

c-erbB-2 Mitogen-activated Protein Kinases

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Mitogen-activated Protein Kinases Activities and c-erbB-2 Expression in Breast Cancer Carcinogenesis and Progression

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Purpose: Increased level mitogen-activated protein kinase (MAPK) and activation of MAPK have been reported in human breast cancers, especially in breast cancers with HER2/neu overexpression. To understand the relationship between the MAPK protein expressions and other clinicopathological parameters, we examined the status of MAPKs in 20 breast cancers compared to those of paired normals.

Methods: A total of 20 breast cancers and paired normal breast tissues were included in this study. Tissues were obtained at the operation room and stored at - 80°C. Tissue proteins were extracted and the concentration was determined by Bio-Rad protein assay method. Western blot analysis were performed to determine the level of MAPKs expressions using 100 ug of tissue protein in 8%, 10%, or 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). MAPK assays were carried out by a non-radioactive method developed by Cell Signaling Tech. as recommended by the manufacturer. Clinico-pathological information was provided from the Breast Cancer Registry of Department of Surgery, Yonsei University College of Medicine.

Results: The levels of MAPKs were higher in 95% of breast

cancers compared to those of paired normals. The levels of ERK1/2 were significantly higher in cancer tissues compared to paired normals but the activated forms were not. The levels of JNK, p38, and MKP1 proteins were significantly increased in the cancer tissue compared to the paired normals. The levels of ERK1/2 and activated ERK1/2 proteins were not different between tumor stages. There were no significant differences of the levels of ERK1/2 and activated ERK1/2 proteins between HER2-negative and HER2-positive cancers. There were significantly higher levels of activated ERK1/2 proteins in ER-positive cancers than those in ER-negative cancers (P < 0.05).

Conclusion: The levels of MAPKs, but not the activated forms, seem to be increased in breast cancer tissues compared to those of paired normals. The levels of activated MAPKs seem to be associated with estrogen receptor expression in cancer tissues. (J Korean Surg Soc 2003;64:6-13)

Key Words: Mitogen-activated protein kinase, HER2/neu, Estrogen receptor, Breast cancer, Stage

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: 2002 9 17 , : 2002 12 30
1999

가
1998
14.1% 가
(I)
가

(apoptosis) (apoptotic pathway) mi-

c-erbB-2 (9) togenic signal

30%

MAPK 가

protein kinases (MAPKs) (2,3) Mitogen-activated 가 MAPK 가

가 가

(renal cell carcinoma) MAPK (constitutive activation)가 MAPK

(4) MAPK mRNA 가 (metastatic potential) 1 6 , 2 9 3 5

MAP kinase 가 tyrosyl residue 5 10 가 -80°C

(5)

MAP kinase (dominant oncogenes) liquid nitrogen 가 (ice-cold lysis buffer) (70 mM B-glycerophosphate pH 7.2, 1 mM each mea- and ortho- vanadate, 2 mM manesium chloride, 1 mM EGTA, 1 mM dithiothreitol (DTT), 0.5% Triton X-100, 0.2 mM phenylmethylsulphonyl fluoride (PMSF) and 5 ug ml⁻¹ each of pepstatin A, shymostatin, leupeptin and peptin)

nase phosphatase (MKP)-1 MAP kinase MAP ki- 20 (sample) 30 (sonicate) 4°C 23,000 g 15

MAPK-mediated mitogenic effect (6) ERK-1 MKP-1

(7) MKP-1 가 , MKP-1

가 가

(loss) ERK-1 MKP-1 5q35-ter locus

MKP-1 (marker) (8) MKP-1

(8) MAP kinase

MAPK 가 MKP-1

ERK MKP-1 mRNA JNK

MKP-1 ERK-1 MKP-1

MKP-1 JNK (9)

3) Western blot analysis

100 ug

8%, 10%, 12% sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) Protran Nitrocellulose Membrane (Schleicher and Schuell Corporation, Dassel, Germany) transfer . Blots blocking buffer (20 mM Tris-Cl, pH 7.5, 15 mM sodium chloride, 0.05% Tween-20 (TBST) containing 5% non-fat Carnation milk)

blot (Immunoblot) TBST

1 TBST milk (Santa Cruze Biotech., CA USA)
 2 4°C . Blots TBST
 2 TBST milk 1 2
 incubation blots Amersham ECL kit
 (Amersham International, Buckinghamshire, UK)
 . Western band
 film GS-690 imaging Densitometer

4) Antibodies

1 Phospho-p44/42 MAP Kinase (Thr202/Tyr204) (Cell Signaling Tech., MA, USA), p44/ 42 MAP Kinase (Cell Signaling Tech., MA, USA), Phospho- p38 MAP Kinase (Thr 180/Tyr182) (Cell Signaling Tech., MA, USA), p38 MAP Kinase (Cell Signaling Tech., MA, USA), Phospho-SAPK/JNK (Cell Signaling Tech., MA, USA), SAPK/ JNK (Cell Signaling Tech., MA, USA), MKP-1 (Santa Cruze Biotech., CA USA) p44/42 MAP Kinase Assay Kit (Cell Signaling Tech., MA, USA) In vitro MAPK assay . 2 Anti-mouse IgG-HRP (Santa Cruze Biotech., CA USA) Anti-rabbit IgG-HRP

5) In Vitro MAPK assays

MAPK assays Cell Signaling Tech. (MA, USA) 7+
 . MAPKs 300 ug phospho-specific ERK 1/2 MAPK (Thr202/ Tyr204) (immunoprecipitation)
 kinase assays ATP substrate Elk-1
 . phosph-Elk-1 phospho-specific Elk-1 (Ser 383) Western blot analysis
 phospho Elk-1 Ser383 Elk-1

6)

SPSS 10.0
 SPSS
 independent sample t-test
 crosstab . P < 0.05



Fig. 1. Levels of MAPKs in the representative breast cancers and paired normals. The ERK 1/2 proteins were detected by Western blot analysis by using ERK 1/2 polyclonal antibody (upper panel). The phospho-ERK 1/2 proteins were detected by Western blot analysis by using phospho-specific MAPK (pERK 1/2) antibody (lower panel). The levels of ERK 1/2 were significantly higher in cancer tissues compared to paired normals but the activated forms were not. N and C represent normal and carcinoma, respectively.

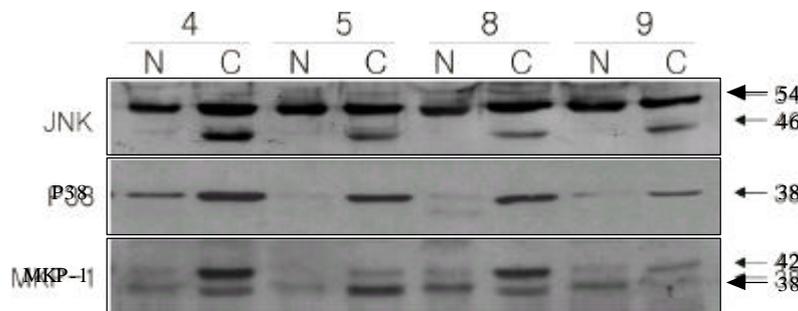


Fig. 2. Levels of JNK, p38, and MKP1 in the representative breast cancers and paired normals. Each protein was detected by Western blot analysis by using JNK 1/2 polyclonal antibody (uppermost panel), p38 polyclonal antibody (middle panel), and MKP1 polyclonal antibody (lowest panel). The levels of JNK, p38, and MKP1 proteins were significantly increased in the cancer tissue compared to the paired normals. N and C represent normal and carcinoma, respectively.

1) MAPKs proteins and activated ERK1/2 in cancer tissues and paired normals

ERK1/2 1
(5%) 가 4.3 가(0.7) MAPKs 3 ERK1/2 가
12.2)
ERK1/2 가
ERK1/2 6 (30%) 가
8 (40%) 가 6 (30%) 가
1.17
(0.21 5.6) 가
MAPK Western blot
MAPK (Data not shown).
JNK 1.89 (0.83 3.34) 가
가 , p38 5.06 (0.97 25.34) 가
MAPK MKP1
1.71 (0.5 3.26) 가 MAPK
가 (Table 1, Fig. 1, 2).

2) Levels of ERK1/2 and activated ERK1/2 according to the axillary lymph node status and tumor stages

MAPK
(Table 1, Fig. 3)

3) Levels of ERK1/2 and activated ERK1/2 according to HER2 and ER expressions

HER2 MAPK
(Table 1, Fig. 4), ER ER ERK1/2
가 ERK1/2 (P < 0.05) 가 (Table 1, Fig. 5).

4) Levels of ERK1/2 and activated ERK1/2 according to histologic grade and MKP1 levels

ERK1/2

Table 1. Clinico-pathological characteristics and the ratios of MAPKs in cancer tissues over paired normals

Sample	LN	Stage	HG	ER	HER2	ERK	pERK	JNK	p38	MKP1
1	0	1	2	0	1	1.1	0.8		1.5	2.7
2	1	2	2	1	0	1.9	0.9		1.8	2.4
3	0	1		1	0	1.8	1.5		1.0	0.5
4	2	2		1	1	2.6	1.4	3.1	2.3	1.8
5	0	2	3			6.6	1.0	1.5	9.6	1.9
6	1	2	2	1	0	7.0	2.5	2.4	8.4	1.2
7	2	2	2	1	0	2.5	1.2	1.7	3.5	2.1
8	0	2	2	1	1	12.2	1.0	2.3	5.5	1.8
9	0	2	2	0	0	4.3	0.9	1.4	1.3	0.8
10	26	2	2	0	1	3.6	0.9	2.9	2.1	
11	0	1	3	0	0	4.2	0.6	2.0	14.6	3.3
12	1	2	1	1	0	1.1	0.8	1.2	1.0	
13	18	3	3	0	1	1.0	0.5	1.2	1.3	1.0
14	15	3	3	0	1	2.2	0.2	1.0	1.7	1.8
15	0	2	2	1	1	11.1	5.6	3.3	4.1	0.7
16	0	1	3	0	0	9.1	0.4	1.7	3.1	1.2
17	11	3	2	1	0	0.7	1.5	0.8	1.0	
18	0	1	2	0	1	3.7	0.3	2.0	25.3	2.0
19	4	3	3	1	0	6.8	1.0	2.2	9.0	2.0
20	0	1	2	1	1	2.5	0.4	1.3	3.0	1.8

LN, HG, and ER represents the number of metastatic lymph nodes in the axilla, histologic grade, and estrogen receptor, respectively. The values of MAPKs were calculated by denomination of the optical density of Western blots of Cancer tissue over that of paired normal tissue.

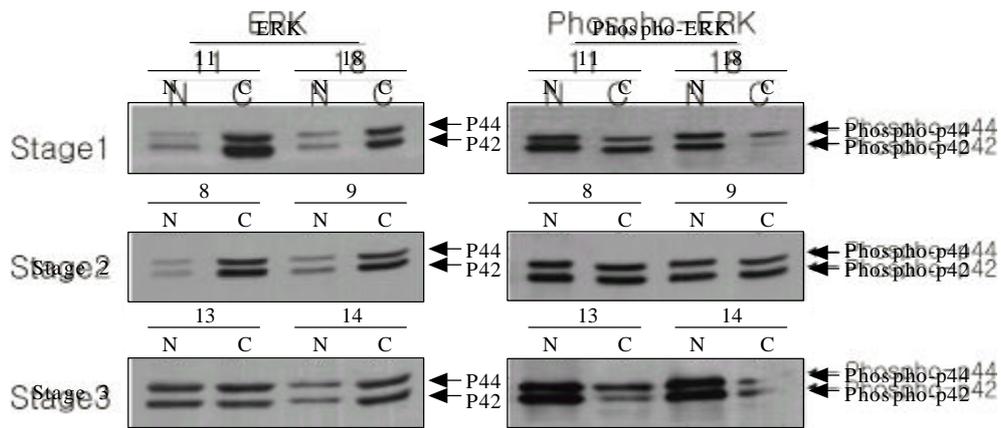


Fig. 3. Levels of MAPKs in the representative breast cancers and paired normals according to tumor stages. The ERK 1/2 proteins were detected by Western blot analysis by using ERK 1/2 polyclonal antibody (left panel). The phospho-ERK 1/2 proteins were detected by Western blot analysis by using phospho-specific MAPK (pERK 1/2) antibody (right panel). Uppermost panel represents stage 1, middle one represents stage 2, and lowest panel represents stage 3. The levels of ERK 1/2 and activated ERK 1/2 proteins were not different between stages. N and C represent normal and carcinoma, respectively.

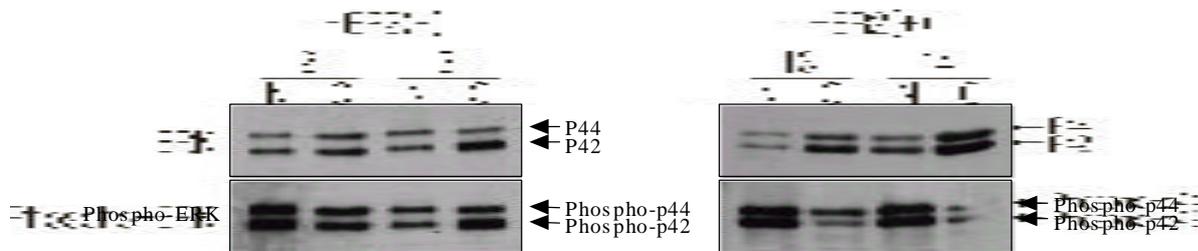


Fig. 4. Levels of MAPKs in the representative breast cancers and paired normals according to HER2 expressions. The ERK 1/2 proteins were detected by Western blot analysis by using ERK 1/2 polyclonal antibody (upper panel). The phospho-ERK 1/2 proteins were detected by Western blot analysis by using phospho-specific MAPK (pERK 1/2) antibody (lower panel). There were no significant differences of the levels of ERK 1/2 and activated ERK 1/2 proteins between HER2-negative (left panel) and HER2-positive cancers. N and C represent normal and carcinoma, respectively.

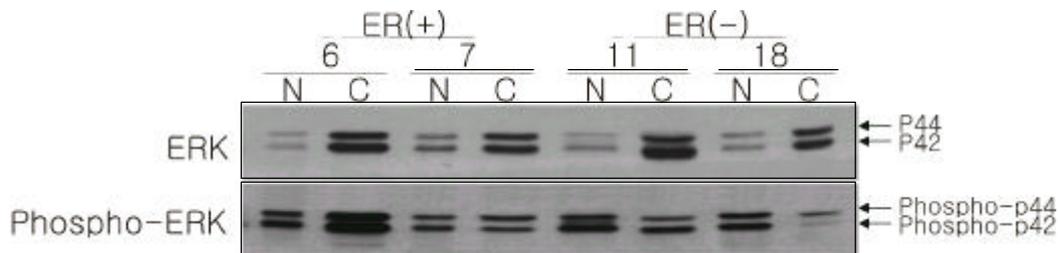


Fig. 5. Levels of MAPKs in the representative breast cancers and paired normals according to ER expressions. The ERK 1/2 proteins were detected by Western blot analysis by using ERK 1/2 polyclonal antibody (upper panel). The phospho-ERK 1/2 proteins were detected by Western blot analysis by using phospho-specific MAPK (pERK 1/2) antibody (lower panel). There were significantly higher levels of activated ERK 1/2 proteins ER-positive (left panel) cancers than those in ER-negative cancers ($P < 0.05$). N and C represent normal and carcinoma, respectively.

(Table 1) MKP1 가 HER2 ERK 1/2 가 (P=0,086) (Table 1, Fig. 6).
 (60% vs 33%) ERK

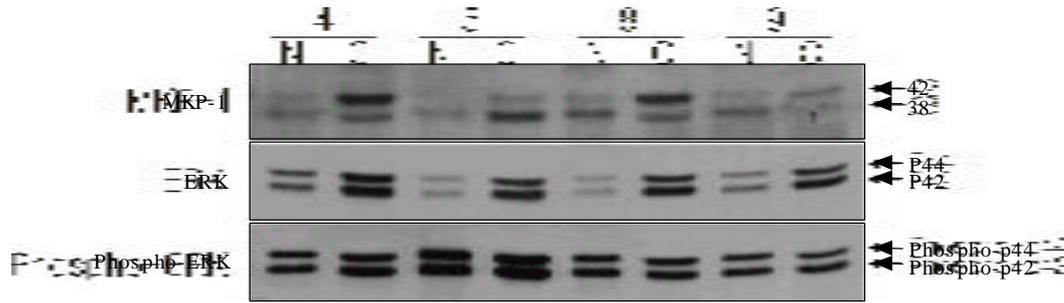


Fig. 6. Levels of MAPKs in the representative breast cancers and paired normals according to MKP1 expression. There were no significant differences in ERK1/2 and activated forms between higher MKP1 (samples 4, 5, and 8) and Lower MKP1 groups (sample 9). N and C represent normal and carcinoma, respectively.

(20-23) MAPK
 가
 ERK1/2
 MAPK (fibroblast) MAPK kinase (MEK)
 MAPK oncogenicity
 가 HER2
 ER (P < 0.05),
 (10) MAPK
 가 MAPK (activated form) 가 가 ER
 MAPK HER2 MAPK
 MAPK MAPK
 MAPK MAPK 가
 MAPK , MAPK ER MAPK ; ,
 MAPK 가 ERK JNK p38 ER MAPK 가;
 knase activated form MAPK (19) (24-26)
 Mueller(11)
 MAPK MAPK 가 ERK MAPK
 (11)
 MAPK 가
 MAPK MAPK 가
 MAPK (12-15) ERK JNK
 (hydrogen peroxide)
 (16) ER ERK1/2 가 가
 ERK1/2 (major regulator) (17) HER1 p38 JNK
 HER2 ras MAPK
 가 (18) (an ERK1/2
 aggressive tumor phenotype) (14) JNK
 MAPK 가
 (19) MAPK 가 ERK1/2
 (18) , potent mi- ERK1/2
 togenic stimuli 가 가 (4.31 : 20

19 10.1 12.21 가) ERK 1/2 ERK 1/2
 ER (P < 0.05) ERK 1/2 MKP1 가
 MKP1 MAPK 가
 HER2 (8)
 MKP1 가 ERK 1/2
 MAPK (P=0.086)
 가
 stress-activated protein kinase p38 JNK
 가
 MAPK
 mapk
 mapk 5 10 MAPK 가 (5)
 5 20 가
 mRNA (metastatic potential) (5) MAPK
 가 (27) MAPK
 (4) ERK 1/2
 MAPK
 MAPK
 MAPK
 mitogenic signal
 MAPK mitogenic effect
 (6) (block differentiation).(7) MKP 1
 mitogenic signals, MAPK
 (28) MKP 1
 가
 가
 MKP 1
 MKP 1
 ERK 1
 MKP 1
 MKP 1
 (8) MKP 1 mRNA
 가 ERK 1, 2 가
 MKP 1
 (high-grade
 tumor) MKP 1 ERKs
 MAPK (MAPK
 independence) (29,30)
 MAPK
 (8) MKP 1 HER2 , ER
 , ERK 1/2

ERK 1/2
 MKP1
 MKP1
 가
 ERK 1/2

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