Original Article

Correlation of FOXA1 protein expression and ER, PR and Her2 expression in breast cancer patients

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Abstract

Forkhead box protein A1 (FOXA1) is pioneer factor has a dual role either as growth stimulator or a repressor and the significance role of this protein as a therapeutic biomarker still controversial, so the aim of this study was to identify the precise role of FOXA1 protein expression in treatment of BC patients by immunohistochemical (IHC) staning method. In this study, seventy three tissues samples embedded in wax block were taken from BC patients with (52.71+11.55) years who had surgery between 2014-2020 and thirty eight of normal (non tumoral) breast tissues samples with (54.17+10.84) years as a control group.

There was a significant association between positive FOXA1 protein expression and Luminal A and Luminal B of BC (P=0.011). Moreover, FOXA1 positivity was elevated significantly in patients with positive Estrogen receptors (ER) expression (P=0.0001), positive Progesterone receptor (PR) expression (P=0.016). Furthermore, there was no significant correlation between FOXA1 expression in BC patients and Her2 expression (P=0.764). FOXA1 positive expression can be employed as a therapeutic marker to predict hormone responsiveness.

Keywords: FOXA1, IHC, ER, PR, Breast cancer.

1. Introduction

The most prevalent kind of cancer in women worldwide is BC. It makes up 25% of all cancers, and the number of cases has more than doubled in the last 25 years (Ghoncheh et al., 2016). ERs are expressed in most of BC. Two-thirds of all BCs are luminal subtypes, and ER activity is essential for their growth (Carroll et al., 2005). Because estrogen signaling is necessary for the progression of ER-positive BC, ER inhibitors can be used to treat the condition (Ali and Coombes, 2002).

Tamoxifen, for example, is a particular ER modulator that has been applied to the treatment of BC patients. However, ER-positive BC frequently develops tamoxifen resistance after long-term treatment; thus, overcoming tamoxifen resistance with a novel therapeutic strategy is required to treat BC patients (Rondón-Lagos et al., 2016). Even though we don't fully understand how tamoxifen resistance works at the molecular level (Yde et al., 2012). Approximately 70% of BC patients who are positive for ER / positive for PR response to hormonal therapy, while the remaining 30% do not.

Additional markers that can be used to improve hormone predictive response may significantly advance the management proposal for a large number of BC patients (Parmar et al., 2021).

Expression of FOXA1 correlates with ER positivity. Good prognosis in luminal (A) BC which responds well to anti-estrogen treatment (Thorat et al., 2008). Additionally, there was a negative correlation between recurrence and FOXA1 expression (Ademuyiwa et al., 2010).

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It has been hypothesized that treatments increasing FOXA1 expression or activity, when combined with antiestrogens, may prevent the progress of estrogen independence (Nakshatri and Badve, 2007). There have been other studies that looked at FOXA1 predictive value, but their scope was limited (Habashy et al., 2008; Albergaria et al., 2009). So, this study carried out to investigate the relationship between FOXA1 expression and ER, PR and Her2 expression in BC patients.

2. Materials and Methods:

This research was carried out between September 2021 and May 2022 at the University of Kufa's Faculty of Medicine, Middle Euphrates Unit for Cancer Research.

2.1. Collection of Samples:

2.1.1. Patients Group:

This study is carried out on seventy three cases of BC their Paraffin blocks gathered from surgical BC patients between 2018 and 2022. The archives of two private laboratories were used to collect the data retroactively. Each pathologic specimen was histologically reevaluated by two pathologists. After getting the BC paraffin block from the private laboratories, the data of death and hormone therapy intake followed up for these blocks from middle Euphrates Center of oncology and obtained from it.

2.1.2. Control group:

Thirty eight blocks of normal non-tumoral breast tissue has been gathered randomly from archives of three private laboratories during the collection of BC tissue blocks, and also, re-evaluated by two pathologist to ensure their normality.

2.2. IHC Procedure:

One hundred eleven blocks of Paraffin were prepared for FOXA1 by Labeled Streptavidin Biotin (LSAB) method, Five µm thickness sections have been cut from paraffin-embedded blocks and set on positively charged slides.

2.2.1. Results of Staining:

Only tumor cells that have nuclear positivity are included for assessment. Evaluation takes into account both staining ratio (the percentage of stained cells) and intensity. The staining ratio is recorded as 0 (0%), 1 (>0% to 25%), 2 (>25% to 50%), 3 (>50% to 75%), and 4 (>75%), whereas the intensity was marked as 0 (negative), 1 (weakly positive), 2 (moderately positive), or 3 (strongly positive). The overall protein expression score = staining intensity score × staining extent score, which was graded and divided into five scores according to this equation, 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%) (Yuan et al.,2020).

2.3. Statistical Analysis:

The data were analyzed using SPSS version 21. Numeric variables were reported as mean ± SD and nominal variables as number and percent when variables were regularly distributed, the Student t-test is used to compare means between two groups, whereas the Mann Whitney U test was used to do so, when the distribution of the variables was not normal. When the chi-square test cannot be used because it is invalid, the corrected chi-square test was utilized. When the P-value was less than 0.05, it was considered significant.

3. Results

3.1 BC Molecular Subtypes FOXA1 protein expression:

There was a significant association between positive FOXA1 protein expression and Luminal A 22 patients (62.9%) and Luminal B 16 patients (80.0%) of BC in comparison with HER2/neu Subtype 3 patients (30%) and 2 patients (25.0%) Triple Negative Subtype (P=0.011) as showed in (Table 3-1).

Table 3-1: BC Molecular Subtypes and FOXA1 protein expression.

Molecular Subtypes	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Luminal A	Count	22	13	35
	%	62.9%	37.1%	100 %
Luminal B	Count	16	4	20
	%	80.0%	20.0%	100%
HER2/neu type	Count	3	7	10
	%	30.0%	70.0%	100%
Triple negative type	Count	2	6	8
	%	25.0%	75.0%	100%
Total	Count	43	30	73
	%	58.9%	41.1%	100%

P=0.011

3.2 Association of ER, PR and Her2 with FOXA1 protein expression:

Positive FOXA1 expression in BC patients is increased significantly in ER positive patients 38 patients (71.7%) in comparison with ER negative 5 patients (25.0%) (P=0.0001) as seen in (Table 3-2).

Table 3-2: Estrogen receptor and FOXA1 protein expression.

ER Receptor	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Positive	Count	38	15	53
	%	71.7%	28.3%	100%
Negative	Count	5	15	20
	%	25.0%	75.0%	100%
Total	Count	43	30	73
	%	58.9%	41.1%	100%

P=0.0001

Positive FOXA1 expression in BC patients was increased significantly in PR positive patients 32 patients (69.6%) in comparison with PR negative 11 patients (40.7%) (P=0.016) as seen in (Table 3-3).

Table 3-3: Progesterone receptor and FOXA1 protein expression.

PR Receptor	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Positive	Count	32	14	46
	%	69.6%	30.4%	100%
Negative	Count	11	16	27
	%	40.7%	59.3%	100%
Total	Count	43	30	73
	%	58.9%	41.1%	100%

P=0.016

There was no significant correlation between FOXA1 expression in BC patients and Her2 expression (P=0.764) as seen in (Table 3-4).

Table 3-4: Her2 receptor and FOXA1 protein expression.

Her2 Receptor	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Positive	Count	17	13	30
	%	56.7%	43.3%	100%
Negative	Count	26	17	43
	%	60.5%	39.5%	100%
Total	Count	43	30	73
	%	58.9%	41.1%	100%

4. Discussion:

The result of this study revealed that significant association between positive FOXA1 protein expression and Luminal A and Luminal B while Her2 and TNBC subtypes were inversely correlated with positive FOXA1 expression. Similarly, numerous studies have shown the same results (Thorat et al., 2008; Mehta et al., 2012). Luminal A has a better outlook than subtype B, and it makes more ER. It might be that ER works differently in luminal A cancers than in luminal BC. This could be because other factors such as transcription factors, co-activators, and co-repressors affect how ER works (Thorat et al., 2008). According to Dai et al. (2019), FOXA1 transcriptionally suppresses the production of SOD2 and IL6, making it a subtyping marker that distinguishes triple negative BC from the luminal subtype and can enhance malignancy. When compared to samples of the other subtypes, TNBCs only slightly had a higher expression of SOD2, which is similar to earlier finding that clarified cells proliferated more quickly and underwent, there was reduced apoptosis when FOXA1 was suppressed and the opposite pattern was seen when SOD2 was inhibited.

Histone deacetylase 7 (HDAC7) forms a ternary complex with FOXA1 and ER that is required for estrogen-directed repression of the cell cycle suppressor. Transducin-like enhancer protein 3 (TLE3), a different corepressor, via binding to FOXA1, indirectly interacts with chromatin at ER-responsive genes when estrogens were not present. Suppress gene expression by TLE3 recruits HDAC2. TLE3 was associated with approximately 50% of ER-FOXA1 binding events in the luminal MCF7 cell line, indicating that TLE3 and FOXA1 work cooperatively to suppress expression. In fact, TLE3 was brought in by FOXA1 to the chromatin to suppress the transcription of the estrogen-responsive gene (Jangal et al., 2014). As a result, FOXA1 is a crucial regulator of hormone response in BC, serving as an actual pioneering factor to develop ER binding patterns (Glont et al., 2019).

The result of this study confirmed that significant association between positive FOXA1 protein expression and positive ER and PR protein expression in BC patients while Her2 expression didn't correlated with FOXA1 expression,. In

accordance with the present results, previous studies have demonstrated the same findings (Kawase et al., 2015; Horimoto et al., 2020; Abelzaher et al., 2022). According to Hu et al. (2014), FOXA1 can either stimulate or suppress growth. It works as a stimulator by binding to chromatinized DNA, opening the chromatin, and enhancing (ER) binding to its target genes. FOXA1 does more than just change the way ER works. Additionally, it connects directly to the promoter of the ESR1, which is required for BC cells to produce ER mRNA and protein. Therefore, FOXA1 is necessary for both ER activity and expression. Two potential growth inhibitory repressor mechanisms were discovered: stopping the spread of metastatic disease and differentiating ER pathway regulation. On the other hand, FOXA1 reduced the development of cells and blocked the ER pathway in ER positive cells. In individuals with ER positive BC, FOXA1 positive expression can be employed as a therapeutic marker to predict hormone responsiveness.

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