Lack of Prognostic Value of Insulin-like Growth Factor-1 in Patients with Breast Cancer: Analysis with Tissue Microarray

SHYR-MING SHEEN-CHEN[1], FONG-FU CHOU[1], WAYNE HSU[1], CHAO-CHENG HUANG[2], HOCK-LIEW ENG[2] and REI-PING TANG[3]

Departments of [1]Surgery and [2]Pathology, Chang Gung Memorial Hospital, Kaohsiung Medical Center and [3]Department of Surgery, Chang Gung Memorial Hospital, Linkou College of Medicine, Chang Gung University, Taiwan, R.O.C.

Abstract. *Background: Recent reports on the risk of prostate, breast, colorectal and lung cancer suggested that high circulating insulin-like growth factor-1 (IGF-1) concentrations are associated with an increased risk of cancer. The power of the tissue microarray (TMA) technique is the ability to perform a series of analyses of thousands of specimens in a parallel fashion with minimal damage to the original blocks. Materials and Methods: Archival tissue specimens from 106 patients with primary invasive breast cancer were selected and IGF-1 expression was analyzed by immunohistochemical staining of tissue microarrays. The data regarding primary tumor staging, age, estrogen receptor status, lymph node status, histological grading and TNM staging were also collected. Results: There were 2 patients (2%) with grade 1 expression for IGF-1, 39 patients (37%) with grade 2 expression and 65 patients (61%) with grade 3 expression. There was no significant relationship between IGF-1 expression and age (p=0.256), estrogen receptor status (p=0.921), histological grading (p=0.815), primary tumor staging (p=0.455), or TNM staging (p=0.194). No survival difference was noted among the three groups with different IGF-1 expression (p=0.462). Conclusion: Immunohistochemical staining of the tissue microarray was convenient and feasible for the analysis of IGF-1 expression in breast cancer, yet its expression did not show any significant correlation with the overall survival rate.*

Insulin-like growth factors (IGFs) are multifunctional peptides that appear to be important in the regulation of the growth of normal cells and in the resistance to apoptosis in neoplastic tissue (1, 2). Unlike most other growth factors, IGF peptides appear in high concentrations in the circulation and show systemic, hormonal and local paracrine effects on cell behavior (3). Much epidemiological evidence supports a role for IGFs in the induction and progression of various types of cancer (4, 5). Recent reports on the risk of prostate, breast, colorectal and lung cancer suggested that high circulating IGF-1 concentrations are associated with an increased risk of cancer (6-9). Such a phenomenon is supported by laboratory evidence which showed that IGF-1 is both mitogenic and antiapoptotic (5).

The creation of tissue microarrays (TMA) allows for the rapid immunohistochemical analysis of thousands of tissue samples in a parallel fashion with minimal damage to the original blocks (10, 11).

This study was designed with the application of TMA to analyze the IGF-1 status in breast cancer and with the hope of elucidating the possible relationship between IGF-1 expression and breast cancer.

Materials and Methods

Specimen selection and data collection. Archival tissue specimens from 106 patients with primary invasive breast cancer were selected from the pathology files of Chang Gung Memorial Hospital at Kaohsiung between January 1994 and December 1998. All the patients underwent modified radical mastectomy due to invasive breast cancer, defined as carcinoma with invasion to or beyond the basement membrane regardless of histological classification (ductal or lobular) (12). The data regarding primary tumor staging, age, estrogen receptor status (13-18), lymph node status, histological grading and TNM staging were also collected. The hematoxylin-eosin-stained slides of the paraffined-embedded tumor specimens were reviewed by our pathologists to confirm the accuracy of the histological diagnoses and lymph node status.

Tissue microarray assembly. Representative areas of both tumor and non-tumor parts for each case were selected and circled to match the blocks for the tissue microarray. The tissue blocks matching the circled slides were retrieved to prepare the recipient block for the microarray. To assure the representativeness of the selected cores,

Correspondence to: Shyr-Ming Sheen-Chen, MD, Professor of Surgery, Department of Surgery, Chang Gung Memorial Hospital, Kaohsiung, 123, Ta-Pei Road, Niao-Sung Hsiang, Kaohsiung Hsien, Taiwan, R.O.C. Tel: +88 677317123, Fax: +88 677318762, e-mail: smsheen@yahoo.com

Key Words: Breast cancer, tissue microarray, insulin-like growth factor-1, IGF-1.

three areas each for both tumor and non-tumor parts per case were determined for assembling the recipient blocks. Each target area on the selected blocks was punched to form a 0.6-mm diameter tissue core and placed consecutively on the recipient blocks of approximately 3 cm x 2 cm with a precision instrument (Beecher Instruments, Silver Spring, MD, USA) as described elsewhere (19).

Immunohistochemical analysis. Rabbit polyclonal antibodies against human IGF-1 were purchased from NeoMarkers Inc (Fremont, CA, USA) and were diluted 1:50 in phosphate-buffered saline (PBS). Five-micrometer sections were cut from the recipient blocks of the tissue microarray, incubated overnight in a 37˚C oven, dewaxed in xylene, and dehydrated in a series of graded alcohols. The sections were then treated with 3% hydrogen peroxide for 10 minutes to neutralize the endogenous peroxidase activity and microwaved in 10 mM citrate buffer (pH6.0) to unmask the epitopes. After antigen retrieval, the sections were incubated with diluted IGF-1 antibody for 1 hour followed by a PBS wash. Horseradish peroxidase/Fab polymer conjugate (PicTure™-Plus kit) (Zymed, South San Francisco, CA, USA) was then applied to the sections for 30 minutes. After washing, the sections were incubated with the peroxidase substrate diaminobenzidine for 5 minutes and counterstained with hematoxylin.

Grading for IGF-1 immunoreactivity. For evaluating the immunoreactivity for IGF-1, staining was classified using a four-grade scale: 0, absence of staining in tumor cells; 1+, weak cytoplasmic and/or nuclear staining in tumor cells; 2+, an intermediate staining intensity between 1+ and 3+ in tumor cells; and 3+, strong cytoplasmic and nuclear staining in tumor cells (Figure 1).

Patients and follow-up. All of the patients were women aged from 26 to 76 years old, with a mean age of 48.8±10.2 years. The mean follow-up was 71.2±25.4 months (range, 10 to 98 months). Follow-up was usually performed every 3 months for the first 2 years and then every 6 months for the next 3 years. After 5 years, follow-up became annual. Chest radiography, serum alkaline phosphatase level and a detailed physical examination were usually performed at follow-up. Annual mammography or breast sonography (for younger patients) were performed. Radionuclide bone scan, abdominal sonography or other image studies were performed if specific symptoms, signs or elevated serum alkaline phosphatase level were noted. Data regarding patient survival, clinical status and clinicopathological factors were obtained from medical records, contact with the patients at the outpatients clinics or by telephone, or both.

Statistical analyses. Comparisons between groups were performed using the Chi-squared test. For survival analyses, the end-point was overall survival. Overall survival rates were calculated by the Kaplan-Meier method and the differences were assessed with the log rank test. Statistical analyses were conducted using SPSS software (version 11.0 SPSS, Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant. All *p*-values were estimated from two-sided tests.

Results

There were 2 patients (2%) with grade 1 IGF-1 expression, 39 patients (37%) with grade 2 expression and 65 patients (61%) with grade 3 expression (Table I). Using the Chi-Squared test, comparisons between groups were performed. There was no significant relationship between IGF-1 expression and age ($p=0.256$), estrogen receptor status ($p=0.921$), histological grading ($p=0.815$), primary tumor staging ($p=0.455$), or TNM staging ($p=0.194$). For survival analyses, the end-point was overall survival. Survival differences were compared using the log rank test. No survival difference was noted among the three groups with different IGF-1 expression ($p=0.462$, Figure 2).

Table I. *IGF-1 expression in relation to clinicopathological variables.*

IGF-1 expression	1	2	3	*P*-value
Age (years)				
<50	0 (0%)	23 (39.7%)	35 (60.3%)	0.256
≥50	2 (1.9%)	16 (36.8%)	30 (62.5%)	
Estrogen receptor status				
Negative	1 (1.5%)	25 (35.9%)	41 (61.5%)	0.921
Positive	1 (1.9%)	14 (36.8%)	24 (61.3%)	
Histological grading				
1	0 (0%)	5 (35.7%)	9 (64.3%)	
2	2 (3.2%)	22 (35.5%)	38 (61.3%)	0.815
3	0 (23.33%)	12 (40.0%)	18 (60.0%)	
Primary tumor staging				
T1	0 (0%)	10 (50.0%)	10 (50.0%)	
T2	2 (3.6%)	20 (35.7%)	34 (60.7%)	
				0.455
T3	0 (0%)	4 (45.5%)	15 (54.5%)	
T4	0 (0%)	5 (36.8%)	6 (61.3%)	
Lymph node status				
N0	1 (2.1%)	21 (43.8%)	26 (76.2%)	
N1	0 (0%)	5 (23.8%)	16 (76.2%)	0.008
N2	0 (0%)	3 (13.6%)	19 (86.4%)	
N3	1 (1.9%)	10 (36.8%)	4 (61.3%)	
TNM staging				
I	0 (0%)	8 (61.5%)	5 (38.5%)	0.194
II	1 (2.0%)	18 (36.7%)	30 (61.2%)	
III	0 (0%)	11 (27.5%)	29 (72.5%)	

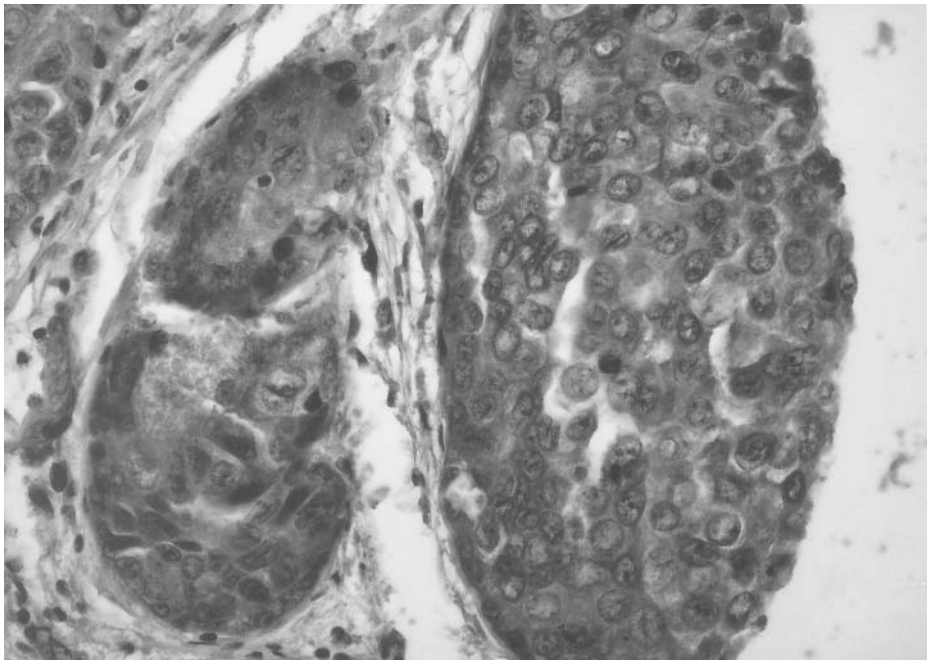

Figure 1. *Immunostaining with the IGF-1antibody on the tissue microarray slides of the breast cancer specimens. The representative 3+ case shown here reveals strong cytoplasmic and nuclear immunoreactivity in the tumor cells. Original magnification, x400.*

Discussion

Kononen J *et al.* (20) recently described an array-based high-throughput technique that facilitates analysis of very large numbers of tumors at once, either at the DNA, RNA, or protein level. As many as 1000 cylindrical tissue biopsy specimens from an individual tumor can be arrayed in a single tissue microarray (TMA) block. The power of the TMA technique is the capability of performing a series of analyses of thousands of specimens in a parallel fashion with minimal damage to the original blocks (10, 11, 20). In contrast to immunohistochemical analyses on large sections, the TMA allows a high level of standardization for immunohistochemical staining because all tumor sections are pretreated and stained under exactly the same conditions. Being different from the reading of large sections which always is an attempt to integrate the observations in multiple different regions of a tissue section, the morphological classification and interpretation of immunoreactivity are based on the findings within one small, highly defined tissue area in TMAs. The criteria for diagnostic decisions are therefore much easier to establish between the individual samples on the array and to compare among different observers (10, 11, 20).

Nevertheless, criticism of TMAs arises as to whether such small specimens (diameter 0.6 mm) are really representative of their donor tumors. It has been reported that some alterations are not detected if the analysis of heterogenous tumors is restricted to samples measuring 0.6 mm (21). However, Moch *et al.* (10) pointed out that the TMA approach has been designed to examine tumor populations and not to survey individual tumors. They (10) have analyzed the impact of tissue heterogeneity on TMA data comparing results obtained from TMA with results from large sections in multiple different studies and found that the results did show heterogeneity within tumors but suggested that this heterogeneity did not influence the identification of prognostic parameters. The reliability of TMAs in detecting protein expression and gene amplification in breast cancer has been confirmed (22, 23). Our study analyzed IGF-1 expression in breast cancer by

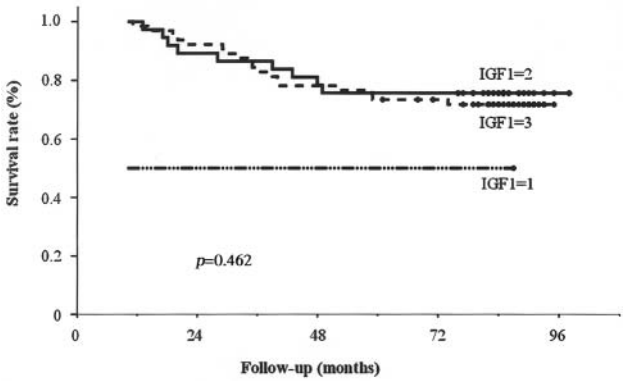

Figure 2. *No survival difference was noted among the three groups with different IGF-1 expression (p=0.462).*

immunohistochemical staining with TMA and the results were obtained without difficulty. To the best of our knowledge, this is probably the first report with long-term follow-up regarding IGF-1 expression in breast cancer analyzed by using TMAs.

Some reports demonstrated that patients with breast cancer had higher serum IGF-1 concentrations when compared with healthy people (24, 25). In contrast, Favori *et al.* (26) found no significant differences in IGF-1 concentration between cancer patients and a control group matched for age and menopausal status. Sancak *et al.* (27) also found no association between serum levels of IGF-1 and well-known clinicopathological characteristics of breast carcinoma including age, tumor size, axillary lymph node and ER/PR status, disease stage and tumor grade. In our study, a TMA technique was used and we also found that there was no significant relationship between IGF-1 expression and well-known clinicopathological parameters including age, estrogen receptor status, histologic grading, primary tumor staging and TNM staging. Furthermore, no survival difference was noted among the three groups with different IGF-1 expression ($p=0.462$, Figure 2).

In conclusion, immunohistochemical staining with tissue microarray was convenient and feasible for the analysis of IGF-1 expression in breast cancer, yet its expression did not show significant correlation with the overall survival rate.

References

1 Holdaway IM, Mason BH and Lethaby VS: Serum insulin-like growth factor-1 and insulin-like growth factor binding protein-3 following chemotherapy for advanced breast cancer. ANZ J Surg *73*: 905-908, 2003.

2 Khandwawala HM, McCurcheon IE, Flyvbjerg A and Friend KE: The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev *21*: 215-244, 2000.

3 Jones JI and Clemmons DR: Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev *16*: 3-34, 1995.

4 Yu H and Rohan T: Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst (Bethesda) *92*: 1472-1489, 2000.

5 Khandwala HM, McCutcheon IE, Flyvbjerg A and Friend KE: The effect of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev *21*: 215-244, 2000.

6 Chan JM, Stampfer MJ and Giovannucci E: Plasma insulin-like growth factor-1 and prostate cancer risk: a prospective study. Science *279*: 563-566, 1998.

7 Hankinson SE, Willett WC and Colditz GA: Circulating concentrations of insulin-like growth factor-1 and risk of breast cancer. Lancet *351*: 1393-1396, 1998.

8 Ma J, Pollak MN and Giovannucci E: Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-1 and IGF-binding protein-3. J Natl Cancer Inst *91*: 620-625, 1999.

9 Yu H, Spitz MR and Mistry J: Plasma levels of insulin-like growth factor-1 and lung cancer risk: a case-control analysis. J Natl Cancer Inst *91*: 151-156, 1999.

10 Moch H, Kononen J, Kallioniemi OP and Sauter G: Tissue microarrays: what will they bring to molecular and anatomic pathology. Adv Anat Pathol *8*: 14-20, 2001.

11 Rijn MVD and Gilks CB: Applications of microarrays to histopathology. Histopathology *44*: 97-108, 2004.

12 The World Health Organization Histological Typing of Breast Tumor, 2nd ed. Am J Clin Pathol *78*: 808-816, 1982.

13 Sheen-Chen S-M, Chen W-J, Eng H-L and Chou F-F: Serum concentration of tumor necrosis factor in patients with breast cancer. Breast Cancer Res Treat *43*: 211-215, 1997.

14 Sheen-Chen S-M, Eng H-L, Chou F-F and Chen W-J: The prognostic significance of proliferating cell nuclear antigen in patients with lymph node-positive breast cancer. Arch Surg *132*: 264-267, 1997.

15 Sheen-Chen S-M, Chen W-J, Eng H-L and Chou F-F: Serum-soluble interleukin-2 receptor concentrations in patients with breast cancer: A preliminary report. Chang Gung Med J *21*: 133-138, 1998.

16 Sheen-Chen S-M, Chou F-F, Eng H-L and Chen W-J: An evaluation of the prognostic significance of HLA-DR expression in axillary-node-negative breast cancer. Surgery *116*: 510-515, 1994.

17 Sheen-Chen S-M, Eng H-L, Sheen C-W and Cheng Y-F: Serum levels of circulating intercellular adhesion molecule-1 in patients with breast cancer. Anticancer Res *17*: 282-236, 1997.

18 Sheen-Chen S-M, Chen W-J and Eng H-L: Evaluation of the prognostic value of serum soluble CD44 in patients with breast cancer. Cancer Invest *17*: 581-585, 1999.

19 Perrone EE, Theoharis C, Mucci NR and Hayasaka S: Tissue microarray assessment of prostate cancer tumor proliferation in African-American and white men. J Natl Cancer Inst *92*: 937-939, 2000.

20 Kononen J, Bubendorf L and Kallioniemi A: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med *4*: 844-847, 1998.

21 Schraml P, Kononen J and Bubendorf L: Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res *5*: 1966-1975, 1999.

22 Torhorst J, Bucher C and Kononen J: Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol *159*: 2249-2256, 2001.

23 Zhang D, Salto-Tellez M, Putti TC, Do E and Koay ES: Reliability of tissue microarray in detecting protein expression and gene amplification in breast cancer. Modern Pathol *16*: 79-84, 2003.

24 Peyrat JP, Bonneterre J, Hecquet B and Vennin P: Plasm insulin-like growth factor-1(IGF-1) concentrations in human breast cancer. Eur J Cancer *29A*: 492-497, 1993.

25 Bruning PF, Bonfrer JM, van Noord PA, Hart AA, de Jong-Bakker M and Nooijen WJ: Insulin resistance and breast-cancer risk. Int J Cancer *52*: 511-516, 1992.

26 Favoni RE, de Cupis A, Perrotta A, Sforzini S, Amoroso D, Pensa F and Miglietta L: Insulin-like growth factor-1(IGF-1) and IGF-binding proteins blood serum levels in women with early-and late-stage breast cancer: mutual relationship and possible correlations with patients' hormonal status. J Cancer Res & Clin Oncol *121*: 674-682, 1995.

27 Sancak B, Coskun U, Gunel N, Onuk E, Cihan A, Karamercan A, Yildirim Y and Ozkan S: No aoociation between serum levels of insulin-like growth factor-1, vascular endothelial growth factor, prolactin and clinicopathological characteristics of breast carcinoma after surgery. Inter Med J *34*: 310-315, 2004.

Received June 4, 2007
Revised July 30, 2007
Accepted August 2, 2007