Original Article

The evaluation of hepatoma-derived growth factor in determining of prognosis and estimating of invasive probability of tumoral cells, recurrent, and metastasis of lymphatic glands in breast carcinoma

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Abstract

Introduction: Recently, hepatoma-derived growth factor (HDGF) has been considered as a significantly important factor in determining the prognosis and estimating the probability of tumor cell invasions, recurrence, and lymph node metastasis in different cancers, including breast malignancies. **Materials and Methods:** Immunohistochemistry (IHC) study for HDGF was performed on paraffin-embedded blocks of patients with breast carcinoma in Modarres hospital, Tehran, Iran, since 1387–1390 (74 cases); three separate pathologists read the slides after complete IHC staining. Thereafter, necessary information was recorded from patient files, and eventually, findings were analyzed by SPSS program. **Results:** Expression of nuclear HDGF has significant statistical correlation with tumor grade according to Nottingham grading scheme; this correlation is also seen with nuclear pleomorphism of tumor cells and mitotic count. No correlation between age and tumor size with expression of HDGF is found. Lymph node metastasis is in inverse ratio to nuclear HDGF staining. **Conclusion:** Nuclear expression of HDGF in tumor cells is increased concordantly to tumor grade, which implies us to the role of this marker in determining the prognosis and choosing the most suitable treatment plan.

Keywords: Breast carcinoma, hepatoma-derived growth factor, immunohistochemistry

Introduction

The invasive breast carcinoma is the most common noncutaneous cancer among women that has 2nd grade in mortality after lung cancer. In 2007, almost 178,000 cases of invasive breast carcinoma and 62,000 cases of carcinoma were diagnosed at once and about 40,000 women died because of disease. It was predictable that there was a 1.3% increase in the number of infected women for the next 20 years.[1] There are a lot of studies for achieving an appropriate treat pattern to determine the prognosis that the most common of them are biological and genetic markers such as HER2/neu P53, BCL2, BRCA1, estrogen receptor, and progesterone receptor. Recently, a marker used for evaluating the tumor status in different organs is hepatoma-derived growth factor (HDGF). This heparin-bind growth factor can transfer to nucleolus and help the growth stimulation and increase the number of different cells such as HuH7, fibroblasts, smooth muscle cells, and endothelium.[2-7]

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There are mountain evidences for proofing the HDGF rule in progress of the bulk of tumors. This marker is found in almost all fetal tissues, and it seems that there is a function in maturation of liver,[8] kidney,[9] cardiovascular system,[5] and lung.[10] The high expression of HDGF is along with negative prognosis,[11] and it was known as an independent biomarker for determining the prognosis of several malignancies such as stomach cancer,[12,13] hepatocellular carcinoma,[14-16] lung cancer,[17,18] pancreatic cancer,[19] esophageal cancer,[20] breast cancer,[21-24] nasopharyngeal carcinoma,[25] glioma,[26] gastrointestinal stromal tumor,[27,28] cholangiocarcinoma,[29] and oral cancer.[30] The expression of this marker predicted the probability of tumoral cell invasion,[22,23] lymph node metastasis,[12,21,22,24] and recurrence.[20-22] Few studies have been conducted on this major and there is no related literature on the subject in Iran. Furthermore, according to the increase in the prevalence of cancer, it is hoped to achieve an applicable treatment pattern and prognosis by evaluating this marker.

Materials and Methods

In this descriptive study, the information of 74 patients suffering from breast cancer such as age and paraffin block number were extracted according to pathologic archive of Modarres hospital, Tehran, Iran, in 1387–1390. The pertaining slides were signed out and some slides were achieved from paraffin blocks including tumoral tissues with using 3 micron thickness slicing.

1. One day before conducting immunohistochemistry (IHC), the tissues were sliced based on an applicable thickness (1–2 μm), and they were attached on slides with positive charge and conserved in 37° for 24 h. If this step had not been done, the tissues would have removed during Ag retrieval
2. The slides were put in xylose for 7 min and alcohol 70%–100% and phosphate-buffered saline (PBS) or tryptone soya broth (every step for 5 min). In this step, the slides were degreased and discharged with water again because the slides were dried and water free (H_2O) in the respect of fixation; PBS led to remaining on osmotic pressure and prevented cell damage (isotonic ambiance)
3. We prepared applicable PH buffer according to on-studied marker. For example, the most of nuclear markers needed high PH = 9 and the membrane of nucleus and cell was permeable and the marker joined to target easily
4. The slides were put in suitable package for preventing evaporation. These were put in microwaves in 900 Watt for 5–7 min because of getting buffer to boiling point; Then, the slides were retrieved for 35–45 min. In this step, the linkages of proteins made by formalin in fixation time were broken and our respective antigens hiding into net were appeared again. It is considerable that this step is the most important part of IHC and the time of Ag retrieval can be changed according to tissue processing
5. The slides lost amount of fatty in this step after boiling and retrieval; it is necessary to be washed with distilled water in 25°
6. H_2O_2 blocking:
   - In this part, the slides were dipped into H_2O_2 with 3.5%–5% concentration about 7–10 min for preventing indogenous peroxidase. The importance of this step is significant in hepatic and renal tissues. The direction of using 35% stock of H_2O_2: the 30 cc of H_2O_2 were added to 270 cc of distilled water or methanol and the final volume was 300 cc (3.5% H_2O_2). The methanol is a fixative and can inhibit the inner peroxidase
7. After H_2O_2 block, the slides were washed again with distilled water for omitting the extra H_2O_2. Then, the PBS solution was poured on slides for getting PH = 7.2–7.4. In this step, the surroundings of the tissues were drawn with DAKO pen. Then, the target antibody having applicable concentration and directed time was poured, and it was incubated in 37°
8. First step washing: There are 2 solutions; the first one is IHC wash buffer that the slides were moved slowly into it for 5 min, and then, they were dipped in PBS for 5 min. It is better that slides were moved slowly several times in this step
9. Envision (second antibody): The incubation time was 30 min in 37°. We used SureFISH H chr1:156684584-156923809 kits for HDGF from DAKO Company
10. Second step washing: It is precisely similar to 8th part. The importance of washing was considerable and it prevented "color-base" of slides
11. The DAB solution included 20–50 λ of chromogen, and 1000 λ of buffer was poured on slides and washed with distilled water for omitting extra DAB
12. Counter staining: After washing with distilled water, the slides were dipped into hematoxylin for nucleus. About 1–2 min was enough and washing with distilled water was necessary.

In the final dewatering step for mounting, the slides were dipped into 70%–100% alcohol and xylose about 5 min for every step, respectively. Then, the slides were mounted and ready for observing.

Statistical analysis

The information was analyzed by sorting in SPSS, (IBM SPSS, Armonk, NY, USA) from related forms and some tests such as Fisher's exact test aspect the expression of HDGF, age, pathologic diagnose, grade, tumor size, and lymph node metastasis.

Results

There is not any significant relation between age and cytoplasmic and nuclear HDGF among 74 women patients with 48.9 ± 11.2 mean age and 48 middle age ($P = 0.37$ and $P = 0.576$). The patients are sorted in 4 groups according to pathologic diagnosis [Table 1]. There are 2 cases of invasive lobular carcinoma (8.7%) and the others are invasive ductal carcinoma (91.3%), among 23 cases suffering from *in situ* carcinoma. There are 4 low-grade cases (17.4%) and 19 high-grade cases (82.6%). There is a positive significant relation between the intensity of nuclear

HDGF of low grade and high grade among *in situ* carcinomatous component ($P = 0.025$). In the present study, there is not any significant relation between the incidence of cytoplasmic and nuclear HDGF and tubule formation ($P = 0.105$ and $P = 0.469$). However, there is not any significant relation between cytoplasmic HDGF and mitotic count ($P = 0.072$), but a significant relation about nuclear HDGF was seen ($P = 0.012$). This status exists about cytoplasmic and nuclear HDGF and tumor grade based on Nottingham method ($P = 0.036$ and $P = 0.009$). There is not any significant relation between cytoplasmic HDGF and lymph node metastasis ($P = 0.486$), but there is a negative significant relation between nuclear HDGF and lymph node metastasis ($P = 0.008$). In the evaluation of tumor size, there are 31 cases smaller than 2 cm (41.9%), 35 cases between 2 and 5 cm (47.3%), and 8 cases more than 8 cm (10.8%). There are not any significant relation between cytoplasmic and nuclear HDGF and tumor size ($P = 0.251$ and $P = 303$). In the evaluation of pathologic slides, the lymphocytic infiltration was seen in 11 cases (14.9%) and the other 63 cases left did not have this feature (85.1%). There is not any significant relation between cytoplasmic HDGF and lymphocytic infiltration ($P = 1.000$), but a positive significant relation was seen about nuclear HDGF ($P = 0.021$). The summary of results is shown in following Table 2.

Discussion

In the evaluation of IHC's results aspect to HDGF marker, we found that the 65 cases of samples (88%) showed nuclear staining with different intensity and 17 cases of samples (23%) showed weak cytoplasmic staining. These results are leading to this point that cytoplasmic HDGF staining of cancer cells for achieving functional goals is not sensible, that is in line with the study done in China.[22] The intensity of HDGF staining in different age groups was not significant, according to the positive result of this marker in other studies corroborated with poor prognosis in breast cancer;[21-24] it cannot be extracted that poor prognosis is more considerable in specific age group. The combination of IHC stain results as well tumor grading based on Nottingham method showed a significant relationship between nuclear HDGF and tumor grade that it was consistent with the findings of other studies.[22-24] In the evaluation of Nottingham method, components with IHC staining results of the nuclear polymorphism had a significant relation with cytoplasmic and nuclear HDGF staining; also, the mitotic count had a significant relation with nuclear HDGF that is mentioned in other studies;[22] but there was not any significant relation between tubule formation and IHC staining for HDGF. Of course, there was not any significant relation between tumor size and the intensity of positive-HDGF that was not in line with the results of the study done in China, it considered a significant relation between intensity of nuclear HDGF staining and tumor stage.[22] There was not any statically correlation between lymph node metastasis and the incidence of cytoplasmic HDGF, but there was a significant relation about nuclear HDGF that was in line with study done in China.[22,24] Furthermore, the samples were evaluated aspect of *in situ* carcinoma and it was positive for 23 cases, and statistical analysis showed a positive significant relation between *in situ* grade and intensity of nuclear HDGF staining; whereas there was increase in intensity of HDGF staining in high-grade cases. The lymphocytic infiltration was seen in 11 cases that had significant relation with nuclear HDGF, and the intensity of nuclear HDGF staining increased in lymphocytic infiltration-positive cases. In the respect of lymphocytic infiltration, exist in the breast cancer (except medullary carcinoma) caused poor prognosis; it can be extracted that increase in the intensity of staining was along with poor prognosis. The evaluation of the intensity of *in situ* carcinoma staining aspect of HDGF marker and also the relation of lymphatic infiltration with the marker was not done in any study, and our present is a pioneer. In the study performed by Tsang Ty in 2008, Hong Kong,[11] the effect of HDGF marker on apoptosis pathway controlled by bad protein was evaluated. The inhibition of HDGF led to not only inducted expression of preapoptosis protein "Bad" and inhibition of Akt and extracellular signal-regulated kinase but also the stimulation of interior apoptosis. Since the inhibition of HDGF not only made inhibited growth but also caused inducted apoptosis in cancer cells; it can be concluded that it is an effective agent in living through cancer cells and a potential target in treatment of malignancies. Chen *et al.* evaluated the value prognosis of HDGF staining in cytoplasm and nucleus in 86 breast cancer. The results of their study in the cases showed more staining in higher grade and stage of tumor, more mitotic activity (Ki-67 index >20%), and more common invasive and recurrence into lymph node, that increased expression of nuclear HDGF had a rule in progression of cancer, and it was used as prognostic marker in breast cancer.[22] Chen *et al.* studied transgenic in the evaluation of expression and function of HDGF in cancer genesis of breast for evaluating the malignancy behavior and changing epithelial–mesenchymal transition (EMT) of breast

Table 1: The frequency of pathologic diagnosis in studied cases

Index	Groups	Frequency (%)
Pathologic diagnosis	Invasive ductal carcinoma	68 (1.80)
	Invasive lobular carcinoma	4 (5.40)
	Invasive ductal and lobular carcinoma	1 (1.40)
	Metaplastic carcinoma	1 (1.40)
Total		74 (100)

Table 2: The summary of results

	HDGF nuclear	HDGF cytoplasmic
Age	Negative	Negative
Tubule formation	Negative	Negative
Nuclear polymorphism	Positive	Positive
Mitotic count	Positive	Negative
Tumor grade, Nottingham method	Positive	Negative
Lymph node metastasis	Negative	Negative
Tumor size	Negative	Negative
Lymphocytic infiltration	Positive	Negative
Carcinoma *in situ* tumor grade (low or high)	Positive	Negative

HDGF: Hepatoma-derived growth factor

cancer cells.[21] The increase in the expression of HDGF caused increase in the expression of EMT in cancer cells with negative feedback in E-cadherin and positive feedback in Vimentin. In comparison, the HDGF suppression caused by RNA interfere in MDA-MB-231 cells led to weakness in malignancy behavior and stimulating of EMT reversing with increase in E-cadherin expression and decrease in Vimentin expression. In the mentioned information, it can be elicited that increase in HDGF expression might be prognosis agent in metastasis and recurrent tumor through EMT regulation in breast cancer. The expression of mRNA related to HDGF was evaluated in 24 breast cancer individuals and surrounded tissues by real-time polymerase chain reaction, and IHC was performed for evaluating the expression of HDGF in 75 breast cancer cases and surrounded tissues. The results showed that mRNA expression related to HDGF in breast cancer was vividly more than normal tissue, and there is considerable decrease in HDGF expression in breast normal tissue in comparison with cancer tissue. The level expression of HDGF in breast cancer with high stage is more than the low stage one; of course, the HDGF expression in malignancy cases with lymph node metastasis was more than nonmalignancy cases. Hence, the increase in HDGF expression can be effective in pathogens and metastasis of breast cancer.[24]

Conclusion

It can be elicited from the present study that the positive-HDGF marker was corroborated with increase in tumor grade and absolutely positive prognosis. Furthermore, the HDGF can induct the apoptosis, prognosis rule for metastasis and recurrence of lymph node and potential marker for treatment of cancer cells.

To complete the findings of the present study, it is suggested that, in an applicable time range, the new breast cancer cases referred for treatment be evaluated for HDGF marker and achieve the more functional goals through monitoring their answer to treatment and long age. If needed, it is better to use this marker for getting applicable treatment schedule as a routine procedure.

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Conflicts of interest

There are no conflicts of interest.

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