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ORIGINAL ARTICLE

Do Interferon Regulatory Factors 3 and 7 Play Role in Breast Cancer?

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ABSTRACT

Background*: Breast cancer is a disease whose progression varies from atypical ductal hyperplasia to ductal carcinoma in situ and invasive ductal carcinoma. Interferon regulatory factor-3mediated apoptosis is independent of IFN or p53 and a big debate about whether IRF3 is considered as a tumor suppressor gene or not. The IRF7 level was shown to be silent in some metastatic breast cancer cell lines as it was proved that silencing of IRF7 in breast cancer cells promotes bone metastasis.* ***Objective****: We conducted this work to study the effect of IRF3and IRF7 in disease progression of cancer breast.* ***Methodology****: IRF3 and IRF7 were measured in 48 cancer breast patients and 48 normal control persons by using ELISA technique. Paraffin block for the breast tumor was done for all cases to assess the stage and the grade of cancer. Distant metastasis was diagnosed through chest x-ray, abdominal sonography and bone scan.* ***Results:*** *It was found that IRF3 and IRF7 were higher in advanced breast cancer compared with normal control group.* ***Conclusion****: To assess the effect of IRF3 and IRF7 in breast cancer is still in need for more researches.*

INTRODUCTION

Breast cancer is a major health problem that is regulated by several mediators which vary from atypical ductal hyperplasia (ADH), ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC)[1]. These signals were produced whether from the tumor itself and/or adjacent stroma, promote tumor vasculature, tumor proliferation, survival, and metastasis to different organs[2].

Several researches reported that interleukins (ILs), interferons (IFNs) and interferon regulatory factors (IRFs) are present in both tumor microenvironments and in metastatic sites as some of these cytokines stimulate while others inhibit breast cancer proliferation[3]. The role of these cytokines in disease progression, as markers of disease stage, and as novel treatment strategies requires further attention.

IRFs are group of transcription factors responsible for the regulation of interferon expression [2]. All these factors contain N-terminal which constitute of 120 amino acids. By consequence, these amino acids fold in order to the interferon consensus sequence (ICS) that is present on the interferon genes. For the remaining parts of these factors, their function depend upon the specific function of the protein[4]. It is very obvious that IRFs play an important role in the immune system[4,5], the immune modulation[5-7] and the haematopoietic differentiation[6-8].

These factors had DNA-binding domain (DBD)[9] responsible for binding to the IRFs present in the IFN-β promoter[10]. The mammalian IRF family is comprised of 9 members: IRF-1, 2, 3, 4/PIP/LSIRF/ICSAT, IRF-5, 6, 7, 8/ICSBP and 9[11-12].

IRF-3 had several functional domains such as the DBD, the C-terminal IRF association domain as well as the several regulatory phosphorylation sites [13]. Usually, IRF3 is found in an inactive cytoplasmic form that formed a complex with CREB binding protein [14]. By turn, this complex is translocated to the nucleus and lead to the activation of the interferons alpha and beta and other interferon-induced genes [15-16].

There was a big homology between IFN3 and IRF7. Type I IFN is induced through IFN3 after activation upon engagement of Pattern Recognition Receptors in response to invading pathogens [17].

It was found that it has a major role in apoptosis in response to the DNA damaging agents and in the absence of viral infection[18-19]. Therefore, the mechanism of IRF3-mediated apoptosis is independent of IFN or *p*53 but dependent on (TRAIL/ Apo2L) tumor necrosis factor-related apoptosis-inducing ligand or Apo 2 ligand that is transcriptionally activated by IRF3 [20-21]. The over expression of IRF3 can inhibit the growth of cancer cell lines *in vitro* and *in vivo* thus, blocking DNA synthesis& inducing apoptosis [22-23].

There was a big debate about whether IRF3 can be considered as a tumor suppressor gene or not. A study reported the normal IRF3 expression in lung epithelial

cells and its altered expression in lung cancer according to the two variants IRF3 protein which has not been established [24] and still this point under study.

For the relationship between serum IRF3 expression and the prognosis of malignancy, several studies were conducted . They found that the higher IRF3 expression in patients, the better prognosis after curative resection of non-small cell lung cancer, in comparison to those with a poor prognosis [25]. Also, IRF3 can induce tumor suppression by transcriptional reprogramming of macrophages [26-28].

IRF7 had a strong regulation of many interferon-alpha genes as well as its strong role in the transcriptional activation of virus-inducible cellular genes, including the type I interferon genes [29]. Also, the expression of IRF7 is restricted to the lymphoid tissue, whereas it is inducible in many tissues [30].

For the relationship between IRF7 level and malignant cells, it was found that it is silenced in some metastatic breast cancer cell lines, because it can help these cells to avoid the host immune response. In animal models, restoring IRF7 to these cell lines reduced metastases and increased host survival time [31].

Moreover, IRF7 has antitumor effects due to the induction of type I IFN where the growth of the tumor suppresses IFN pathway as an early event in the development of cancer [32]. IRF-7 is a transcriptional target of the tumor-suppressor gene BRCA1 mutation that is one of the predisposing factor for breast and ovarian cancer, also, BRCA1 is one of the essential process in cellular DNA repair, cell-cycle regulation and chromatin remodeling [33-35]

In addition, it was proved that the silencing of IRF7 in breast cancer cells promotes bone metastasis and these findings were confirmed in a study conducted on 800 patients in which high expression of IRF7 regulated genes in primary tumors was associated with prolonged bone metastasis free survival [36].

The over-expression of IRF7 in macrophages, induced the production of type I IFN and increased the expression of genes encoding TRAIL, IL-12, IL-15, and CD80 and negatively regulated the transcription of pro-tumorigenic genes .Furthermore, IRF7 transduced macrophages express a cytostatic activity on different cancer cell lines [37-38]. The aim of the work is to study the effect of IRF3 and IRF7 in disease progression of cancer breast.

METHODOLOGY

This study was conducted in the Clinical Pathology Department Faculty of Medicine Suez Canal University Hospital; Ismailia, Egypt from January 2018 to January 2019.

The studied population was 48 cancer breast patients and 48 healthy persons as a control group. Blood samples were collected from all patients. IRF3 and IRF7 levels were measured by ELISA Kit purchased from CUSABIO, China. The test was performed on PVC microliter plate. The plate was read within 30 min after adding the stopping reagent at 450 nm. The ELISA reader is STAT FAX, USA.

Also, a paraffin block for the breast tumor was done for all cases to assess the stage and the grade of cancer by examining the skin, the lymph node invasion & the breast tissue. Each block was stained by Heamotoxin and Iodine (H &I) and was evaluated by a pathologist to confirm the diagnosis .

Moreover, assessment of distant metastasis was mediated through chest x-ray, abdominal sonography and bone scan.

Ethical consideration

Consent for an interview was taken from each participant, who was assured about the confidentiality of his/her information.

RESULTS

The studied population was 48 cancer breast patients with an age range 43.24 ± 11.63 (Mean ± SD)

Table 1: The demographic data of the studied population

Parameter	Patients number n=48	
Age/ years (Mean ± SD)	43.24 ± 11.63	
Tumor size cm	3.34 ± 1.5	
Lymph Nodes Metastasis	**Negative** (-ve node involvement) 11	**Positive** (+ve node involvement) 37
Pathology type	**Invasive ductal carcinoma** 46	**Invasive Lobular carcinoma** 2
Distant Metastasis	**Present** 3	**Absent** 45

As shown in table(1) the total number of patients were 48 cancer breast patients with an age range 43.24 ± 11.63 and the tumor size of 3.34 ± 1.5(Mean ± SD) respectively. The pathology type was invasive duct carcinoma in 46 patients and invasive lobular carcinoma in 2 patients. 37/48 cases have malignant involvement of lymph nodes with 3 recorded cases suffering from distant metastasis.

As shown in table (2), the levels of IRF3 and IRF7 in breast and control groups were 72.54±6.7, 27.77±9.7 for the breast cancer group and 4.3±3.8 & 3.7±0.8 for the control group with a statistical significance.

Table 2: The mean ± SD of IRF3 and IRF7 levels in breast cancer and normal controls groups.

Group	IRF3	IRF7	*P value*
Breast cancer group	72.54±6.7	27.77±9.7	0.004*
Control group	4.3±3.8	3.7±0.8	0.004*

**P<0.05 significant*

Table 3: Mean ± SD of IRF3 and IRF7 levels in different stages of breast cancer

Stages of breast cancer	No. of patients	IRF3	IRF7
I (Mean ± SD)	5	22.60±10.31	18.60±6.46
IIa/Iib (Mean ± SD)	29	45.78±15.6	20.12±7.9
IIIa/IIIb (Mean ± SD)	11	51.1±18.6	26.6±20.30
IV (Mean ± SD)	3	85.3±13.7	43.3±16.90

**P value between groups is significant <0.05*

Table (3) showed the levels of IRF3 and IRF7 in cancer breast patients according to the pathological stages. In stage I, the levels of their IRF3 and IRF 7 were 22.60±10.31 and 18.60±6.46 respectively. In groups IIa/IIb, the IRF levels of the 29 patients were 45.78±15.6 for IFR3 and 20.12±7.9 for IRF7. In groups IIIa/IIIb, the IRF3 and IRF7 were 51.1±18.6 and 26.6±20.30 respectively. Lastly, for group V, the levels of IRF3and IRF7 in the 3 patients were 85.3±13.7 and 43.3±16.90 respectively.

Table 4: Factors associated with poor prognostic markers in female breast cancer patients

Factors	Statistical Significance	
	Univariate analysis *p-value*	**Multivariate analysis *p-value***
Age (years)	<0.05	NS
IRF3	<0.05	NS
IRF7	<0.05	<0.05

*p<0.05 significant

DISCUSSION

Cancer occurs when there is an imbalance between cell growth and death. In the present study, we tried to express the role of IRF3and IRF7 as anti-tumor factors activity which suppress distant metastasis in patients suffering from cancer breast with different stages and grades. More than 20 years, since the discovery of IRF are playing big roles in regulating/initiating host immunity downstream danger sensors through regulation of hematopoietic differentiation and controlling of cell-cycle and apoptosis and thus oncogenesis and antitumor immunity in several cancers are their main effects [39-40].

Although IRF3 is a part of a family of IFN regulatory transcription factors that has a potential antioncogenic functions, so the expression of the dominant negative IRF3 mutant which inhibits the expression of IRF3 target genes induced oncogenic transformation *in vitro* and *in vivo* [23]. In normal cells, IRF-3 inhibits cellular proliferation and induces silencing through the *p*53 function. Also, it was found that IRF3 inhibits tumor growth through the expression of chemokines such as MIP-1, CCL5/RANTES, and CXCL10/IP-10 *in vivo* & IFN-β, tumor necrosis factor-α, and IL 6 *in vitro* [39].

For instance, IRF3 was highly expressed in early (stage I) and advanced (stage II and III) cancers who survived which suggests that this gene may suppress tumor progression and metastasis [37] . It has been demonstrated that this factor shows a relatively higher expression in tumors with favorable outcome [38].And this is coincident with our study as IRF3 is expressed in stage I and highly expressed in advanced stages (II,III&IV) as compared with the control groups.

On the other hand, activation of IRF7 is crucial for the induction of IFN-I production [34] where they control clearance of apoptic cells, clearance &angiogenesis [26] as when they are activated induce tumoricidal activity through phagocytosis and ADCC [33].Macrophages and tumor cells are known that they have the power to release certain pro-tumorigenic factors, used for macrophage adoptive transfer in cancer therapy. Interestingly, it was observed that the IRF7 active mutants in macrophages down-regulate the transcription of pro-angiogenic and metastatic genes (such as *VEGF*) as IRF7 may increase the antitumor properties of macrophage to reduce their protumorigenic effects [36]

In vivo, primary macrophages transduced with Ad-IRF7 mediate their antitumor effects through the secretion of type I IFN (for type I IFN-sensitive tumors), via recruitment and polarization of other immune cells and down-regulation of expression of the genes which promote metastasis and angiogenesis [30].

The immune system plays a major role in suppression metastasis as human cancer correlates with disease progression [34].As it was found that low expression of IRF7 in primary breast tissue increase the risk of metastasis in those patients [32-34]. All of these studies are consistent with our study as it was expressed in our work that IRF7 play a major role in breast cancer rather than IRF3and was expressed in higher level in advanced stages in breast cancer rather than the normal control group .

CONCLUSION

IRF3 and IRF7 play an important role in the immune system and have great implication in tumor development/progression. From our results, they play a consistent role in disease progression of cancer breast with different stage and metastasis especially IRF7. Finally, the relationship between IRF and oncogenesis are still in need for more research.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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