions and pathological conditions like inflammatory diseases have been widely reviewed elsewhere [22].

Although tumor promoting activities of IL-10 are known, it is predominantly showed to have anti-tumor property. Some of the proposed mechanisms of anti-cancer activity of IL-10 includes- activation of NKcells [23], synergistic activation of cytotoxic T-lymphocyte for maintenance of $CD8^+$ and CD4+ mediated [24] anti-tumor response, enhancement in surface expression of MHC antigen for maintaining susceptibility of cancer cells to NK-cells [25], enhancement in tumor infiltration by neutrophil and macrophages, and finally prevention of angiogenesis and invasiveness through induction of metalloproteinase inhibitor [26]. Kundu et al. [27] studied anti-tumor and anti-metastatic properties of IL-10 in murine model showed that tumerogenicity in immunocompetent mice was significantly abrogated by IL-10. Later, it was found that mice subjected to immunization with IL-10 expressing tumor cells promoted the loss of tumorigenicity and induced a protective anti-tumor immune response which was mediated either by NKcells or $CD8^+$ T-cells [28].

CONCLUSION

IL-10 may serve as a useful biomarker with potential prognostic value as there is statistically significant association of high IL-10 mRNA levels and the breast tumour tissue when compared with peri-tumoral tissue. Evidence from various studies suggest that IL-10 within tumour tissue has an important role in initiation and progression of breast carcinoma. This is also supported by high serum IL-10 levels noticed in such patients, although the mechanisms involved in the process are not exactly known.

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