한국유방암학회지 2000;2:152-161

Clinical implication of altered expression of Mad1 protein in human breast cancer

Department of Surgery[1], Pathology[2], Internal Medicine[3], and Radiation Oncology[4], Inje University Sanggye Paik Hospital, Seoul, Korea

Sehwan Han, M.D[1]., Kyeong mee Park, M.D[2]., Hong-Yong Kim, M.D[1]., Myung-Soo Lee, M.D[1]., Hon-Joo Kim, M.D[1]., Young-Duck Kim, M.D[1], Young Jin Yuh, M.D[3]., Sung Rok Kim, M.D[3]. and Hyun Suk Suh, M.D[4]

= Abstract =

유방암에서 Mad1 단백 발현의 임상적 의의

인제대학교 상계백병원 외과학교실, 병리학교실, 내과학교실, 방사선종양교실

한세환 · 박경미 · 김홍용 · 이명수 · 김홍주 · 김영덕 · 유영진 · 김성록 · 서현석

Background: Mad1 protein is known to repress Myc target genes and antagonize Myc function. We underwent this study to investigate the clinical implication of Mad1 expression in human breast cancer. **Materials and Methods:** We performed immunohistochemical assay for Mad1 protein together with Myc in human breast cancer, along with tissues from normal and benign diseases. The data from protein assay were merged with clinical and biologic parameters of the patients. **Results:** Of 66 patients with invasive ductal cancer, Mad1 expression was detected in 22(33.3%). Intensity and area of Mad1 expression significantly decreased in DCIS and invasive cancers while high levels of Mad1 expression were persistent in benign breast lesions. Mad1 expression was significantly reduced in poorly differentiated tumors($p<0.001$). Expression of Mad1 was not associated with tumor size, lymph node status, and stage of the disease. We could not observe any correlation between S-phase and expression status of Myc or Mad1. Mad1 expression was closely linked to differentiation of the cancer cells and inversely correlated with Myc expression($p=0.042$). In survival analysis, Mad1 possessed a prognostic significance to predict recurrence of the disease but not overall survival after CMF chemotherapy. **Conclusions:** In human breast cancer cells, expression of Mad1 seems to be downregulated while expression of Myc is amplified. Altered expression of Mad1 may play a role in malignant transformation of human mammary epithelial cells and represent an aggressive phenotype in human breast cancer. **(Journal of Korean Breast Cancer Society 2000;2:152~161)**

Key Words: Differentiation, Myc, Prognosis, Proliferation

Corresponding Author: Sehwan Han, MD., Department of Surgery, Inje University Sanggye Paik Hospital, 761-1, Sanggye-Dong, Nowon-Gu, Seoul, Korea
Tel: 82-2-950-1018, Fax: 82-2-938-4109
E-mail: shwhan@unitel.co.kr

Introduction

Mad or Myc family proteins belong to the basic-helix-loop-helix-zipper(bHLHZ) class of transcriptional factor[1]. HLHZ region is known to mediate DNA-binding and protein-protein interaction by formation of heterodimers with Max protein[2]. Max protein is highly stable and expressed at essentially equivalent levels in resting and proliferating cells. Max protein preferentially heterodimerizes with Myc or Mad protein, which self-associate poorly and fail to bind DNA on their own[1]. Both Myc:Max and Mad:Max hetero-complexes are favored over Max homodimers, and have similar binding specificity and apparent stability[3]. Myc:Max complexes have transcriptional activity while Mad:Max complexes repress transcription regulated through the Myc:Max E-box site[1,4,5]. Levels of Myc or Mad in the cell determine whether the reporter gene is under positive or negative transcriptional control[2].

Differentiation is frequently accompanied by down-regulation of Myc expression, while expression of Mad proteins has been closely linked to terminal differentiation[2,6]. Myc drives cell proliferation by stimulation of cyclin/cdk(cyclin-dependent kinase) complexes activity and inhibits function of cdk inhibitors such as p27Kip1[7]. Activation of cdks leads to Rb inactivation by phosphorylation, consequently leads to cell-cycle progression. Expression levels of Myc and the various members of the Mad family generally are inversely correlated in cell culture systems. Recently, we have reported that Madl expression inversely correlated with Myc protein expression in human gastric cancer[8]. Mad proteins are known to directly repress Myc target genes and antagonize Myc function by heterodimerizing with Max protein competitively[6]. Consequently, Mad protein is considered to function as a tumor suppressor. A survey of Mad protein may provide novel information for biology of human breast cancer if it works as tumor suppressor.

We underwent this study to investigate the regulatory effect of Madl on cancer progression using immunohistochemical analysis in human breast cancer. Correlation between expression of Myc and Madl in the same cancerous tissues were also analyzed, and the changes in expression levels of Myc and Madl according to degree of differentiation of the cancer cells along with benign breast disease were also described.

Materials and Methods

Patient population and statistical methods: Medical records and archival pathology tissues from 66 primary breast cancer patients who underwent modified radical mastectomy or partial mastectomy with axillary dissection at Inje University Sanggye Paik Hospital between January 1994 and December 1995 were evaluated. Important selection criteria for entry to the study were feasible freshness of cancer tissues for flow cytometry analysis and immunohistochemical assay of Myc and Madl proteins. Mean age of studied patients was 42 years ranging from 23 to 75 years. Patients with stage I(n=10), stage II(n=34), stage III(n=14), and stage IV(n=4) included in the study and there were 4 patients with pure ductal carcinoma in situ.(DCIS) Postoperative adjuvant systemic chemotherapy, which was mainly applied to the patients except those who had DCIS and stage I disease, included 5-FU,(600 mg/m^2) methotrexate,(40 mg/m^2) and cyclophosphamide(600 mg/m^2) every 3 weeks for 6 cycle. Patients who received antiestrogen hormonal therapy were excluded from entry of the study.

In addition to 66 breast cancer tissues, 10 normal breast tissues and tissues from 24 patients with benign ductal hyperplasia were analyzed for expression of Madl protein. Correlation between

clinical/biologic data of the patients and expression of Mad1 and Myc protein was estimated by chi-square test. Survival of the patients was plotted by Kaplan-Meier method and statistical analysis was performed by log-rank test.

Cell Cycle analysis: Flow cytometry analysis was performed on cell suspensions from breast cancer tissues obtained by mechanical disaggregation of tumor materials. After centrifugation, supernatants were discarded, and the cell pellets were resuspended in 250 μl of Buffer solution(10mM Citrate, pH 7.5, 20mM NaCl, 20mM MgCl2. After adding 10 μl/ml of trypsin, trypsin inhibitor and DNase-free RNase, nuclei were incubated at room temperature for 30 minutes. DNA staining was obtained with 500 μl of propidium iodide solution(PI; Molecular Probes, Eugine, OR) in PBS(100 μl/ml PI, 0.1% Triton X-100, 1% FCS) for 1 hour at 4℃ in the dark, followed by flow analysis. The DNA fluorescence was analyzed using a FACScan(Becton-Dickinson, Bedford, MA). Data acquisition was performed using the Cell Fit software(Becton-Dickinson) and data analysis using the Phoenix Flow System Multicycle AV software. The results were expressed as the frequency distribution of DNA cell content; normal DNA histograms were characterized by a peak corresponding to the DNA content of G0/G1 diploid cells. Clonal DNA abnormality(aneuploidy) was identified by the presence of an accessory peak generally shifted to the right of the G0/G1 diploid peak. The percentage of aneuploid cells was defined as the percentage of cells in the G0/G1 aneuploid peak with respect to those in the G0/G1 diploid peak. Diploid tumors were considered as those with 0% aneuploid cells.

Immunohistochemical assay: The paraffin blocks for breast cancer patients were retrieved and neoplastic tissues of these breast cancers were examined for expression of Myc and Mad1 proteins using the avidin-biotin complex(ABC) immunoperoxidase method. We used commercially available monoclonal antibody; NCL-cMYC(1:200 dilution) for Myc protein assay(Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), anti-Mad1 antibody(1:500 dilution) for Mad1 protein assay(Santa Cruz Biotechnology, Inc.). Immune staining was performed as described previously[9]. Counterstaining with hematoxylin was done after ABC immune staining and two pathologists evaluated immunohistochemical staining separately without information of patients' outcome data. Two pathologists reviewed the slides if interpretation of the immunohistochemical analysis was different. Three separate blocks containing malignant cells were stained and scored by calculating the stained cancer cells in percentage. Sections of breast cancer observed to express homogenous and/or intense cytoplasmic immunohistochemical staining for the Myc protein in more than 10% of the observed field were considered to be positive for overexpression(Fig. 1A). Immune staining of Mad1 protein in more than 10% of nuclei of observed cancer cells was regarded as positive expression of Mad1(Fig. 1B, 1C, 1D).

Results

Of 66 patients with invasive ductal cancer of the breast, Myc expression was detected in 44(66.7%) and expression of Mad1 was well preserved in 22 patients(33.3%) with breast cancer. Expression rate of Myc was 75%(3 of 4) while Mad1 was not expressed in 4 patients with DCIS. Intense cytoplasmic staining of Myc was apparent(Fig. 1A) while immune staining of Mad1 was limited to the nuclei of the cells(Fig. 1B, 1C, 1D). Expression of Myc was prominent in cancerous tissues and growing cells, whereas Mad1 expression located mainly in non-proliferating cells.

Results of immunohistochemical assay for Mad1 and Myc protein were merged with clinical and

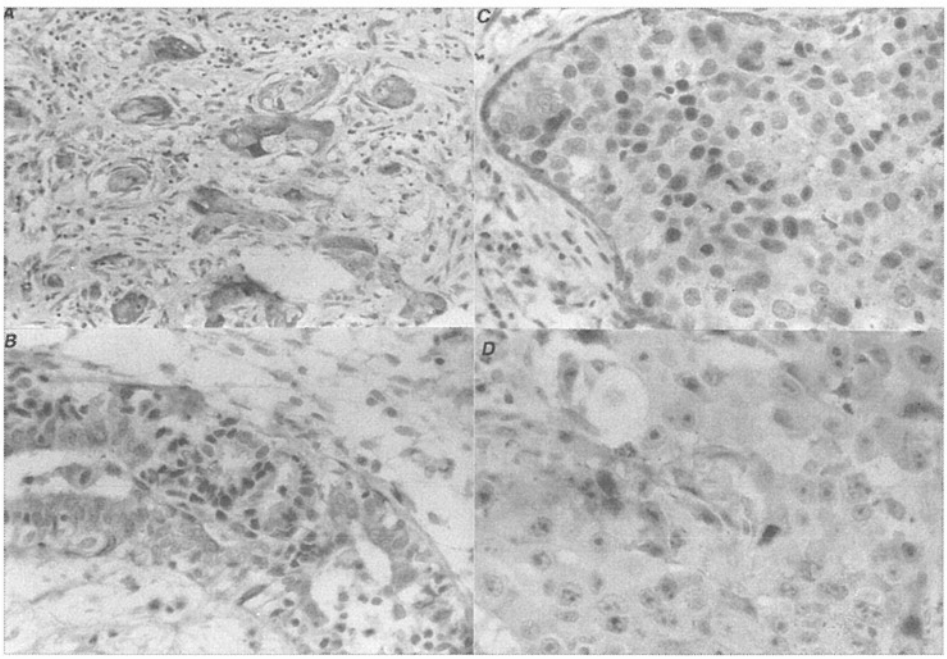

Fig. 1. Immunohistochemical staining of Myc and Mad1 protein. A, intense cytoplasmic staining of Myc in cancer cells and growing cells(x400). B, Homogenous imunohistochemical reaction of Mad1 is visible in cells of benign ductal hyperplasia(x400). C, positive immune staining of Mad1 is heterogeous in ductal carcinoma in situ(x400). D, Mad1 immune staining in infiltrating ductal cancer(x400). Intensity and proportion of positive immunoreactivity of Mad1 markedly decreased in ductal carcinoma in situ and invasive cancers compared with benign ductal hyperplasia.

biological parameters of the patients. A significant correlation was observed between Myc expression and ER expression of cancer cells(Table 1). Myc expression increased significantly in ER positive tumors($p=0.036$). When we analyzed the pattern of Myc expression according to cellular differentiation, expression rate of Myc increased significantly in the poorly differentiated tumors($p=0.018$). There was no significant correlation between expression of Myc and tumor size, lymph node metastasis, and stage of the disease(Table 1).

Mad1 expression correlated with histologic and nuclear grade of the tumors and expression of Mad1 was significantly reduced in poorly differentiated tumors($p<0.001$). Nuclear grade I tumors expressed Mad1 in 62.5%(10 of 16) while Mad1 was expressed in only 4% of nuclear grade III tumors(1 of 25). Expression of Mad1 increased significantly in premenopausal women(Table 1). Expression of Mad1 was not associated with size of the tumors, lymph node status, and stage of the disease. An interesting finding of present study was that we could not observe any correlation between S-phase and expression status of Myc or Mad1.

In normal breast tissues, Mad1 expression was apparent and diffuse in entire observed fields and expression of Mad1 was well preserved in cells of benign breast disease(Fig. 1B). However, intensity and area of Mad1 expression significantly decreased in DCIS components of invasive cancer and invasive cancers(Fig. 1C, 1D). We summarized the change of Mad1 expression according to progress of the disease in Table 2. Expression of Myc was analyzed according to the expression of Mad1 in the same

Table 1. Correlation between clinicopathologic data and expression of Myc and Mad1

Variables	Myc expression		p-value	Mad1 expression		p-value
	<10%	>10%		<10%	>10%	(%)
Tumorsize(cm)			NS*			NS
<2	6(33.3)	12(66.7)		10(55.6)	8(44.4)	
2-5	10(50.0)	10(50.0)		14(70.0)	6(30.0)	
>5	6(21.4)	22(78.6)		20(71.4)	8(28.6)	
L/N metastasis			NS			NS
negative	9(28.1)	23(71.9)		18(56.3)	14(43.7)	
positive	13(38.2)	21(61.8)		26(76.5)	8(23.5)	
Stage			NS			NS
I	3(30.0)	7(70.0)		4(40.0)	6(60.0)	
II	13(38.2)	21(61.8)		22(64.7)	12(35.3)	
III	5(35.7)	9(64.3)		11(78.6)	3(21.4)	
IV	0(.0)	4(100)		3(75.0)	1(25.0)	
ER status			0.036[a]			NS
Negative	14(46.7)	16(53.3)		17(56.7)	13(43.3)	
positive	8(22.2)	28(77.8)		27(75.0)	9(25.0)	
PR status			NS			NS
negative	7(35.0)	13(65.0)		11(55.0)	9(45.0)	
positive	15(32.6)	31(67.4)		33(71.7)	13(28.3)	
Nuclear grade			0.018[b]			< 0.001[b]
I	5(20.0)	20(80.0)		24(96.0)	1(4.0)	
II	8(32.0)	17(68.0)		14(56.0)	11(44.0)	
III	9(56.3)	7(43.8)		6(37.5)	10(62.5)	
Ploidy			NS			NS
Diploid	11(34.4)	21(65.6)		21(65.6)	11(34.4)	
Aneuploid	10(34.5)	19(65.5)		18(62.1)	11(37.9)	
S-phase			NS			NS
<10%	9(34.6)	17(65.4)		18(69.2)	8(30.8)	
>10%	13(32.5)	27(67.5)		26(65.0)	14(35.0)	
Menopausal status			NS			0.045[a]
Pre	14(32.6)	29(67.4)		25(58.1)	18(41.9)	
Post	8(34.8)	15(65.2)		19(82.6)	4(17.4)	

*NS: not significant

[a]Chi-square test

[b]Spearman correlation test

tumor tissues. The result demonstrated that expression of Myc was significantly reduced in the tumors presenting high levels of Mad1 expression while expression rate of Myc significantly increased in the tumors which did not express Mad1(Fig. 2). We could observe inverse correlation between expression of Myc and Mad1 in human breast cancers(p=0.042).

In survival analysis of 58 patients excluding 4 patients with DCIS and another 4 patients with stage

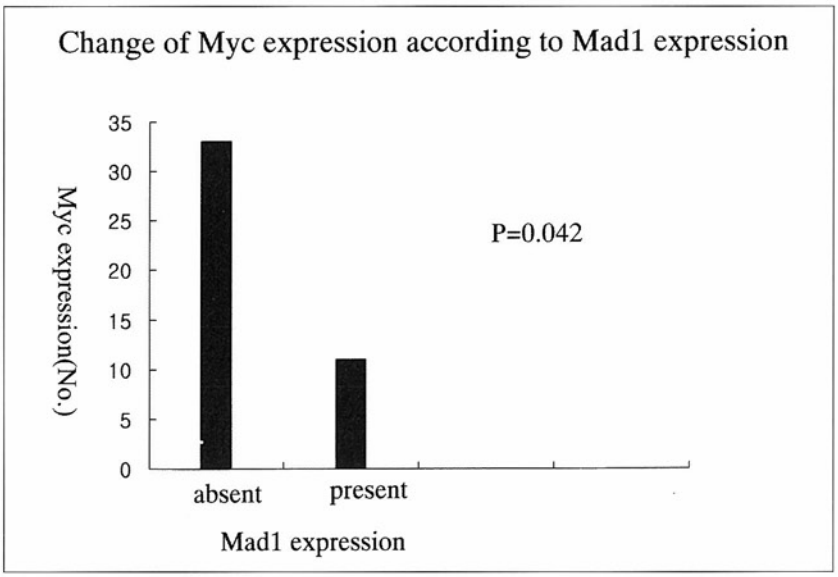

Fig. 2. Change of Myc expression according to Mad1 expression status. Expression of Myc was significantly reduced in the tumors presenting high levels of Mad1 expression while expression rate of Myc significantly increased in the tumors which did not express Mad1(p=0.042)

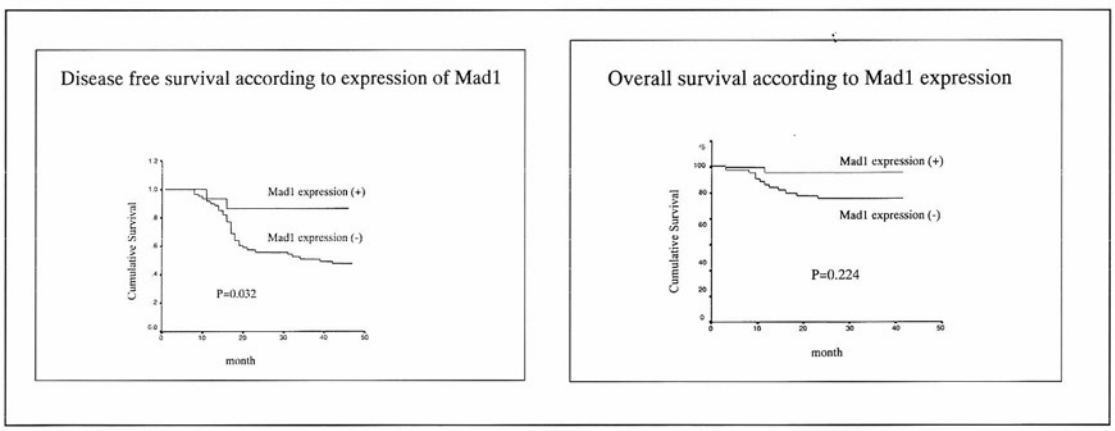

Fig. 3. Cumulative survival of the patients with invasive breast cancer according to Mad1 expression. A, Patients who exhibited reduced expression of Mad1 shows poorer disease free survival than other patients(p=0.032 by log-rank test). B, Overall survival of the patients was not different according to expression status of Mad1

IV disease, 16 patients(27.6%) had recurrent disease and 12 patients(20.7%) died of recurrent disease. Of 16 patients who had recurrent disease, twelve patients(75%) showed reduced expression of Mad1 protein and 10 patients(62.5%) showed high expression level of Myc protein. Reduced expression of Mad1 was statistically significant to predict recurrence of the disease but not patient survival with median follow-up period of 32 months, ranging 26-48 months(Fig. 3).

Table 2. Change of Mad1 expression according to progression of breast disease.

Mad1 expression	normal	BDH	DCIS	IDC
	9/10 (90.0%)	18/24 (75.0%)	0/4 (0 %)	22/66 (33.3%)

BDH: benign ductal hyperplasia
DCIS: ductal carcinoma in situ
IDC: invasive ductal cancer

● Expression rate of Mad1 protein decreased significantly in DCIS and IDC comparing with benign breast disease.

Discussion

In the results of present study, expression of Mad1 was reduced in a significant proportion of early stage breast cancer cells and the finding was persistent through the progress of breast cancer. To the contrary, expression of Mad1 was well preserved in normal breast epithelial cells and cells of benign ductal hyperplasia(Table 2). Consequently, loss of Mad1 expression may play a role in malignant transformation of human mammary epithelial cells. In normal epidermis and colonic mucosa Myc expression is restricted to proliferating cell layers, while Mad1 expression is restricted to differentiating cell layers. Mad1 induction occurs only in those cells that retain a differentiation response and increasing malignancy correlates with loss of both Mad1 and capability to differentiate[6].

Myc and Mad expression are tightly coupled to the transition from proliferation to differentiation of epithelial cells and it seems likely that restriction of Mad expression may be associated with loss of normal differentiation capability and with carcinogenesis. In the results of numerous in vitro studies, differentiation is frequently accompanied by down-regulation of Myc expression and a shift from Myc:-Max complexes to Mad:Max complexes has been detected as a rather early response to the induction of differentiation in cell culture system[2,6,10-13].

Result of the present study is similar to those from aforementioned studies[2,6,10-13] in that Myc expression was high in poorly differentiated cancer cells while Mad1 was expressed at high levels in differentiated cancer cells. Mad1 expression correlated with nuclear grade of the tumors and was significantly reduced in high grade tumors($p<0.001$). Furthermore, we could observe inverse correlation between expression of Myc and Mad1 in human breast cancers. The result demonstrated that expression of Myc was significantly reduced in the tumors expressing high levels of Mad1 while expression rate of Myc significantly increased in the tumors which did not express Mad1(Fig. 2). We have already reported a similar findings in primary gastric cancer[8]. Expression of Myc was apparent whereas expression of Mad1 was significantly reduced in cells of DCIS. This finding was also observed in DCIS components of studied invasive cancer tissues. In human breast cancer cells, expression of Mad1 seems to be downregulated while expression of Myc increased. Consequently, Myc and Mad1 seem to be closely linked to cellular differentiation and carcinogenesis in human breast epithelial cells as well as in cultured cell systems. If Mad1 is capable of antagonizing the biological effects of Myc, which is involved in malignant transformation of numerous human cancers, Mad1 could function as

a tumor suppressor[14]. However, we could not define a role of Mad1 as tumor suppressor in breast cancer at this point because there was no significant correlation between expression of Mad1 and extent of the disease in the results of current study. Mad1 expression was well preserved in a significant proportion of early stage breast cancer cells and was persistent through the progress of breast cancer.

An interesting finding of present study is that we could not observe any correlation between S-phase and expression status of Myc or Mad1. The heterocomplex switch from Myc:Max to Mad:Max is responsible for a switch in the transcriptional activities of a subset of genes involved in the proliferation program[2,15]. Incorporation of Mad1 into heterocomplex with Max may serve to down-regulate genes activated by Myc:Max complexes and permit cells to arrest growth in preparation for differentiation[16,17]. Myc expression is highest in proliferating cells and increased Myc expression is associated with the proliferative phases of development[18-20]. Reduction of Myc level lengthens G1 phase[3,17,21], while ectopic expression of Myc shortens G1 phase in cell culture systems[22,23]. Initial burst of Myc expression is likely to be a critical event for cell-cycle entry[24,25]. Inhibition of Myc expression blocks cell cycle progression and leads to G1 arrest in serum-depriven fibroblast[24]. Myc expression is often down-regulated in conjunction with differentiation and Myc expression can block differentiation in some cell type[26]. Myc is thought to play a central role in normal growth and development, as well as in cellular transformation and carcinogenesis. If the Mad1 functions as a repressor of transcriptional signal induced by Myc, cell proliferation may be closely linked to expression of Mad1. However, we could not find any relationship between expression of Myc or Mad1 and S-phase while Mad1 expression was closely related with proliferative index of tumor cells in primary gastric cancer[8]. The findings may be explained by heterogeneity of human cancer, and expression of Mad family proteins may be tissue type specific. Human cancers are composed of a number of different clones and there were 11 breast cancers which did not express neither Myc nor Mad1 in the result of present study. Another possible explanation is heterogeneity of intratumor proliferative activity[27]. It seems likely that biologic behavior of cancer cells in vivo is quite complex comparing with isolated environment using cultured cells.

One of remarkable findings of present study is that Myc expression significantly increased in ER positive tumors. It has been well characterized that Myc transcription is regulated by estrogen in breast tissues. Induction of Myc requires DNA binding region of the ER, and P2 promoter region of Myc gene contains an estrogen reactive element(ERE) half site and CG-rich Sp1 binding site. Sp1 binding site is known to cooperate to induce transcription[28]. Several studies have indicated that growth inhibition of ER positive breast cancer cells by antiestrogens is also accompanied by a decrease in Myc expression, both in vivo[29] and in vitro[30-32]. The results suggest that biologic modification to reduce the expression of Myc could potentiate the effect of antiestrogen therapy in breast cancer patients. Furthermore, in the result of current study, there was a tendency that expression of Mad1 is repressed in ER positive tumors and Mad1 expression decreased in postmenopausal women. Expression of Mad1 and Myc in breast cancer might be under the regulation of estrogen. The results suggest that biologic modification incorporating with Mad1 to reduce the expression of Myc could enhance the effect of antiestrogen therapy in breast cancer patients.

In survival analysis, reduced expression of Mad1 protein was significant to predict recurrence of the disease but not patient survival. The reason seems to be a relatively small population of studied patients

and relatively short period of patient follow-up. However, expression of Mad1 was inversely correlated with Myc expression in human breast cancer and is closely linked to cellular differentiation. Loss of Mad1 expression may have a role in carcinogenesis of human breast since expression of Mad1 was significantly lower in breast cancer than in normal and benign breast disease. Furthermore, patients with reduced expression of Mad1 protein had increased risk for recurrence of breast cancer after CMF chemotherapy, thus reduced expression of Mad1 may represents an aggressive phenotype in breast cancer. However, we can not postulate the clinical utility of Mad1 precisely in breast cancer at this point. Additional study to characterize the biologic implication of change in expression levels of Mad1 along with Myc or other proliferation markers may provide new therapeutic targets in the management of breast cancer patients.

References

1) Ayer DE, Kretzner L, Eisenman RN, Mad: A heterodimeric partner for Max that antagonizes Myc transcriptional activity. Cell 72:211-22, 1993
2) Ayer DE, Eisenman RN. A switch from Myc: Max to Mad:Max heterocomplexes accompanies monocyte/macrophage differentiation. Genes Dev 7:2110-9, 1993
3) Blackwood EM, Kretzner L, Eisenman RN. Myc and Max function as a nucleoprotein complex. Curr Opin Genet Dev 2:227-35, 1992
4) Kretzner L, Blackwood EM, Eisenman RN. Myc and Max proteins possess distinct transcriptional activities. Nature 359:426-9, 1992
5) Amati B, Dalton S, Brooks MW, Littlewood TD, Evan GI, Land H. Transcriptional activation by the human c-Myc oncoprotein in yeast requires interaction with Max. Nature 359:423-6, 1992
6) Hurlin PJ, Foley KP, Ayer DE, Eisenman RN, Hanahan D, Arbeit JM. Regulation of Myc and Mad during epithelial differentiation and HPV-associated tumorigenesis. Oncogene 11:2487-501, 1995
7) Zornig M, Evans GI. On target with Myc. Curr Biol: 1553-6, 1996
8) Han S, Park K, Kim HY, Lee MS, Kim HJ, Kim YD. Expression of Mad1 protein inhibits proliferation of cancer cells and inversely correlated with Myc protein expression in primary gastric cancer. Oncol Rep 6:569-574, 1999
9) Han S, Yun IJ, Noh DY, Choe KJ, Song SY, Chi JG. Abnormal expression of four novel molecular markers represents a highly aggressive phenotype in breast cancer. Immunohistochemical assay of p53, nm23, erbB-2, and cathepsin D protein. J Surg Oncol 65:22-7, 1997
10) Chin L, Schreiber-Agus N, Pellicer I, Chen K, Lee HW, Dudast M, et al: Contrasting roles for Myc and Mad proteins in cellular growth and differentiation. Proc Natl Acad Sci USA 92:8488- 92, 1995
11) Delgado MD, Lerga A, Canelles M, Gomez-Casares MT, Leon J. Differential regulation of Max and role of c-Myc during erythroid and myelomonocytic differentiation of K562 cells. On- cogene 10:1659-65, 1995
12) Larsson LG, Pettersson M, Oberg F, Nilsson K, Luscher B. Expression of mad, mxi1, max and c-myc during induced differentiation of hematopoietic cells: opposite regulation of mad and c-myc. Oncogene 9:1247-52, 1994
13) Vastrik I, Kaipainen A, Penttila TL, Lymboussakis A, Alitalo R, Parvinen M, et al K. Expression of the mad gene during cell differentiation in vivo and its inhibition of cell growth in vitro. J Cell Biol 128:1197-208, 1995
14) Koskinen PJ, Ayer DE, Eisenman RN. Repression of Myc-Ras cotransformation by Mad is mediated by multiple protein-protein interactions. Cell Growth Differ 6:623-9, 1995
15) Ayer DE, Laherty CD, Lawrence QA, Armstrong AP, Eisenman RN. Mad protein contain a dominant

transcription repression domain. Mol Cell Biol 16: 5772-81, 1996

16) Cerni C, Bousset K, Seelos C, Burkhardt H, Henriksson M, Luscher B. Differential effects by Mad and Max on transformation by cellular and viral oncoproteins. Oncogene 11:587-96, 1995

17) Blackwood EM, Eisenman RN. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science 251:1211-7, 1991

18) Luscher B, Eisenman RN. New light on Myc and Myb. Part I. Myc. Genes Dev 4:2025-35, 1990

19) DePinho RA, Schreiber-Agus N, Alt FW. myc family oncogenes in the development of normal and neoplastic cells. Adv Cancer Res 57:1-46, 1991

20) Marcu KB, Bossone SA, Patel AJ. myc function and regulation. Ann Rev Biochem 61:809-60, 1992

21) Blackwood EM, Luscher B, Eisenman RN. Myc and Max associate in vivo. Genes Dev 6:71-80, 1992

22) Meichle A, Philipp A, Eilers M. The functions of Myc proteins. Biochim Biophys Acta 1114:129-46, 1992

23) Henriksson M, Luscher B. Proteins of the Myc network: essential regulators of cell growth and differentiation. Adv Cancer Res 68:109-82, 1996

24) Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks et M, al. Induction of apoptosis in fibroblasts by c-myc protein. Cell 69:119-28, 1992

25) Hanson KD, Shichiri M, Follansbee MR, Sedivy JM. Effects of c-myc expression on cell cycle progression. Mol Cell Biol 14:5748-55, 1994

26) Nguyen HQ, Selvakumaran M, Liebermann DA, Hoffman B. Blocking c-Myc and Max expression inhibits proliferation and induces differentiation of normal and leukemic myeloid cells. Oncogene 11:2439-44, 1995

27) Paradiso A, Mangia A, Barletta A, Fusilli S, Marzullo F, Schttulli F, et al. Heterogeneity of intratumour proliferative activity in primary breast cancer: Biological and clinical aspects. Eur J Cancer 31A:911-6, 1995

28) Dubik D, Shiu RPC. Mechanism of estrogen activation of c-myc oncogene expression. Oncogene 7: 1587-94, 1992

29) Le Roy X, Escot C, Brouillet JP, Theillet C, Maudelonde T, Simony-Lafontaine J et al: Decrease of c-erbB-2 and c-myc RNA levels in tamoxifen-treated breast cancer. Oncogene 6:431-7, 1991

30) Musgrove EA, Hamilton JA, Lee CS, Sweeney KJE, Watts CKW, Sutherland RL. Growth factor, steroid, and steroid antagonist regulation of cyclin gene expression associated with changes in T-47D human breast cancer cell cycle progression. Mol Cell Biol 13:3577-87, 1993

31) Vink-van Wijngaarden T, Pols HAP, Buurman CJ, van den Bemd GJ CM, Dorssers LCJ, B:rkenhager JC et al: Inhibition of breast cancer cell growth by combined treatment with vitamin D3 analogues and tamoxifen. Cancer Res 54:5711-7, 1994

32) Wilken NR, Sarcevic B, Musgrove EA, Sutherland RL. Differential effects of retinoids and antiestrogens on cell cycle progression and cell cycle regulatory genes in human breast cancer cells. Cell Growth Differ 7:65-74, 1996