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A Study of Breast Cancer in Young Women: Prognosis and Prognostic Factors

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Introduction

The prognosis of the young women with primary breast cancer remains controversial. It has been considered that the prognosis of young women with primary breast cancer is more unfavorables compared with that of the older patients[1,2]. Some investigators reported that breast cancer in young women grow more rapidly and metastasize to nodes more easily than in older women[3-6]. The specific features and biological behavior which make the prognosis of breast cancer worse in young women should be discussed.

Recent advances in molecular biology has made the function of many oncogenes and growth factors clarified. PERREN, IWAYA and RAVDIN et al. regarded some of them as prognostic factors in breast cancer[7-9]. Are some oncogenes or growth faactors useful to predict prognosis of young women with breast cancer? The aims of this study were first to clarify the biological nature of breast cancer in young women by analyzing the clinical characteristics and histopathological findings, second to determine the differences between young women with primary breast cancer and older patients with regard to disease free survival (DFS), and finally to evaluate what is the most valuable prognostic factor for young patients.

In this study, expression of c-erbB-2 protein (*c-erbB-2*), and of p53 protein (*p53*) and Ki-67 labeling index (Ki-67 L.I.) were examined immunohistochemically and evaluated as prognostic factors in young women with breast cancer. *C-erbB-2* and *p53* are well known oncoprotein and tumor suppressor gene products. The Ki-67 L.I. indicates cell proliferative ability.

To clarify prognosis and prognostic factors of young women with breast cancer will greatly help the dicision regarding of therapeutic strategy in these patients.

Patients and Method

Patients

From April 1970 to March 1993, 47 patients who were 35 years old or younger underwent mas-

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tectomy for primary breast cancer in our clinic. Among them, 44 patients who underwent curative operation are examined in this study. Curative operation was defined according to the 11th edition Japanese general rules for clinical and pathological recording of breast cancer, (General rules)[10]. The other 3 patients were excluded because they had distal metastasis when diagnosis were made.

"Breast cancer in young women" was defined as cancer occuring in women 35 years old or younger according to the criteria established by the Japanese breast cancer study group in 1965.

The age of patients, size of tumor, presence or absence of nodal metastasies and prognosis were examined. The tumors were categorized based on their size according to the UICC classification[11]. Furthermore, the clinical stages were defined according to General rules. As postoperative adjuvant therapy, all patients were administrated oral estrogenic antagonist (tamoxifen citrate 20 mg/body/-day), and oral fluorouracil (300 mg/body/day) administration were added for the patients of stage II or III disease. The patients with nodal metastasis were underwent irradiation of Co60 (tatal 50 Gy) after surgery.

From April 1976 to May 1981, sequential 32 patients with primary breast cancer who were 36 years old or older at the time of surgery, and did not have metastases at diagnosis. They were examined as older group for comparison.

Method

Analysis of characteristics: A younger group and an older one were compared with regard to tumor size, nodal metastases, clinical stage, histological type, mitotic index (M.I.), histological grade, expression of *c-erbB-2, P53* and Ki-67 L.I..

Analysis of prognosis: Differences regarding outcome between the two groups were compared by based on D.F.S..

Evaluation of prognostic factors: The relationship between D.F.S. and classical prognostic factors, tumor size, nodal metastasis, stage, M.I. and histological grade was analyzed in younger group. The relationships between *c-erbB-2, p53*, Ki-67 L.I. and D.F.S. were analyzed, and evaluated what is the most valuable factor to predict D.F.S. among them.

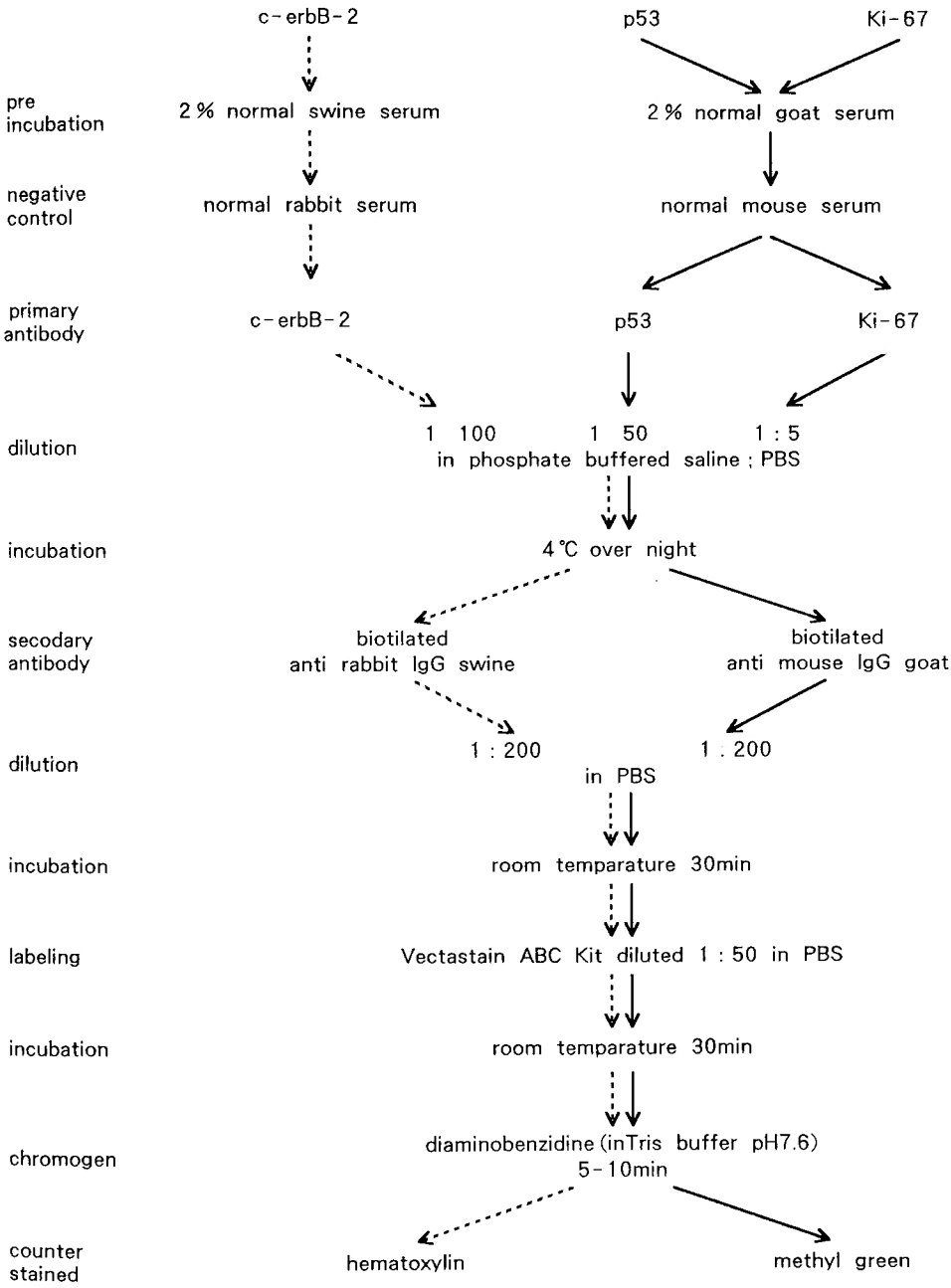
Histopathological examination

The samples of the tumor had been obtained by surgery, fixed in formalin and embedded in paraffin. After staining with Hematoxylin and eosin, the tumors were examined and classified histopathologically. Histological grading was performed based on TSUDA's criteria which were a modification of BLOOM and RICHARDSON's criteria[12,13].

Immunohistological examination

The samples of 35 young women were able to be used for immunohistological examination; the other 9 samples were in bad condition. The samples were cut into 4 μm sections and paraffin was re moved with xylene. After deparaffinization, the sections were passed through ethanol series with descending concentration of ethanol. The sections were processed with microwave 3 times for 5 minutes each at 500 watt in 0.01 M citrate buffer. To avoid endogenous peroxidase activity, each sections was incubated for 30 minutes with 0.3% hydrogen peroxide and methanol. Then, they were stained by the standard avidine-biotin method (Fig. 1)[14]. The primary antibody for *c-erbB-2* was polyclonal rabbit anti-human c-erbB-2 oncoprotein (DAKO, Glostrup, Denmark). Monoclonal mouse anti-human p53 protein DO-7 (DAKO) was used for *p53* immunostaining. Monoclonal mouse anti-

body anti-Ki-67 clone MIB-1 (Immunotech, Marseille, France) was used for Ki-67 immunostaining. *C-erbB-2* was judged as positive when nothing but the membrane was immunostained (Fig. 2a).



* between each steps. the slides were washed 3 times in PBS for 5 min

Fig. 1 Flow chart of immunostaining

P53 was regarded positive when nuclei was strongly and/or widespreded immunostained, while weak and/or focal staining with less than 5% of tumor cells showing a positive staining reaction was regarded as negative (Fig. 2b). MIB-1 staining was judged positive when the nuclei was strongly

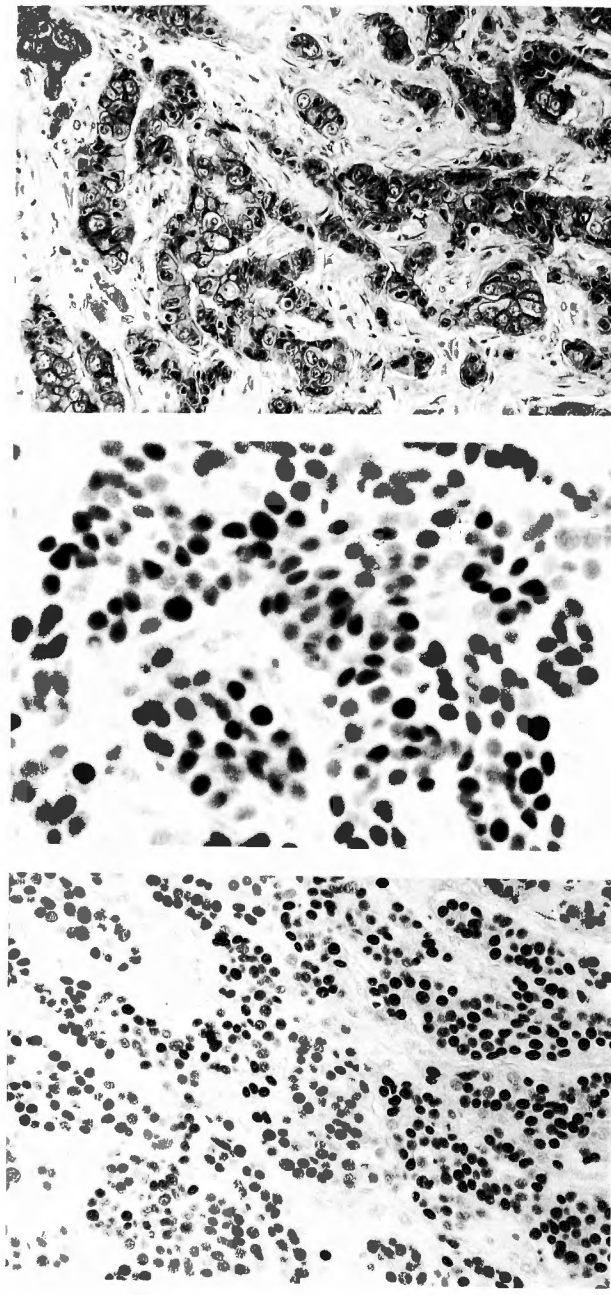


Fig. 2 Microscopic findings of primary breast cancer of young women: immunostained by c-erbB-2 antibody (2a), p53 antibody (2b). ×200. immunostained by MIB-1 antibody (2c). ×40.

stained (Fig. 2c). Ki-67 L.I. was caluculated as the percentage of MIB-1 positive nuclei per 1000 nuclei. The cutt-off level of Ki-67 L.I. was decided according to mean Ki-67 L.I. of younger group.

Statistics

Differences between the two groups regarding characteristics and prognostic factors were examined statistically using the Chi-square test or Mann-Whitney non-parametric test. The curves of D.F.S. were drawn according to the Kaplan-Meier method for each factor. Differences regarding of each curve were analyzed using generalized Wilcoxon test. A level of $p<0.05$ was taken as being significant. The dominant value of prognostic factors for D.F.S. were evaluated by Cox's proportional hazzard model[15].

Result

Characteristics

The characteristics of patients are shown in Table 1. There was no statistical differences in tumor size, nodal metastases, stage or duration of follow up between two groups. Histopathplogical findings are shown in Table 2. There were significantly more patients with tumors of higher histological grade among younger group than older one. No significant difference was observed between the two groups in histological type and M.I.. *C-erbB-2* was more frequently detected in samples from the younger group than in those from older one. On the other hand, *p53* was detected more frequently in samples from older group than in those from younger group. The Ki-67 L.I. was higher in those from younger group than in older one and showed statistical significance.

Prognosis

The percentage of 15-year D.F.S. of the young group was 70.64%, while that of older grup was 71.28%, showing no statistical difference between the two groups (Fig. 3). Local relapse or distant

Table 1 Characteristics of patients

characteristics	=<35 yr (n=44)	>35 yr (n=32)	p
age, yr: median (range)	31.23 (22–35)	50.25 (36–72)	
tumor size			
T1	14	11	0.4717
T2	18	16	
T3	12	5	
nodal metastasis			
negative	17	20	0.0684
positive	27	12	
stage			
I	12	9	0.1211
II	16	19	
III	16	4	
reccurrences, No. (%)	11 (25.0)	7 (21.9)	
follow up, M. (SE)	2711 (404.2)	3257 (388.4)	0.1408

metastases were not seen in the patients with stage I disease. Among the patients with stage II disease, there were 4 patients who had local relapse or distal metastases in younger group, and 5 in older group. Among those with stage III disease, There were 3 patients who had local relapse or distal metastases in younger group, and 2 in older group. There were no statistical differences in D.F.S. between two groups for patients with stage II and those with stage III disease (Fig. 4a, 4b).

Table 2 Distributions of histological and immunohistological details

	=<35 yr (n=35)	>35 yr (n=32)	p
histological type, No.			
ductal	4	9	0.0903
invasive	28	19	
special	3	4	
M.I., No. (%)			
1	18 (51.4)	25 (78.1)	
2	12 (34.3)	4 (12.5)	0.0634
3	5 (14.3)	3 (9.4)	
Grade, No. (%)			
I	8 (22.9)	16 (50.0)	
II	19 (54.3)	5 (46.9)	0.0009
III	8 (22.9)	1 (3.1)	
c-erbB-2 (+), No. (%)	18 (51.4)	8 (25.0)	0.032
p53 (+), No. (%)	14 (40.0)	26 (81.3)	0.0011
Ki-67 L.I., median	30.1	21.0	0.0148
(range)	(2.8–70.4)	(3.2–59.3)	

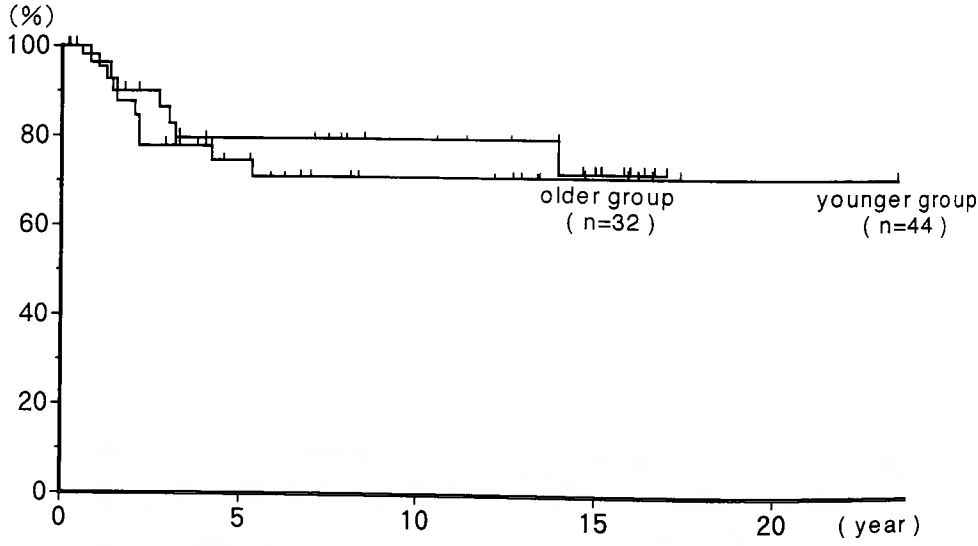


Fig. 3 D.F.S. of younger group and older one with primary breast cancer.

Prognostic factors

Significant differences regarding D.F.S. in related to tumor size, nodal metastases and stage were found in the univariate analysis. Higher histological grade, *c-erbB-2* positive and higher Ki-67 L.I. correlated with a shorter D.F.S. but there was no statistical significance (Table 3). In the multivariate analysis among tumor size, nodal metastases, histological grade, *c-erbb-2, P53* and Ki-67 L.I., *c-erbB-2* positive was significantly associated with a shorter D.F.S. (Table 4a). Furthermore in the multivariate analysis among nodal metastases and *c-erbB-2*, node positive and *c-erbB-2* positive showed statistical significance (Table 4b).

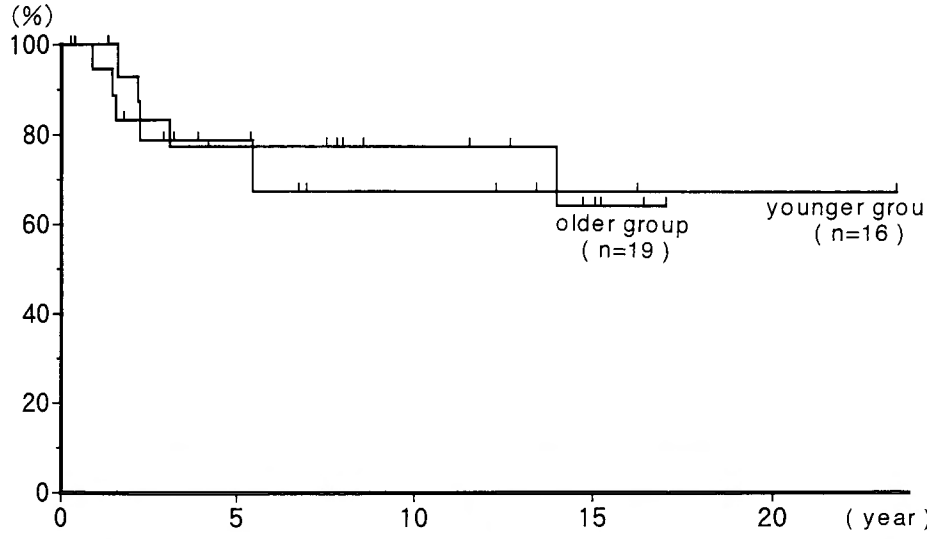


Fig. 4a D.F.S. of younger group and older one in stage II

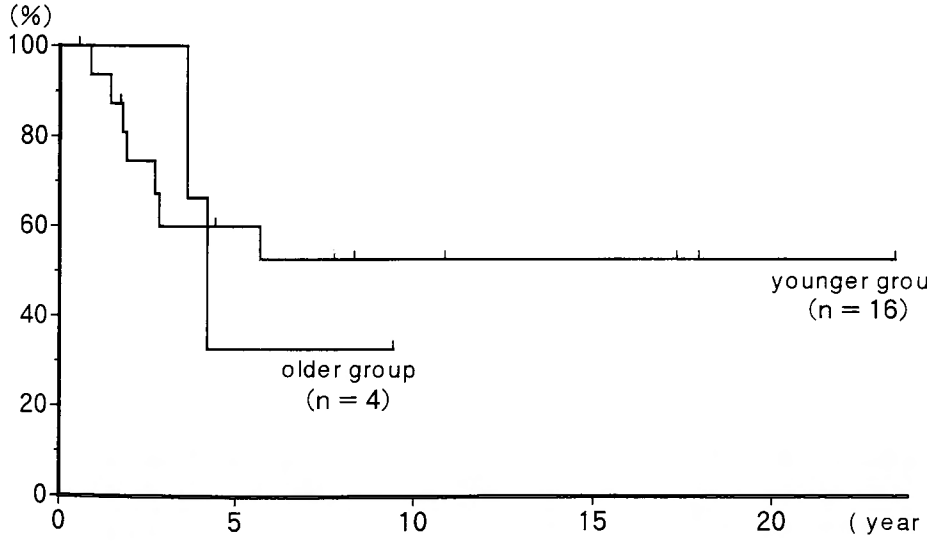


Fig. 4b D.F.S. of younger group and older one in stage III

Table 3 Univariate analysis of the prognostic factors in younger group

	D.F.S. (day)	S.E.	p
Tumor size			
1 or 2	7021.8	624.9	0.0287
3	3514.7	818.3	
nodal metstasis			
positive	4088.3	555.7	0.0399
negative	7988.9	535.8	
Stage			
I or II	7279.7	587.1	0.0047
III	3248.0	799.4	
mitotic index			
1 or 2	5652.6	793.3	0.8409
3	5108.2	1096.4	
histological grade			
I or II	5991.5	815.1	0.4686
III	4172.0	988.4	
c-erbB-2			
positive	4790.6	1094.2	0.1113
negative	5238.3	568.1	
p53			
positive	3754.2	436.4	0.3767
negative	5374.6	1119.5	
Ki-67			
$<=30$	6884.1	787.0	0.7159
>30	4992.3	739.8	

Table 4 Multivariate analysis of the prognostic factor in younger group

a.

	hazard ratio	95% confidential interval	p
T	1.279	(0.528–3.099)	0.598
n	2.253	(0.965–5.258)	0.070
Grade	1.360	(0.567–3.266)	0.496
c-erbB-2	5.165	(1.281–20.843)	0.028
p53	1.203	(0.162–8.906)	0.858
Ki-67 L.I.	0.439	(0.050–3.858)	0.466

b.

n	2.331	(1.059–5.129)	0.0438
c-erbB-2	4.963	(1.296–19.012)	0.0258

Discussion

It has been considered that the prognosis of young women with primary breast cancer is unfavorable[1,2]. However, recently some investigators have contradicted to conventional opinion like that[4,16,17]. The prognosis of the young women with primary breast cancer remains controversial. If it is unfavorable, the specific factors which make the prognosis of young women with breast cancer worse should be discussed.

For examining whether two groups were just for study, distributions of background factors between two groups were analyzed. Tumor size, nodal metastasis, stage and duration of following up between two groups showed no statistical difference in distributions. The result showed that these groups were thought to be suitable for study.

Recent progress in molecular biology revealed that tumor cells with overexpression of *c-erbB-2* and higher Ki-67 L.I. had higher proliferate potential[18,19]. Compareing histological and immunohistological details including *c-erbB-2, p53* and Ki-67 L.I., younger group showed different distributions in some details. The number of cases with higher histological grade, *c-erbB-2* positive and higher Ki-67 L.I. was bigger in younger group than in older one. These findings suggested that breast cancer in young women was biologically aggressive. And *p53* showed statistical difference in distribution between two groups. *P53* was more frequently observed in older group. P53 is well known tumor suppressor gene and *p53* is mutant p53 gene products, as detected by immunohistochemistry. Mutation of p53 gene is thought to be closely related development and progression of some neoplastic disease[21,22]. Discrepancy of overexpression of p53 between younger group and older one suggested that mutation p53 gene may be caused accompanied with aging and the mechanism of oncogenesis in young women may have been different from older women.

In spite of being infered aggressive behavior of breast cancer in young women, there was no differences in D.F.S. between younger group and older one. The D.F.S. rate for younger group was higher compared to the data of previous studies that showed poor prognosis for young women[16,17]. The reasons why our cases showed longer D.F.S. were thought that firstly all the patients in this study underwent curative operation. ROSEN et al.[23] also found 10-year survival rate of young women with operable disease were not appreciably different from those of women treated for breast cancer at a later age. Secondary the cases in this study were analyzed with stratification. There has been a few studies which analyzed the prognosis by stratification to correct the biases among cases in previous studies which showed poor prognosis for young women[2,3]. In other studies which were analyzed data by staratification, most of them commented that prognosis of young women was not poor compared to older women[4,23–25]. Prognosis of young women with breast cancer was thought to be not poor when they had underwent curative operation.

Providing that breast cancer in young women was biologically aggressive, factors that proved it must be closely related to prognosis of young women with breast cancer. Conventional histological factors, histological grade, and oncogene products-*c-erbB-2, p53*, Ki-67 L.I. which showed significant difference in distributions were examined. Univariate analysis revealed that tumor size, nodal metastasis and stage were valuable, but histological grade, *c-erbB-2, p53* and Ki-67 L.I. did not show statistical significance. Conventional prognostic factors were though to be represented the position in the time course of tumor progression. And they are able to predict patients prognosis. But they are not able to explain prognostic differences among patients with same stage disease. On the other hand, oncogene products and growth factors seemed to be free from the time course of tumor progression

and were thought to be able to explain prognostic differences among younger patients with same stages[23,24]. And univariate analysis is not able to explain the interactions among many prognostic factors. To evaluate what is the most valuable prognostic factor for young patients, the multivariate analysis was performed. Stage and M.I. were excluded because stage depended on tumor size and nodal metastasis, M.I. and histological type consisted historogical grade. *C-erbB-2* showed statistical significance as an independent prognostic factor. The hazzard ratio of *c-erbB-2* was higher than that of nodal metastasis. There has been reported more important prognostic factor than nodal metastasis in young women[25,26]. *C-erbB-2* has a structure highly homologous to that of the epidermal growth factor receptor, it has been postulated that overexpression of *c-erbB-2* might be associated with faster tumor proliferation[18]. Preoperative analysis of *c-erbB-2* using biopsy material will help to make the dicision of therapeutical strategy in young patients.

P53 and Ki-67 L.I. were not powerful prognostic factors in multivariate analysis. Number of patients with *p53* positive were smaller in younger group than in older one. These results suggested different mechanism of oncogenesis in young women with breast cancer, but did not attribute to prognostic value. Higher Ki-67 L.I. in younger group emerged higher proliferate potential of breast cancer in young women, but could not predict D.F.S. of young women.

In conclusion, the prognosis of young women with breast cancers was not poorer than that of older women who underwent curative operation. And *c-erbB-2* appeared as a strong prognostic factor equal to nodal metastasis in young women with breast cancer.

Preoperative analysis of *c-erbB-2* will contribute to the establishment of therapeutical strategies for young women with breast cancer leading to a better survival.

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References

1) Ewing J: neoplastic disease, 3rd ed., WB Saunders, Philadelphia, pp 579, 1934.
2) Taylor G: Carcinoma of the breast. Surg Clin N Am 27: 1151–1155, 1947.
3) Humphrey L, Swerdlow M: Factors influencing the survival of patients with carcinoma of the breast. Am J Surg **106**: 440–444, 1963.
4) Wallgren A, Silversward C, Hultborn A: Carcinoma of the breast in women under 30 years of age. Cancer **40**: 916–923, 1977.
5) Haagensen CD, Miller E, Harvey RB: Treatmant of early mammary carcinoma. Ann Surg **170**: 875–878, 1969.
6) Gaos J, Skaikes G: Prognosis of mammary carcinoma in young women under 30 years of age. Surgry **78**: 339–342, 1975.
7) Perren TJ: C-erbB-2 oncogene as a prognostic marker in breast cancer. Br J Cancer **63**: 328–332, 1991.
8) Iwaya K, Tsuda H, Hiraide H, et al: Nuclear p53 immunoreaction associate with poor prognosis of breast cancer. Jpn J Cancer Res **82**: 835–840, 1991.
9) Ravdin PM: Evaluation of cathepsinD as a prognostic factor in breast cacner. Breast Cancer Res Treat **24**: 219–226, 1993.
10) Japanese breast cancer society: General rules for clinical and pathological recording of breast cancer, the 11th edition. Kaneharashuppan, Tokyo, 1993.
11) UICC International Union Against Cancer: TNM classification of malignant tumors, fourth, fully revised edirtion. Springer-Verlag, Berlin, Heiderberg, New York, London, Paris, Tokyo, 1987.

12) Bloom HIJ, Richardson WW: Histological grading and progression in breast cancer. Br J Cancer **11**: 359–377, 1957
13) Tsuda H, Hirohashi H, Shimosato Y, et al: Correlation between histologic grade of malignancy and copy number of c-erbB-2 gene in breast carcinoma. Cancer **65**: 1794–1800, 1990.
14) Hsu SM, Raine L, Fanger H: Use of avidine-biotin-peroxidase complex (ABC) in immunoperoxydase tecqhniques. J Histochem Cytochem **29**: 577–580, 1981.
15) Cox DR: Reggression models and life-tables. J R Stat Soc **34**: 187–220, 1972.
16) Rosen PP, Lessserr ML, Kinne DW: Breast carcinoma in women 35 years of age or younger. Ann Surg **199**: 133–142, 1984.
17) Treaves N, Holleb AL: A report of 549 cases of breast cancer in women 35 years of age or younger. S G O **107**: 271–283, 1958
18) Coussens l, Yang TL, Liao YC: Tyrosinekinase receptor with extensive homology to EGF receptor shares chromosomal locarion with neu oncogene. Science **230**: 1132, 1985.
19) Gerdes J, Lemke H, Baisch H, et al: Cell cycle analysis of a cell priliferation associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol **133**: 1710–1715, 1984.
20) McGurrin J, Dori M, Dawson P, Karrison T, Stein H, Franklin W: Assessment of tumor cell kinetics by immunohistochemixtry in carcinoma of breast. Cancer **59**: 1744–1750, 1987.
21) Cattoretti G, Rilke R, Andoreolas, et al: P53 expression in breast cancer. Int J Cancer **59**: 1744–1750, 1987.
22) Hollstein M, Sidrnsky D, Vogelstein B: P53 mutation in human cancers. Science **253**: 49–54, 1991.
23) Noyes RD, Spanos WJ, Montague AE: Breast cancer in women ages 30 and under. Cancer 49: 1302–1307, 1982.
24) Arriagada R, Rutqvist EL, Skoog L, Johanson H, Kramar A: Prognostic factors and natural history in lymph node negative breast cancer patient. Breast Cancer Res Treat **21**: 101–109, 1992.
25) Weidner N, Folkman J, Pozza F, et al: Tumor angiogenesis: A significant and independent prognostic indicator in early stage breasr carcinoma. J Natl Cancer Inst **84**: 1875–1887, 1992.
26) Rochefordiere A, Asselain B, Campana F, et al: Age as prognostic factor in premenopausal breast cancinoma. Lancet **341**: 1039–1043, 1933.
27) Nixon A, Neuberg D, Hayes D, et al: Relationship of patients age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer. J Clin Oncol **12**: 888–894, 1994.

和文抄録

若年者乳癌の予後と予後因子についての研究

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【目的】若年者乳癌の予後と予後因子について検討した．
【方法】35歳以下の原発性乳癌患者35名を若年群，36歳以上の32名を年長群とした．全ての症例は原発性乳癌に対し治癒切除術を受けている．各症例の背景因子，病理組織学的所見，免疫組織学的所見 (c-erbB-2, p53, Ki-67 L.I.) について調べ，健存率を比較した．
【結果】若年群に未分化な組織型，c-erbB-2 蛋白過剰発現および高い Ki-67 L.I. を示す症例が有意に多かった．若年群の15年健存率は年長群と比べ有意差を認めなかった．若年群において，リンパ節転移と c-erbB-2 蛋白過剰発現は，多変量解析で独立した予後因子であると認められた．
【結語】若年者乳癌は生物学的な悪性度が高いと考えられた．しかし治癒切除術がなされた症例では，若年者と年長者の予後に差を認めなかった．c-erbB-2 蛋白過剰発現は，若年者乳癌においてリンパ節転移と同等の強力な予後因子であると認められた．