The THAI *Journal of* SURGERY

Official Publication of the Royal College of Surgeons of Thailand

Vol. 20 January - March 1999 No. 1

p53 Mutation and MDR1 Overexpression in Local and Distant Failures of Breast Cancer Treated by Lumpectomy and Radiation

Rumpa Amornmarn, MD*
Thongbliew Prempree, MD., Ph.D., FACR.**
Marilyn Bui, MD., Ph.D.***
Shahla Masood, MD., FCAP, MIAC†

**Associate Professor, Dept. of Radiation Oncology, University of Florida Health Science Center, Jacksonville, Florida, USA.*
***Professor, University of Florida, Currently Chief of Radiation Oncology, Samitivej Srinkarin Hospital, Bangkok. Thailand.*
****Clinical Instructor, Dept. Pathology University of Florida Health Science Center, Jacksonville, Florida, USA.*
†Professor & Associate Chair, Dept. Pathology, University of Florida Health Science Center, Jacksonville, Florida, USA.

Correspondence to : Thongbliew Prempree, MD., Ph.D., FACR
Department of Radiation Oncology, Samitivej Srinakarin Hospital,
488 Srinakarin Road, Bangkok, Thailand

Abstract: **To search for the risk factors associated with local recurrence and distant metastasis of breast cancer treated by lumpectomy and irradiation, 112 cases treated during a period of eleven years (1983-1994) at University Hospital, Jacksonville were studied. Among those studied, 4(4%) patients recurred locally within one year of treatment; 10(9%) cases presented with distant metastasis within 3 years. No obvious clinical risk factors were identified for local recurrence, however, positive node status appeared to be associated with distant metastasis. The primary tumors of these cases were then studied using immnohistochemical staining to evaluate the potential prognostic value of tumor markers including estrogen receptor(ER), progesterone receptor(PR), tumor suppressor gene p53, HER2/neu oncogene and multidrug resistance gene (MDR1). The overexpression of p53 was associated with all local recurrence while 50 per cent was associated with distant failures. The overexpression of MDR1 gene was observed in 80 per cent of all distant failure cases. This interesting findings may warrant further studies on a larger scale to assess the predictive value of p53 and MDR1 in the overall management of breast cancer.**

Lumpectomy and radiation has been a treatment of choice for women with early breast cancer for a number of years.[1-13] The combination of lumpectomy and radiation has proven to be as effective as a radical or modified radical mastectomy without disfiguring the patients.[2,3,8,14] The usage of chemotherapy and or hormonal therapy in a high risk group of patients in conjunction with surgery and or radiation has added more value to the overall management of breast cancer.[15-27] While the search for an improved method of treatment has always been in the forefront, there continues to be a need for molecular biomarker as predictive and prognostic factors to evaluate the response to various treatments including chemotherapy and radiation therapy.[28-34,35-41]

This study was intended to evaluate two following goals.

1. To evaluate the efficacy of radiation treatment after lumpectomy by using local and distant failures as an end point of study.
2. To identify the potential molecular biomarkers as predictive and prognostic factors of response to radiotherapy.

Materials and Methods

Patients

One hundred and twelve patients with primary breast cancer treated with lumpectomy followed by definitive radiotherapy at the University Florida Health Science Center/Jacksonville, Florida, between 1983-1994 were studied. Surgical treatment consisted of complete removal of primary tumor with wide local excision or quadrantectomy. All cases underwent post lumpectomy mammography to detect any residual tumors. Three of six cases with positive margins were re-excised. Node dissection was performed in 103 patients. Approximately one third of the patients (38 cases) also received adjuvant chemotherapy. Another third (42 cases) received hormonal therapy with tamoxifen. Chemotherapy consisted of 5-fluorouracil, Cyclophosphamide, Methotrexate (CMF) or Cytoxan and Adriamycin for women who were considered as high risk (e.g. young age, positive nodes) by medical oncologists. The endpoint of observation was local recurrence and distant metastasis of breast cancer. The follow up period ranged from 3 to 14 years. For the 95 disease-free survivors, the median follow-up was 7 years.

Radiation Therapy

The treatment plan is summarized in Table 1. Briefly, the breast was irradiated using two opposed medial and lateral tangential portals encompassing the whole breast with small portion of the lung. The total tumor dose of 5000-5040 cGy was administered in 25-28 fractions to 90 per cent isodose. After whole breast irradiation, additional 1000/5 fractions were administered to the original tumor site. Supraclavicular and axillary irradiation were performed in all patients with axillary metastasis and advanced T stage with a tumor dose of 5000/25 fractions to the depth of 3 cm. Posterior axillary irradiation was used to supplement radiation to the mid plane of the axilla after anterior supraclavicular-axillary irradiation to bring the dose to 5000 cGy. No attempt was made to treat internal mammary node except a few with T3 or T4 in the early years.

Immunohistochemical Analysis

Standard immunohistochemical analysis was performed. The procedures were summarized as follows:

1. Slide preparation: Four micrometer sections

Table 1 Radiation treatment plan.

Field	Dose/Fractions	Depth	Technique	Machine
Breast	5000-5054/25-28	90%	Opposed Tangential	20meV/6MeV/Cobalt 60
Boost to tumor bed	1000/5	90%	AP	20 MeV
Supraclavicular & Axilla	5000/25	3 cm	AP	6MeV/Cobalt 60
Post axilla boost	5000 mid axilla	Mid axilla	PA	20 Mev/6MeV/Cobalt 60

Table 2 Specification of antibodies to 5 molecular biomarkers.

	Clonality	Vender	Dilution
Estrogen receptor (ER)	6F11	Novacastra	1:15
Progesterone receptor (PR)	PR-2C5	Zymed	1:25
Anti-human p53 protein	DO-7	Dako	1:30
HER-2/neu oncogen protein	TAB250	Zymed	1:20
Multiple-drug-resistance gene protein (MDR)	UIC2	Immunotech	1:60

were cut from the formalin-fixed paraffin-embedded tissure blocks. Sections were placed on silanized glass slides. After being dried in an oven for one hour at 65°C, the slides were deparaffinized with three changes of xylene, then rehydrated with graded ethanol and distilled water.

2. Pretreatment: The slides subjected to ER, PR, and p53 analysis were pretreated by standard high temperature antigen recovery in 1 mM citrate buffer at pH 6.0. The slides for HER-2/neu analysis were pretreated enzymatically (with 0.1% Ficin) according to the protocol. MDR analysis did not require any pretreatment. Endogenous peroxidase was blocked using 3 per cent hydrogen peroxide for 5 minutes.

3. Primary antibody reaction: The specifications of antibodies used in our study are listed in Table 2. ER, PR, p53, and HER-2/neu assays were performed using Ventana ES automated immunohistochemical staining system (Ventana Medical Systems, Tucson, AZ 85705). Primary antibodies were inclubated on the slides for 32 minutes at 42°C. MDR antibody was applied manually. The incubation was at room temperature for 30 minutes.

4. Secondary antibody reaction: A standard labeled streptavidin-biotin detection method (LSAB2, catalog number KO675, DAKO, Carpinteria, CA (93013) was used with diaminobenzidine (DAB) as a substrate.

5. Counterstaining and interpretation: ER, PR, and p53 slides were counterstained with ethyl green. Brown nuclear staining indicated immunoreactivity. The counterstain for HER-2/neu and MDR was hematoxylin. Membrane and cytoplasm staining brown indicate HER-2/neu and MDR overexpression. Each run of assay was performed in parallel with a negative and a positive control slide.

Results

Biographic and Biological Characteristics of the Patients

Sixty six cases (59%) were Caucasians, 44 were blacks. One was Asian and one was hispanic. Their ages range from 28 to 86 years with a median age of 59. As demonstrated in Table 3, most of the tumors were T1, T2 and stage I and II without lymph node involve-

Table 3 Clinical and histolgoical characteristics of the tumors.

	Lumpectomy (112)
Tumor size	
T1	61 (54%)
T2	43 (39%)
T3	5 (4%)
T4	3 (3%)
Positive nodes	
0	73 (65%)
1-3	11 (10%)
4-9	12 (11%)
> 10	7 (6%)
No node dissection	9 (8%)
Stage	
I	50 (45%)
II	50 (45%)
III	12 (10%)
Histological types	
Infil. ductal	65 (58%)
Grade II	5 (4%)
Grade III	30 (27%)
Others	12 (11%)

ment. The majority of the tumors were infiltrating ductal carcinoma.

Follow-up Status

All 112 patients were followed-up very well after surgery and radiation treatment (Table 4). There were 4 cases of local recurrence within a year of treatment. Ten cases developed distant metastasis within 3 years. Ninty-five cases survived free of breast cancer. Four patients died of intercurrent disease which were not related to breast cancer. The follow-up periods range from 3 to 14 years. For the 95 tumor free survivors, the median follow-up was 7 years. 5-years disease-free survival was 92 per cent for stage I; 85 per cent for stage II and 83 per cent for stage III respectively (Table 4).

Risk Analysis

No obvious clinical risk factors were identified for 4 local recurrent and 1 regional cases (Table 5). However, node positivity was more frequently observed in metastasis cases (Table 6). In order to investigate the potential predictive value of molecular tumor markers, the expression of 5 proteins were analyzed using immunohistochemical method. No obvious pattern of expression was found regarding to ER, PR, and HER-2/neu. However, p53 immuno-reactivity was found in all local recurrence cases as well as 50 per cent of the metastatic cases. Table 7 summarizes the clinical and molecular characteristics of the local recurrence cases, indicating among all the risk factor investigated, p53 positivity was the only factor observed in all four cases. Among the 5 distant metastatic cases that were

Table 4 Summary of follow-up status of breast cancer patients.

Stage	Patients	Local Recurrence	Distant Metastasis	Disease-free Survivors
I	50	2 (4%)	3 (6%)	44 (92%)*
II	50	2 (4%)	6 (12%)	41 (85%)*
III	12	1 (8%)	1 (8%)	10 (83%)
Total	**112**	**5 (4%)**	**10 (9%)**	**95 (87%)**

***2 Patients died of intercurrent diseases**

Table 5 Clinical risk factors analysis of locally recurrent cases.

	T1	T2	T3	T4	Total
N0	2/50	1/22	0/1	0/3	3/76 (4%)
N1	0/10	1/17	1/2	0	2/29 (7%)
N2	0/1	0/4	0/2	0	0/7
Total	**2/61 (3%)**	**2/43 (5%)**	**1/5 (20%)**	**0/3**	**5/112 (4%)**

Table 6 Clinical risk factors analysis of metastatic cases.

	T1	T2	T3	T4	Total
N0	3/50	1/22	0/1	0/3	4/76 (5%)
N1	1/10	3/17	1/2	0	5/29 (17%)
N2	0/1	1/4	0/2	0	1/7 (14%)
Total	**4/61 (7%)**	**5/43 (12%)**	**1/4 (20%)**	**0/3**	**10/112 (9%)**

Table 7 Summary of clinical and molecular characteristics of local/regional recurrence cases.

	Clinical	Molecular	Recurrence	Salvage Treatment	Outcome
1	41 y/o, T2 N0, IQ, Margins (+)	ER (+), PR (+), HER-2 (–), MDR (+), p53 (+)	9 months Breast & Node Inflammatory	Chemo (CAF)	Distant metastasis Death
2	41 y/o, T1N0, IQ	ER (–), PR (–), HER-2 (–), MDR (–), p53 (+)	7 months Breast	Surgical (MRM)	NED
3	57 y/o, T1 N0, CIS	ER (+), PR (–), HER-2(+), MDR (–), p53(+)	1 year Breast	Surgical (MRM)	NED
4	36 y/o T2 N0, IQ poorly, vascular invasion	ER (–), PR (+), HER-2(–), MDR (+), p53 (+)	1 year Breast	Surgical (MRM) Chemo (Taxol)	Living
5*	33 y/o T3 N1, 9x10 cm	Not available	9 years Internal mammary node	XRT Chemo	Pleural involvement Death

***A case of regional recurrence. Also a consultation case.**

available for retrospective immuno-histochemical analysis, 4 of them were MDR positive. This may suggest a potential role of MDR as a risk factor of resistance to multiple therapy modality. No obvious differences were observed regarding to the clinical risk factors and molecular markers between the group receiving adjuvant chemotherapy versus the group not receiving adjuvant chemotherapy.

Discussion

Our treatment appeared to be effective as compared to the other series in the literatures.[1,2,5,7,8,10,12] As shown in Table 4, the local recurrence occurred at the rate of 4 per cent. These figures fared very well with many published data or even better in some national protocols.[1,2,5,7,8,11,12] Factors contributed to our results perhaps were due to 1) Lumpectomy followed by irradiation is the very effective treatment reducing not only local recurrence but also distant metastasis; 2) Uniformed radiation treatment policy; 3) Most, if not all, our cases had clear surgical margins prior to initiating radiation treatment; 4) Most of our patients had negative node (stage I and II) (Table 3).

Our data showed lymph node metastasis was a clinical risk for distant metastasis (Table 6) but not for local recurrence (Table 5). Our findings confirmed the data from previous study by other group.[1,2,5,7,8,10-14] P53 overexpression was observed in all local recurrent cases and 50 per cent of distant metastatic cases. This finding raised the importance of the findings relative to significant predictive value of p53 immunoreactivity for local recurrence of breast cancer.[38,39,42,43] It was important also to know that in invasive breast cancer without treatment about 20-30 per cent showed p53 immunoreactivity.[39] In published literatures, others have reported controversially on the expression of p53 immunoreactivity regarding to recurrent and metastatic breast cancer cases, ranging from no significant predictive value to significant in both groups.[38,39,42,43,52]

The protein product of the Multidrug Resistance Gene (MDR 1) was known to be predictive in breast cancer refractory to chemotherapy.[44-47] The P-glycoprotein (MDR 1) was also known to be overexpressed in Glioblastoma multiforme brain tumors which were resistant to both irradiation and chemotherapy and in both patients and cell cultures.[48,49]

Eventhough little is known about the association of MDR 1 gene overexpression and radiation resistance in breast cancer, our data showed that overexpression was observed in 80 per cent of breast

cancer tissue from patients who later on developed distant metastasis and 50 per cent of breast cancer tissue from patients who later on developed local recurrence. These findings may be important and significant in recognizing the tumor resistance to chemotherapeutic agents and or radiation prior to initiating the treatment.

Recently the study was underway to investigate the blocker of MDR 1 protein in order to obviate the effect of MDR 1 protein towards the radiation or chemotherapeutic agents in order to improve the response rate of locally advanced breast cancer.[50] We are still awaiting the results of those studies.

In regard to Estrogen and Progesterone receptor (ER,PR) status, it has been accepted in general that the presence of ER and PR would indicate a favorable prognosis.[23,24,35,51] In the ER and PR negative breast cancer, the behavior of the tumor would be similar to those of non hormone dependent carcinoma of the other parts of the body.[23,24,35,51]

HER-2/neu overexpression or amplification has been detected in 25-50 per cent breast cancer and the amplification of HER-2/neu gene was always associated with shorter overall survival and rapid relapse.[40,41,52] Our data on HER-2/neu overexpression did not seem to be related to local recurrences and distant metastases. This may be due to our small sample size rather than negative correlation with survival, local or distant failures.

P53 alone may not be an effective predictive and prognostic marker to evaluate the response of radiation treatment. However, knowing the state of p53 suppressor gene whether it is in the mutated or wild type state may be very useful in determining the biological behavior of that tumors.[36,38,39,43] It is now known that Apoptosis (programmed cell death) works best when p53 is in its natural state. Most, if not all, chemotherapeutic agents and ionizing radiation work best when p53 is not mutated.[38,39,42] On the other hand, when p53 is in mutated state, its function/ p53 protein product will not work effectively and in turn, the radiation and or chemotherapeutic agents under poor p53 function will not be most effective against the tumors.

Conclusion

1. Lumpectomy and radiation treatment of early breast cancer is an effective treatment when it was done in a good clinical setting.

2. Our data fared very well with most data from national protocols in that local recurrence occurred at the rate of 4 per cent while distant metastasis occurred at the rate of 9 per cent.

3. We were not able to identify clinical risk factors for locol recurrence but positive node status appeared to be associated with distant failures.

4. We studied 5 tumor marker proteins using immunohistochemical staining and found p53 protein to be positive in all local recurrent cases (100%) while only 50 per cent was positive in distant failure cases. MDR1 gene amplification, on the other hand, was found to be positive 80 per cent of distant failure cases.

5. These interesting findings may warrant further studies on a larger scale basis in order to further assess the above data for future clinical usage.

Acknowledgement

The Authors thank Khun Monsinee Yoothana-amorn for her kindness in typing this manuscript.

References

1. Blichert-Toft M, Brincker H, Anderson J, et al. A Danish randomized trial comparing breast preserving therapy with mastectomy in mammary carcinoma. Acta Oncol 1988; 27:671.
2. Srrazin D, Le M, Arriagada R, et al. Ten-year results of a randomized trial comparing a conservative treatment to mastectomy in early breast cancer. Radiother Oncol 1989; 14:177.
3. Valagussa P, Bonadonna G, Veronesi U. Patterns of relapse and survival following radical mastectomy: analysis of 716 consecutive patients. Cancer 1978; 41:1170.
4. Fisher B, Wolmark N, Bauer M, et al. The accuracy of clinical nodal staging and of limited axillary dissection as a determinant of histologic nodal status in carcinoma of the breast. Surg Gynecol Obstet 1981; 152:765.
5. Early Breast Cancer Trialists' Group. Treatment of early breast cancer: world-wide experience, 1985-1990, vol 1. Oxford, U.K.: Oxford University, 1990.
6. Rutqvist L, Pettersson D, Johansson H. Adjuvant radiation therapy versus surgery alone in operable breast cancer: long-term follow-up in a randomized clinical trial. Radiother Oncol 1993; 26:104.
7. Van Dongen J, Bartelink H, Fentimen I, et al. Randomized clinical trial to assess the value of breast-conserving therapy in stage I and II breast cancer, EORTC 10801 trial. J Natl

Cancer Inst Monogr 1992; 11:15.

8. Lichter A, Lippman M, Danforth D, et al. Mastectomy versus breast conserving therapy in the treatment of stage I and II carcinoma of the breast: a randomized trial at the National Cancer Institute. J Clin Oncol 1992; 10:976.
9. Fisher B, Anderson S, Redmond C, et al. Reanalysis and results after 12 years of follow-up in a randomized clinical trial comparing total mastectomy with lumpectomy with and without irradiation in the treatment of breast cancer. N Engl J Med 1995; 333:1456.
10. Fisher B, Redmond C, and others of the National Surgical Adjuvant Breast and Bowel Project. Lumpectomy for breast cancer: an update of the NSABP experience. J Natl Cancer Inst Monogr 1992; 11:7.
11. Fisher B, Constantino J, Redmond C, et al. Lumpectomy compared with lumpectomy and radiation therapy for the treatment of intraductal breast cancer. N Engl J Med 1993; 328:1581.
12. Fowble B, Solin L, Schultz D, et al. Frequency sites of relapse, and outcome of regional node failures following surgery and radiation for early breast cancer. Int J Radiat Oncol Biol Phys 1989; 17:703.
13. Rosen P, Groshen S, Saigo P, et al. A long-term follow-up study of survival in stage I (T1N0M0) and stage II (T1N1M0) breast carcinoma. J Clin Oncol 1989; 7:355.
14. Borger J, Kemperman H, Hart A, et al. Risk factors in breast-conservation therapy. J Clin Oncol 1994; 12:653.
15. Bonadonna G, Valagussa P, Rossi A, et al. Ten-year experience with CMF-based adjuvant chemotherapy in resectable breast cancer. Breast Cancer Res Treat 1985; 5:95.
16. Fisher B, Fisher E, Remond C, Ten year results from the NSABP clinical trial evaluating the use of L-phenylalanine mustard (LPAM) in the management of primary breast cancer. J Clin Oncol 1986; 4:929.
17. Howell A, George W, Crowther D, et al. Controlled trial of adjuvant chemotherapy with cyclophosphamide, methotrexate, and fluorouracil for breast cancer. Lancet 1984; 2:307.
18. Rubens R, Knight R, Fentiman I, et al. Controlled trial of adjuvant chemotherapy with melphalan for breast cancer. Lancet 1983; 1:839.
19. Muss H, Cooper R, Brockschmidt J, et al. A randomized trial of adjuvant chemotherapy (CT) without radiation therapy (RT) for stage II breast cancer: 11-year follow-up of Piedmont Oncology Association protocol 74176. Breast Cancer Res Treat 1989; 14:185.
20. McArdle C, Crawford D, Dykes E, et al. Adjuvant radiotherapy and chemotherapy in breast cancer. Br J Surg 1986; 73:264.
21. Blomqvist C, Tiusanen K, Elomma I, et al. The combination of radiotherapy, adjuvant chemotherapy (cyclophosphamide-doxorubicin-ftorafur) and tamoxifen in stage II breast cancer: long-term results of a randomized trial. Br J Cancer 1992; 66:1171.
22. Bonadonna G, Valagussa P, Zucali R, et al. Feasibility of adjuvant chemotherapy plus radiotherapy in operable breast cancer. In: Harris JR, Hellman S, Silen W, eds. Conservative management of breast cancer. Philadelphia: JB Lippincott, 1983:329.
23. Rutqvist L, Cedermark B, Fornander T, et al. The relationship between hormone receptor content and the effect of adjuvant tamoxifen in operable breast cancer. J Clin Oncol 1989; 7:1474.
24. Nolvadex Adjuvant Trial Organization. Controlled trial of tamo-xifen as a single adjuvant agent in management of early breast cancer: Analysis at six years by the Nolvadex Adjuvant trial Organization. Lancet 1985; 1:836.
25. Wazer D, Joyce M, Chan W, et al. Effects of tamoxifen on the radiosensitivity of hormonally responsive and unresponsive breast carcinoma cells. Radiat Oncol Invest 1993; 1:20.
26. Margolese R. Surgical considerations in selecting local therapy cancer: analysis at six years by the Nolvadex Adjuvant Trial Organization. Lancet 1985; 1:836.
27. Breast Cancer Trials Committee. Scottish Cancer Trials Office: Adjuvant tamoxifen in the management of operable breast cancer: the Scottish trial. Lancet 1987; 2:171.
28. Veronesi U, Luini A, Galimberti V, et al. Conservation approaches for the management of stage I/II carcinoma of the breast: Milan Cancer Institute Trials. World J Surg 1994; 18:70.
29. Vilcoq J, Calle R, Stacey P, et al. The outcome of treatment by tumorectomy and radiotherapy of patients with operable breast cancer. Int J Radiat Oncol Biol Phys 1981; 7:1327.
30. Boyages J, Recht A, Connolly J, et al. Early breast cancer: predictors of breast recurrence for patients treated with conservative surgery and radiation therapy. Radiother Oncol 1990; 19:29.
31. Kurtz J, Spitalier J, Amalric R, et al. Mammary recurrence in women younger than forty. Int J Radiat Oncol Biol Phys 1988; 15:271.
32. Matthews R, McNeese M, Montague E, et al. Prognostic implications of age in breast cancer patients treated with tumorectomy and irradiation or with mastectomy. Int J Radiat Oncol Biol Phys 1988; 14:659.
33. Haffty B, Fischer D, Rose M, et al. Prognostic factors for local recurrence in the conservatively treated breast cancer patient: a cautious interpretation of the data. J Clin Oncol 1991; 9:997.
34. Rutqvist L, Pettersson D, Johansson H. Adjuvant radiation therapy versus surgery alone in operable breast cancer: long-term follow-up in a randomized clinical trial. Radiother Oncol 1993; 26:104.
35. McGuire WL. Breast cancer prognostic factors: evaluation guide-lines. J Natl Cancer Inst 1990; 83:154.
36. Clark G. Prognostic and predictive factors. In: Harris J, Lippman M, Morrow M, et al. Diseases of the Breast. Philadelphia: Lippincott-Raven, 1996.
37. Fox S, Smith K, Hollyer J, et al. The epidermal growth factor receptor as a prognostic marker: results of 370 patients and review of 3009 patients. Breast Cancer Res Treat 1994; 29:41.

38. Rosen P, Lesser M, Arroyo C. p53 in node-negative breast carcinoma: an immunohistochemical study of epidemiologic risk factors, histologic features, and prognosis. J Clin Oncol 1995; 13:821.
39. Thor AD, Moore II, DH, Edgerton DM, et al. Accumulation of p53 Tumor suppressor gene protein: An Independent Marker of Prognosis in Breast Cancers. J Natl Cancer Inst 1992; 84:845-5.
40. Andersen J, Thorpe S, King W, et al. The prognostic value of immunohistochemical estrogen receptor analysis in paraffin-embedded and frozen sections versus that of steroid-binding assays. Eur J Cancer 1990; 26:442.
41. Muss H, Thor A, Berry D, et al. c-erbB-2 Expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med 1994; 330:1260.
42. Prosser J, Thompson AM, Cranston G, et al. Evidence that p53 behaves as a tumor suppressor gene in sporadic breast tumors. Oncogene 1990; 5:1573-9.
43. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer. Science 1990; 250:1233-8.
44. Trock BJ, Leonessa F, Clarke R. Multidrug resistance in breast cancer: a Meta-analysis of MR1/GP 170 Expression and its possible functional significance. J Natl Cancer Inst 1997; 89:917-31.
45. Sanfilippo O, Ronchi E, de Marco C, di Fronzo G, Silvestini R. Expression of P-glycoprotein in breast cancer tissue and in vitro resistance ot doxorubicin and vincristine. Eur J Cancer 1991; 27:155-8.
46. Kim R. Expression of the multidrug resistance gene in human tumors. Hiroshima J Med Science 1990; 39:71-7.
47. Dixon AR, Bell F, Ellis IO, Elston CW, Blamey RW. P-glycoprotein expression in locally advanced breast cancer treated by neoadjuvant chemotherapy. Br J Cancer 1992; 66:537-41.
48. Prempree T, Amornmarn R, Nguyen TQ, Arce C, Failace W. Chomosome aberrations, p53 gene mutations and MDR1 amplification in Glioblastoma Multiforme. Presented at Siriraj Scientific Congress, Siriraj Hospital, Bangkok, March 1999:8-12.
49. Shapiro JR, Shapiro WR. Therapy modifies cellular heterogeneity in human malignant glioma. Advances in Oncology 1994; 8:21-9.
50. O'Shanghnessy JA, Cowan KH. Current status of Paclitaxel in the treatment of breast cancer. Breast Cancer Res Treat 1995; 33:27-37.
51. Fugua SAW. Estrogen Receptor Mutagenesis and hormone resistance. Cancer 1994; 74:1026-9.
52. Rozan S, Vincent Salomon A, Zafani B, et al. No significant predictive value of c-erbB2 or p53 expression regarding sensitivity to primary chemotherapy or radiotherapy in breast cancer. Int J Cancer 1998; 79:27-33.