Original Article
Middle East Journal of Cancer 2013; 4(3): 113-118

Concordant Expression of Estrogen and Progesterone Receptors in Primary and Loco-regional Recurrent Breast Cancer

Sedigheh Tahmasebi*, Behnam Dalfardi♦, Abdolrasoul Talei*, Akbar Safaei***, Ahmad Monabati***, Majid Akrami***

*Department of Surgery, Shiraz University of Medical Sciences, Shiraz, Iran
**Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
***Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Malignant neoplastic lesions of the breast are one of the main causes of cancer death among women. Several factors can be influential in the treatment process of breast cancer patients. The expression status of estrogen and progesterone receptors in tumor cells is among the most important determinants affecting the treatment approach and prognosis of these patients. In this paper, we will evaluate changes in the expression of these biomarkers between primary and loco-regional recurrent tumors in breast cancer patients.

Methods: This cross-sectional study included 50 female patients aged 30 to 85 years old who underwent surgical intervention between 1994 and 2011. All experienced loco-regional recurrence of the primary tumor between 6 months and 12 years after the first therapeutic intervention. Detection of estrogen and progesterone receptors was based on the immunohistochemistry staining of tissue samples of malignant neoplastic lesions prepared from tissue biopsies of patients with primary or recurrent breast cancer.

Results: No statistically significant change in the expression of estrogen and progesterone receptors between primary tumors and their matching loco-regional were seen ($P>0.05$).

Conclusion: This research shows no significant changes in estrogen and progesterone receptor expression after loco-regional recurrence of tumors in breast cancer patients. It can be concluded that the assessment of the expression of these biomarkers in primary tumors provides reliable information for the treatment approach of loco-regional tumors.

Keywords: Breast neoplasms, Recurrence, Estrogen receptor, Progesterone receptor

♦Corresponding Author:
Behnam Dalfardi, Medical Student
Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98-913-248-3359
Fax: +98-711-212-2970
Email: Dalfardibeh@gmail.com

Received: March 25, 2013; Accepted: May 14, 2013

Introduction

Malignant neoplastic lesions of the breast are the most common form of malignancy diagnosed in women.[1] Breast cancer (after lung cancer) is the second leading cause of cancer death among women.[2] About 14% of cancer deaths among women are caused by breast cancer.[3]

Despite geographical patterns of breast cancer incidence and mortality, over the past decades, the incidence of breast cancer has increased worldwide. A small part of this geographical variation is related to genetic differences in people of different races, but it is mainly a consequence of diversity in lifestyle in addition to exposure to various environmental carcinogens.[4-6]

Risk factors for breast cancer include a younger age at the time of puberty (early menarche), a more advanced age at the time of menopause (late menopause), obesity, a negative history of pregnancy or breast feeding, first delivery at an advanced age (especially more than 35 years old), excessive alcohol consumption, and insufficient physical activity.[7,8] Noting these risk factors, one realizes that hormonal factors (especially estrogen, endogenous or exogenous) play an important role in breast cancer incidence and the development of its malignant neoplastic lesions.[9,10] One therapy goal in breast cancer patients, therefore, is to cope with the effects of hormones (especially estrogen) in their bodies.[11]

It should be noted that two thirds of breast cancer patients have tumoral tissues with cells that express estrogen receptor (ER). This feature can affect their treatment process.[12] The probability of ER expression increases in older patients.[13] Selective ER modulators (SERMs) such as tamoxifen and selective ER degraders (SERDs) like fulvestrant are among two of the most widely used drug classes in breast cancer patients that influence estrogen activity.[14] Aromatase inhibitors comprise another drug group used in these patients for targeted therapy against estrogen.[15] Hormone therapy for breast cancer patients leads to a delay in both recurrence and distant metastases and increases survival rate.[16] Of note is the fact that expression of the progesterone receptor (PR) can be predictive of a patient's response to hormone therapy against estrogen, and thus affect survival.[17-19] Unfortunately, resistance to hormone therapy may be seen in these patients (from the beginning or during the treatment process).[20]

Considering the significant role of ER and PR in the treatment approach and prognosis of breast cancer patients, our goal in this study is to assess the expression of ER and PR in primary breast cancer tumors and their matching loco-regional recurrent neoplastic lesions. Then, we evaluate phenotypic changes in the expression of ER and PR between primary and recurrent tumors.

Materials and Methods

This cross-sectional study included 50 female patients aged 30 to 85 years old who underwent surgical intervention (modified radical mastectomy or breast conserving surgery) between 1994 and 2011. All experienced loco-regional recurrence (LRR) of the primary tumor. These patients were selected from the database of the Breast Cancer Clinic at Shahid Motahhari Clinic, Shiraz University of Medical Sciences, Shiraz, Iran. Most, in addition to surgery, based on primary tumor characteristics (size, pathology, nodal involvement, etc.) and hormone receptor expression status had a history of radiotherapy and adjuvant treatments with routine chemotherapy that included such chemotherapeutic regimens as doxorubicin, cyclophosphamide, and taxol, among others, and hormone therapy (tamoxifen).

In this study we defined LRR as a recurrence in cutaneous and/or subcutaneous chest wall tissues in the territory of a previously operated breast, axillary and internal mammary lymph nodes, and lymph nodes located in the supra- or infra-clavicular fossa on the same side as the primary neoplastic lesion.[21]

In this study, detection of ER and PR was based on the immunohistochemistry (IHC) staining of paraffin embedded tissue samples of malignant neoplastic lesions prepared from tissue biopsies of patients with primary or recurrent breast cancer.

Study patients experienced LRR between 6

Table 1. Stepwise IHC protocol for ER and PR staining used in this study.

1. Cut paraffin sections at 4.0 µm thickness (on sticky slides).
2. Put 3 times in xylene for 30 minutes.
3. Hydrate sections by placing in decreasing ethanol grades (100%, 96% and 70%).
4. Wash slides in PBS* buffer (5-10 minutes).
5. Incubate in 3% H2O2 for 3 minutes (inactivation of endogenous peroxidase).
6. Wash slides in PBS buffer.
7. Use TE** buffer to boil slides.
8. Cool slides, put in PBS buffer.
9. Use 10% goat serum to block slides, incubate at room temperature for 20 minutes and use a humidified chamber.
10. Incubate slides with primary antibody in a humidified chamber for 1 hour, add 50-100 µl of ready-to-use antibody [Estrogen receptor (ER): 1D5 and progesterone receptor (PR): PgR636, DAKO Company, Denmark)].
11. Wash in PBS buffer (10 minutes).
12. Apply secondary antibody.
13. Wash in PBS buffer (10 minutes).
14. Add DAB chromogen (5 minutes).
15. Counter stain using hematoxylin and eosin.
16. Dehydrate slides using serial grades of ethanol, clear in xylene, then mount.

*PBS: phosphate buffered saline; **TE: tris-EDTA

months and 12 years after the first therapeutic intervention. We excluded cases that experienced distant metastasis, malignant lesions with new pathology, and recurrence outside the above-mentioned zone for LRR from this study.

For the IHC study, ready-to-use antibodies (antibodies with no need to further process before use) were procured from DAKO Company, Denmark. The IHC staining protocol for tissue samples is explained in Table 1. Statistical analysis was performed by SPSS Statistics software, version 11.5 (Chicago, IL, USA).

Results

All 50 selected patients experienced LRR. At the time of primary tumor diagnosis, tissue samples from 29 (58%) patients were ER positive and 28 (56%) patients were PR positive. Evaluation of tissue samples from cases with recurrent tumors compared to primary tumors revealed that 26 (52%) patients were ER positive and only 22 (44%) were PR positive.

At the time of recurrence, ER expression in 33 (66% of patients) cases and PR expression in 36 (72%) patients were the same as primary tumors. ER and PR expression, each one, lost in 10 (20 %) patients. There were 7 (14%) patients who had new ER expression, and 4 (8%) showed new PR expression.

According to these results, we observed no statistically significant change in the expression of ER and PR between primary tumors and LRR ($P>0.05$). There was no significant difference in the concentration of expressed ER and PR by tumor cells between primary and recurrent tumors. Data are summarized in Tables 2, 3 and 4.

Discussion

The immunohistochemical properties of tumor cells in breast cancer patients, such as ER and PR expression, play key roles in tumor responsiveness to hormone therapy, recurrence rate, and patient survival. Thus, it is important to identify patients who are more likely to respond to hormone therapy.[22,23]

Depending on the stage of the disease at the time of diagnosis and the type of treatment received, about 10% to 35% of women with breast cancer will experience LRR.[24] Changes in the expression of ER and PR in recurrent tumors impose limitations on the use of hormone therapy for these tumors.[25] Today, ER and PR expression is routinely assessed in all newly diagnosed breast cancer patients. Re-evaluation of the expression of these biomarkers at the time of recurrence or distant metastasis to achieve effective and successful treatment remains a controversial issue.[26]

Table 2. Estrogen receptor (ER) and progesterone receptor (PR) expression status in cases with primary breast cancer and their loco-regional recurrent tumors.

	Primary tumor N (%)	Loco-regional recurrent tumor N (%)
ER		
Positive	29 (58)	26 (52)
Negative	21 (42)	24 (48)
PR		
Positive	28 (56)	22 (44)
Negative	22 (44)	28 (56)

Table 3. Estrogen receptor (ER) and progesterone receptor (PR) expression status in loco-regional recurrent tumors compared with primary tumors.

	Number of cases (%)
Loss of ER exp.*	10 (20)
Loss of PR exp.	10 (20)
New exp. of ER	7 (14)
New exp. of PR	4 (8)
Stable ER ph.**	33 (66)
Stable PR ph.	36 (72)

*exp.: Expression; **ph.: Phenotype

Tumor cells in breast cancer are heterogeneous and composed of different cell clones. ER expression varies in different cell lines.[27,28] According to one hypothesis, the cause for change in the phenotype of tumor cells in terms of ER and PR expression during disease progression is linked to the selection of cell lines not expressed in ER, but rather phenomena that occur during the treatment process.[29,30]

Our study included a precise review of works previously conducted to assess changes in ER and PR expression status of tumor cells following recurrence or metastasis in breast cancer patients. This review showed different results of previous studies. Some favored the concordance in ER and PR expression between primary and recurrent or metastatic tumors. Studies by Gomez-Fernandez et al., D'Andrea et al., Li et al. and Robertson showed no significant changes in ER and/or PR content of tumor cells after recurrence or metastasis. Among the above mentioned studies, the work of Gomez-Fernandez et al. included 278 breast cancer patients and was the most wide-scale research which supported stability of the ER phenotype in breast cancer patients.[29, 31-33]

By contrast, some researchers have supported the idea that ER and PR expression status can change during tumor progression, recurrence or metastasis. These included works by Rom et al., Pedrini et al., Morimoto et al., Nomura et al. and Idirisinghe et al. Among these, Nomura et al. was the most wide-scale study that assayed ER expression status in 940 breast cancer patients and PR expression status in 773 breast cancer patients who experienced locally recurrent or metastatic tumors.[25, 34-37]

Differences in evaluation methods of biomarker expression are an important factor that can be related to different results in various studies. Of note, some methodological differences, including biochemical assay versus IHC assay of steroid receptors, method of tissue sampling (surgical biopsy, core needle biopsy, or fine needle aspiration), and inter- or intra-laboratory differences may prevent an accurate assessment of ER and PR during the study process.[22, 33, 36] Heterogeneity in receptor expression between different parts of the tumor tissue may also influence results.[13] Another confounding factor is the administration of tamoxifen, which binds to the ER on the surface of tumor cells that express this receptor and causes false-negative results.[30,33,38] In order to prevent such technical problems in this study, only biopsy specimens of tumor tissue were used, of which these were evaluated by an experienced technician. Biomarkers were assessed by the IHC method, an inexpensive, sensitive method for ER and PR detection widely used as the method of choice in most countries.[39]

Overall, the results of the present research were similar to those of studies which suggested no significant changes in tumor cell phenotypes after recurrence or metastasis. The concentrations of ER and PR between primary and LRR tumors also showed no significant changes.

Conclusion

Considering the results of the present study that

Table 4. Patient and primary tumor characteristics.

Mean patient age in years (range)	50.9 (30-85)
Tumor characteristics	**N (%)**
Invasive ductal carcinoma	43 (86)
Invasive lobular carcinoma	1 (2)
Medullary carcinoma	2 (4)
Other tumor types	4 (8)
Grade 1	10 (20)
Grade 2	25 (50)
Grade 3	1 (2)
Grade not reported	14 (28)

suggest no significant changes in ER and PR expression after the LRR of tumors in breast cancer patients, the assessment of the expression of these biomarkers in primary tumors provides reliable information for the treatment approach of loco-regional tumors. But, due to the existence of studies that suggest a significant change in the receptor expression status after recurrence or distant metastasis, it seems reasonable to conduct further studies on a wider scale.

Acknowledgement

Miss A. Dehbozorgi (Department of Pathology, Shiraz College of Medicine, Shiraz, Iran) offered considerable collaboration in the IHC staining of tissue samples for which the authors thank her profusely. This article is the result of a thesis research project by Mr. Behnam Dalfardi, a medical student, proposal code 89-01-01-2359 under the guidance of Dr. S. Tahmasebi and Dr. A. Talei.

Conflicts of interest

The authors certify that they have no conflicts of interest.

References

1. Habib F, Salman S, Safwat M, Shalaby S. Awareness and knowledge of breast cancer among university students in Al Madina Al Munawara Region. *Middle East J Cancer* 2010; 1(4):159-66.
2. Lacey JV JR, Devesa SS, Brinton LA. Recent trends in breast cancer incidence and mortality. *Environ Mol Mutagen* 2002; 39(2-3):82-8.
3. Coughlin SS, Ekwueme DU. Breast cancer as a global health concern. *Cancer Epidemiol* 2009; 33(5):315-8.
4. McCormack VA, Boffetta P. Today's lifestyles, tomorrow's cancers: Trends in lifestyle risk factors for cancer in low- and middle-income countries. *Ann Oncol* 2011; 22(11):2349-57.
5. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010; 19(8):1893-907.
6. McTiernan A. Behavioral risk factors in breast cancer: Can risk be modified? *Oncologist* 2003; 8(4):326-34.
7. Porter PL. Global trends in breast cancer incidence and mortality. *Salud Publica Mex* 2009; 51 Suppl 2:s141-6.
8. Youlden DR, Cramb SM, Dunn NA, Muller JM, Pyke CM, Baade PD. The descriptive epidemiology of female breast cancer: An international comparison of screening, incidence, survival and mortality. *Cancer Epidemiol* 2012; 36(3):237-48.
9. Massarweh S, Schiff R. Resistance to endocrine therapy in breast cancer: Exploiting estrogen receptor/growth factor signaling crosstalk. *Endocr Relat Cancer* 2006; 13 Suppl 1:S15-24.
10. Boyle P. Breast cancer control: Signs of progress, but more work required. *Breast* 2005; 14(6):429-38.
11. Howell SJ, Johnston SR, Howell A. The use of selective estrogen receptor modulators and selective estrogen receptor down-regulators in breast cancer. *Best Pract Res Clin Endocrinol Metab* 2004; 18(1):47-66.
12. Prat A, Baselga J. The role of hormonal therapy in the management of hormonal-receptor-positive breast cancer with co-expression of HER2. *Nat Clin Pract Oncol* 2008; 5(9):531-42.
13. Eppenberger-Castori S, Moore DH Jr, Thor AD, Edgerton SM, Kueng W, Eppenberger U, et al. Age-associated biomarker profiles of human breast cancer. *Int J Biochem Cell Biol* 2002; 34(11):1318-30.
14. Baumann CK, Castiglione-Gertsch M. Estrogen receptor modulators and down regulators: Optimal use in postmenopausal women with breast cancer. *Drugs* 2007; 67(16):2335-53.
15. Rochefort H, Glondu M, Sahla ME, Platet N, Garcia M. How to target estrogen receptor-negative breast cancer? *Endocr Relat Cancer* 2003; 10(2):261-6.
16. Johnston SR. New strategies in estrogen receptor-positive breast cancer. *Clin Cancer Res* 2010; 16(7):1979-87.
17. Cui X, Schiff R, Arpino G, Osborne CK, Lee AV. Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. *J Clin Oncol* 2005; 23(30):7721-35.
18. Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, Gee J, et al. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *J Clin Oncol* 2007; 25(30):4772-8.
19. Osborne CK, Schiff R, Arpino G, Lee AS, Hilsenbeck VG. Endocrine responsiveness: Understanding how progesterone receptor can be used to select endocrine

therapy. *Breast* 2005; 14(6):458-65.
20. Nicholson RI, Johnston SR. Endocrine therapy--current benefits and limitations. *Breast Cancer Res Treat* 2005; 93 Suppl 1:S3-10.
21. Clemons M, Danson S, Hamilton T, Goss P. Locoregionally recurrent breast cancer: Incidence, risk factors and survival. *Cancer Treat Rev* 2001; 27(2):67-82.
22. Ciocca DR, Gago FE, Fanelli MA, Calderwood SK. Co-expression of steroid receptors (estrogen receptor alpha and/or progesterone receptors) and Her-2/neu: Clinical implications. *J Steroid Biochem Mol Biol* 2006; 102(1-5):32-40.
23. Payne SJ, Bowen RL, Jones JL, Wells CA. Predictive markers in breast cancer-the present. *Histopathology* 2008; 52(1):82-90.
24. Rauschecker H, Clarke M, Gatzemeier W, Recht A. Systemic therapy for treating locoregional recurrence in women with breast cancer. *Cochrane Database Syst Rev* 2001; (4):CD002195.
25. Rom J, Aulmann S, Schneeweiss A, Sohn C, Sinn HP. Comparison of immunohistological parameters in primary breast cancers and corresponding locoregional recurrences. *Pathol Res Pract* 2006; 202(3):125-30.
26. Thompson AM, Jordan LB, Quinlan P, Anderson E, Skene A, Dewar JA, et al. Prospective comparison of switches in biomarker status between primary and recurrent breast cancer: The Breast Recurrence in Tissues Study (BRITS). *Breast Cancer Res* 2010; 12(6):R92.
27. Wong NK, Fuller M, Sung S, Wong F, Karsan A. Heterogeneity of breast cancer stem cells as evidenced with Notch-dependent and Notch-independent populations. *Cancer Med* 2012; 1(2):105-13.
28. Bock C, Rack B, Kuhn C, Hofmann S, Finkenzeller C, Jäger B, et al. Heterogeneity of ERα and ErbB2 status in cell lines and circulating tumor cells of metastatic breast cancer patients. *Transl Oncol* 2012; 5(6):475-85.
29. Gomez-Fernandez C, Daneshbod Y, Nassiri M, Milikowski C, Alvarez C, Nadji M. Immunohistochemically determined estrogen receptor phenotype remains stable in recurrent and metastatic breast cancer. *Am J Clin Pathol* 2008; 130(6):879-82.
30. Johnston SR, Saccani-Jotti G, Smith IE, Salter J, Newby J, Coppen M, et al. Changes in estrogen receptor, progesterone receptor, and pS2 expression in tamoxifen-resistant human breast cancer. *Cancer Res* 1995; 55(15):3331-8.
31. D'Andrea MR, Limiti MR, Bari M, Zambenedetti P, Montagutti A, Ricci F, et al. Correlation between genetic and biological aspects in primary non-metastatic breast cancers and corresponding synchronous axillary lymph node metastasis. *Breast Cancer Res Treat* 2007; 101(3):279-84.
32. Li BD, Byskosh A, Molteni A, Duda RB. Estrogen and progesterone receptor concordance between primary and recurrent breast cancer. *J Surg Oncol* 1994; 57(2):71-7.
33. Robertson JF. Oestrogen receptor: A stable phenotype in breast cancer. *Br J Cancer* 1996; 73(1):5-12.
34. Pedrini JL, Pedrini M, Savaris RF, Machado L, Grudzinski M, Zettler CG. Reassessing tumor markers in local recurrences of breast cancer: A new insight. *Med Sci Monit* 2004; 10(12):BR462-7.
35. Morimoto K, Koh M, Yamamoto A, Tamura K, Yamamoto K, Yamamoto K, et al. Changing behavior of estrogen and progesterone receptors with metastasis or recurrence of breast cancer. *Gan To Kagaku Ryoho* 2006; 33(7):925-9. [Abstract]
36. Nomura Y, Tashiro H, Shinozuka K. Changes of steroid hormone receptor content by chemotherapy and/or endocrine therapy in advanced breast cancer. *Cancer* 1985; 55(3):546-51.
37. Idirisinghe PK, Thike AA, Cheok PY, Tse GM, Lui PC, Fook-Chong S, et al. Hormone receptor and c-ERBB2 status in distant metastatic and locally recurrent breast cancer. Pathologic correlations and clinical significance. *Am J Clin Pathol* 2010; 133(3):416-29.
38. Encarnación CA, Ciocca DR, McGuire WL, Clark GM, Fuqua SA, Osborne CK. Measurement of steroid hormone receptors in breast cancer patients on tamoxifen. *Breast Cancer Res Treat* 1993; 26(3):237-46.
39. Zheng WQ, Lu J, Zheng JM, Hu FX, Ni CR. Variation of ER status between primary and metastatic breast cancer and relationship to p53 expression. *Steroids* 2001; 66(12):905-10.