RESEARCH ARTICLE

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Evaluation of LGR5 Cancer Stem Cell Marker Expression in Breast Cancer and Its Relationship with Hormonal Profile and Clinical Pathological Features

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Abstract

Background: Due to the high prevalence of breast cancer and the importance of evaluating new prognostic criteria for effective treatment of these patients, this study was performed to investigate the role of LGR5 in breast cancer and its relationship with hormonal and clinicalopathological features of the disease. **Methods:** This cross-sectional study was performed on breast cancer tissue samples in the archives of the pathology department of Firoozabadi Hospital in Tehran between 2019 and 2021. Inclusion criteria included invasive ductal carcinoma and exclusion criteria were preoperative chemotherapy. Blocks were examined for LGR5 marker expression by IHC method using LGR5 monoclonal antibody kits (Abcam). The expression pattern of LGR5 marker was cytoplasmic and cells presenting brown staining in the cytoplasm were considered positive for this marker and in terms of distribution and severity of staining were divided into three groups: mild, moderate and severe. **Results:** This study was performed on 60 patients with breast cancer with a mean age of 55.5±9.7. Most of the patients (55%) were in grade II. The KI67 marker was positive in 45 cases (75%) and the HER2 marker in 14 cases (23.3%) and 8 cases (13.3%) were triple-negative. The expression severity of staining of LGR5 marker in 41 cases (68.3%) was moderate and the distribution of marker expression in 31 cases (51.7%) was moderate. No significant relationship was observed between LGR5 expression severity and tumor characteristics. **Conclusion:** LGR5 marker is expressed in a remarkable percentage of breast cancer patients and has no significant relationship with tumor characteristics.

Keywords: Breast neoplasm- cancer stem cell- LGR5

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Introduction

Breast cancer, as a common cancer in women, is the second leading cause of cancer death in women aged 20-59 after lung cancer (Ferlay et al., 2004). It accounts for 26% of all newly diagnosed cancers in women and is responsible for 15% of cancer-related deaths in women (IARC, 2006). Risk factors for breast cancer include age, increased exposure to estrogen, older age at first live birth, positive genetics and family history, personal history, radiation exposure, obesity, consumption of fatty foods, and alcohol consumption (Foulkes, 2007; Mirmalek et al., 2010). One of the biomarkers that has received a lot of attention both as a prognostic factor and as a factor in estimating the response to treatment in breast cancer is HER-2 proto-oncogene. HER-2 overactivity occurs in about 20-30% of breast cancers (Anim et al., 2005; Liu et al., 2010; Mofid B et al., 2009). Evidence suggests that most, but not all, malignancies are derived from cancer stem cells (Visvader et al., 2008). The presence of cancer stem cells explains why only a small minority of cancer cells are able to multiply inside the tumor (Jeon et al., 2006; Souza et al., 2008). CSC (Cancer Stem Cell) is a subset of tumor cells that has the characteristic of stem cells, i.e., the ability of self-renewal (self-renewal) and differentiation, first expressed in 1997 for blood malignancies and then in many solid tumors (Sanders et al., 2011; Wilson et al., 2011). Resistance to common cancer therapies and tumor recurrence appears to be related to selective resistance of cancer stem cells (Choi et al., 2009). LGR5 (leucine-rich-repeat containing G-protein-coupled receptor 5) is from the G-protein coupled receptor family which in 2007 is known as the Cancer Stem Cell Maker. This protein activates the Wnt signal (Barker et al., 2007; Becker et al., 2008).

Despite extensive research on molecular markers of breast cancer, differences in the results and the effects of demographic factors on the status of these biomarkers indicate the need for such studies in different regions (Foulkes, 2007; Siziopikou et al., 2006). On the other

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hand, knowing the prognostic factors in breast cancer and knowing the relationship between them helps to more easily estimate the outcome of the disease, so this study was performed to investigate the role of LGR5 in breast cancer and its relationship with clinical and pathological features of the disease.

Materials and Methods

This cross-sectional study with ethical code IR.IUMS.REC.1398.725 was performed on breast cancer tissue samples in the archives of the pathology department of Firoozabadi Hospital in Tehran between 2019 and 2021. The sample size was determined according to P = 0.5 (Hou et al., 2018) and 95% confidence level and d = 0.125 calculated 60 cases.

To prepare immunohistochemical slides, 4 to 5 micron thick sections of the relevant blocks were placed on the slide and then placed at 37°C for 16 hours and then at 60°C for 1 hour. In order to de-paraffinize in three steps, it was placed in xylene, absolute ethanol and ethanol solutions at 96, 80 and 70 degrees (2 times in each solution for 5 minutes in each time) and washed with running water. The tissues were then placed in buffer (PH = 8.7) and transferred to the macrowave at maximum power until the buffer reached boiling point. The macrowave power was then reduced by 40% and after 15 minutes the tissues were removed and placed at room temperature for 15 minutes. After rinsing with running water and TBS buffer (PH = 9) to neutralize endogenous peroxide, peroxidase inhibitor solution was poured on the slides for 10 minutes and then rinsed with running water and TBS buffer (2 times each time 5 minutes) Excess buffer was removed from the surface of the slides and the texture was determined using a DAKO pen. At this stage, the protein block solution was poured on the slides for 5 minutes and after washing the slides were transferred to a wet chamber and using LGR5 monoclonal antibody diagnostic kits (Abcam company) with a dilution of 1/1,000 and the surface of the tissues was completely covered for 45 minutes. Then, after washing with TBS buffer, the surface of the slides was covered with Post primary block solution for 30 minutes and washed with TBS buffer. Then it was covered with DAB working solution for 30 minutes and the TBS buffer was washed and then the surface of the slides was covered with DAB working solution for 5 minutes to develop peroxidase activity and then the slides were washed with water and the slides were dehydrated at increasing alcohol concentration and finally It was placed in xylene.

Slides prepared by two experienced pathologists were evaluated for LGR5 marker expression. The expression pattern of LGR5 marker was cytoplasmic and cytoplasmic brown color was considered positive and in terms of distribution and severity of staining were divided into three groups: mild, moderate and severe.

Statistical software SPSS 20 and Fisher's exact test were used to analyze the data. P <0.05 was considered as a significant level.

Results

This study was performed on 60 patients with breast cancer with a mean age of 55.5 ± 9.7. The age range of the patients was from 33 to 70 years. The pathological characteristics of the studied tumors are shown in Table 1. As shown in the Table 1, most of the examined patients (55%) were in grade II histopathology. The KI67 marker was positive in 45 cases (75%) and the HER2 marker was positive in 14 cases (23.3%) and 8 cases (13.3%) were triple-negative. The expression of LGR5 marker in 41 cases (68.3%) was moderate and its expression distribution in 31 cases (51.7%) was moderate (Figure 1). The of LGR5 expression in terms of molecular classification of breast cancer and tumor characteristics is shown in Table 2 that there was only a significant relationship between triple-negative type and LGR5 expression (P = 0.01) and in other cases no significant difference was observed.

Discussion

The aim of this study was to evaluate the expression of LGR5 as a cancer stem cell marker in breast cancer cases and its relationship with hormonal profile and clinicopathopathological features of the patient. The results of the study showed that LGR5 marker had more

Table 1. Tumor Characteristic of Breast Cancer

Tumor characteristic		Frequency (%)
Grade	I	16 (26.7)
	II	33 (55)
	III	11 (18.3)
T-stage	T1	39 (65)
	T2	21 (35)
N-stage	N0	32 (53.3)
	N1	26 (43.3)
	N2	2 (3.3)
M-stage	M0	57 (95)
	M1	3 (5)
Perineural invasion		4 (6.7)
Vascular invasion		8 (13.3)
KI67	<14 %	15 (25)
	>=14-50 %	34 (56.7)
	>50 %	11 (18.3)
Estrogen receptor		46 (76.7)
Progesterone receptor		42 (70)
HER2		14 (23.3)
Triple-negative		8 (13.3)
Luminal A		15 (25)
Luminal B		31 (51.7)
LGR5 severity	Mild	19 (31.7)
	Moderate	41 (68.3)
LGR5 distribution	Mild (<10%)	17 (28.3)
	Moderate (10-50%)	31 (51.7)
	Severe (>50%)	12 (20)

Table 2. LGR5 Expression Severity in Breast Cancer by Molecular Type and Tumor Characteristic

Molecular type and tumor characteristic		LGR5- Mild N (%)	LGR5- Moderate N (%)	Total N (%)	P-value
Luminal A	Yes	3 (20)	12 (80)	15 (25)	0.346
	No	16 (35.6)	29 (64.4)	45 (75)	
Luminal B	Yes	10 (32.3)	21 (67.7)	31 (51.7)	1
	No	9 (31)	20 (69)	29 (48.3)	
HER2	Yes	-	6 (100)	6 (10)	0.163
	No	19 (35.2)	35 (64.8)	54 (90)	
Triple Negative	Yes	6 (75)	2 (25)	8 (13.3)	0.01
	No	13 (25)	39 (75)	52 (86.7)	
Perineural invasion	Yes	-	4 (100)	4 (6.7)	0.297
	No	19 (33.9)	37 (66.1)	56 (93.3)	
Vascular invasion	Yes	2 (25)	6 (75)	8 (13.3)	1
	No	17 (32.7)	35 (67.3)	52 (86.7)	
Grade	I/II	13 (26.5)	36 (73.5)	49 (81.7)	0.086
	III	6 (54.5)	5 (45.5)	11 (18.3)	
T-stage	T1	12 (30.8)	27 (69.2)	39 (65)	1
	T2	7 (33.3)	14 (66.7)	21 (35)	
N-stage	N0	11 (34.4)	21 (65.6)	32 (53.3)	0.782
	N1/N2	8 (28.6)	20 (71.4)	28 (46.7)	
M-stage	M0	19 (33.3)	38 (66.7)	57 (95)	0.545
	M1	-	3 (100)	3 (5)	

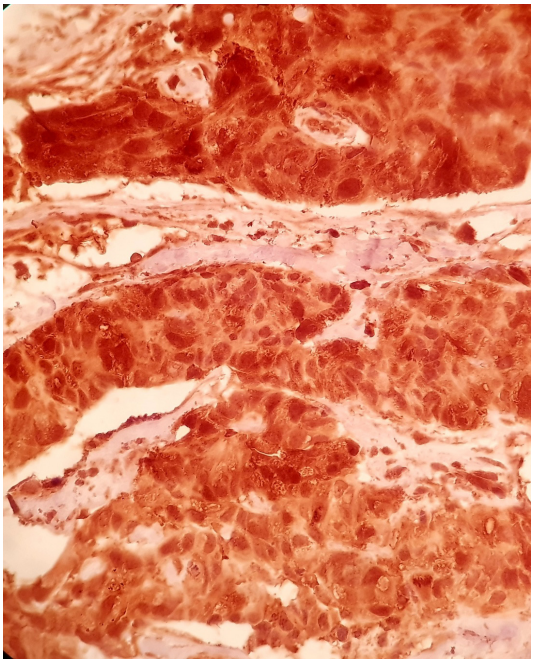

Figure 1. Moderately Cytoplasmic LGR5 Expression in Breast Cancer (x400)

expression than normal in 41 cases (68.3%). In a study by Hou et al., (2018) on 126 breast cancer patients, high expression of LGR5 was reported in 46% of cases or in the study of Shen et al., (2019) high expression of this marker in 61.5%cases of invasive breast carcinoma. Also, in the study of Lee et al. LGR5 was positive in 9 cases (60%) out of 15 cases of Ductal Carcinoma In Situ or in the study of Ogasawara et al., (2020) this marker was positive in 16.2% of 43 triple-negative patients. Comparison of studies shows the important role of this marker in breast cancer and the reported percentage of expression of this marker in studies with acceptable sample sizes, similar to the present study has been reported above. Regarding the role of this marker, Yung et al., (2015) examined the role of LGR5 in the progression of breast cancer and the preservation of stem-like cells by activating the Wnt / β-catenin signaling pathway. They found that activation of LGR5 by the Wnt signal pathway is a possible mechanism for regulating early breast cancer stem cells.

In the present study, there was a significant inverse relationship between triple-negative type and LGR5 expression (P = 0.01), but no significant relationship was found between LGR5 expression and HER2 type of cancer, which may be due to the small number of samples in the present study. Chen and colleagues in 2013 showed that lgr5 increased VEGFR expression, which may activate downstream Her-2 signals despite treatment with herceptin. Therefore, they hypothesized that in cancer patients with high Lgr5 expression, activation of the VEGFA sub-pathway could be one of the reasons for resistance to targeted Her-2 therapies, suggesting a role for these two receptors together.

In conclusion, the results of the present study showed that the LGR5 marker is expressed in a remarkable percentage of breast cancer patients and its value is not high in triple-negative and had no significant relationship with tumor characteristics.

Author Contribution Statement

Study conception and design (FM and RAN); data collection (FM and BB); analysis and interpretation of results (RAN); Draft manuscript preparation (RAN). All authors (FM , BB and RAN) approved the final version of manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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