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Uncoupling Protein 2 (UCP2) and p53 Expression in Invasive Ductal Carcinoma of Breast

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Background : Uncoupling protein 2 (UCP2) is a recently identified mitochondrial inner membrane anion carrier and a negative regulator of reactive oxygen species production. In this study, we evaluated the characteristics and relationships of UCP2 and p53 expression in breast cancer tissues. **Methods :** Tissue microarray slides from 107 cases of invasive ductal carcinoma of the breast were constructed, UCP2 and p53 immunohistochemical staining was conducted, and clinicopathological correlations were investigated. **Results :** UCP2 expression in invasive ductal carcinoma was high in 53 cases (49.5%), while p53 expression in invasive ductal carcinoma was high in 37 cases (34.6%). UCP2 expression was correlated significantly with histological grade ($p = 0.038$) and mitotic count ($p = 0.050$). UCP2 expression was correlated significantly with p53 expression in invasive ductal carcinoma of the breast ($p = 0.045$). UCP2 expression ($p = 0.8308$) and p53 expression ($p = 0.3292$) showed no significant difference for the overall survival rate in patients with invasive ductal carcinoma. **Conclusions :** UCP2 expression in invasive ductal carcinoma increased proportionally with histological grade and mitotic count. High UCP2 expression in invasive ductal carcinoma was observed in conjunction with high p53 expression.

Key Words : Carcinoma, ductal, breast; Mitochondrial uncoupling protein 2; Tumor suppressor protein p53; Reactive oxygen species

Reactive oxygen species (ROS) contribute to the development of cancer. It has been suggested that ROS suppress apoptosis and promote proliferation, invasiveness, and metastasis.[1] ROS and cellular oxidant stress have long been associated with cancer. Transformed cells appear to generate more ROS than normal cells.[2] ROS also promote further genomic instability and stimulate signaling pathways associated with cellular growth and proliferation.[3] Uncoupling protein 2 (UCP2) is a recently identified mitochondrial inner membrane anion carrier, which has emerged as a negative regulator of ROS.[4] UCP1 is expressed exclusively in brown adipose tissue and is a key molecule in thermogenesis.[5] UCP2 is expressed in a variety of tissues, including the brain, lung, spleen, kidneys, liver, adipose tissues, and heart.[6,7] The human *UCP2* gene has been mapped to chromosome 11q13.[8] UCP2 functions in a variety of cell types as a sensor of mitochondrial oxidative stress and may be activated by superoxide or subsequently formed lipid peroxidation products.[9,10] The ability of cancer cells to regulate ROS levels contributes greatly to autonomous growth, the evasion of apoptosis, and other hallmark characteristics of adaptation.[11] Thus, UCP2 activity, a negative regulator of ROS, should be related to cancer development or progression. Actually, Horimoto *et al.*[12] reported that UCP2 expression is correlated with neoplastic changes in human colon cancer. They also suggested that UCP2 expression may increase in colon cancer as a component of tumor adaptation.[12] UCP2 expression may facilitate the adaptation of cancer cells to oxidative stress;[3] however, UCP2 expression and its correlation with clinicopathological factors or outcomes in

human breast cancers have not yet been investigated.

p53, a widely-studied tumor suppressor gene, has also recently been implicated in energy metabolism regulation, and UCP2 inhibits apoptosis of colon cancer cells by interfering with the ROS-mediated phosphorylation of p53 within the transactivating domain.[3]

In this study, we investigated the characteristics and interrelationships of UCP2 and p53 expression in invasive ductal carcinoma of the breast via immunohistochemical analysis in relation to survival and other clinicopathological variables.

MATERIALS AND METHODS

Patients and tissue samples

Tissue samples from 107 cases of invasive ductal carcinoma were utilized. Forty-six cases of normal breast tissues from patients with benign breast disease were used as the control group. All neoplasms were surgically resected at Kyung Hee University Hospital from 1999 to 2006. For each case, two investigators reviewed all of the original hematoxylin and eosin-stained sections. Two representative cores, each 3-mm in diameter, were obtained from a representative area of each paraffin-embedded tumor tissue from which the tissue microarray slides were constructed. Clinicopathological data are summarized in Table 1. The mean patient follow-up duration was 63.1 months (range, 7 to 103 months). Among 107 patients, 17 died of disease, and 89 were alive on the day of the study. One patient was lost during the follow-up period. The ages of the patients ranged from 23 to 71 years (median, 49.4 years). Tumor size ranged from 1 to 12 cm (median, 2.93 cm). Histological grade was classified according to the Nottingham Modification of the Bloom-Richardson system as follows: 26 grade I cases (24.3%), 54 grade II cases (50.5%), and 27 grade III cases (25.2%). Tumor, node and metastasis (TNM) stage was classified according to the American Joint Committee on Cancer 7th ver. as follows: 26 stage Ia cases (24.3%), 31 stage IIa cases (29.0%), 17 stage IIb cases (15.9%), 15 stage IIIa cases (14.0%), five stage IIIc cases (4.7%), and 13 stage IV cases (12.1%).

Table 1. Clinicopathological data of 107 patients with invasive ductal carcinoma

Parameter		Patients
Age (yr)		
	< 49	51 (47.7)
	≥ 49	56 (52.3)
Tumor size (cm)		
	≤ 3	43 (40.2)
	> 3	64 (59.8)
Lymph node metastasis		
	Absent	52 (48.6)
	Present	55 (51.4)
Distant metastasis		
	Absent	94 (87.9)
	Present	13 (12.1)
Estrogen receptor		
	Positive	73 (68.2)
	Negative	34 (31.8)
Progesterone receptor		
	Positive	96 (89.7)
	Negative	11 (10.3)
Tumor grade		
	Grade 1	26 (24.3)
	Grade 2	54 (50.5)
	Grade 3	27 (25.2)
TNM stage		
	Ia	26 (24.3)
	IIa	31 (29.0)
	IIb	17 (15.9)
	IIIa	15 (14.0)
	IIIc	5 (4.7)
	IV	13 (12.1)

Values are presented as number (%).
TNM, tumor, node and metastasis.

Immunohistochemical staining

Immunohistochemistry was conducted on 4 μm tissue sections using the Bond Polymer Intense Detection system (Vision BioSystems, Mount Waverley, VIC, Australia) according to the manufacturer's instructions with minor modifications. Briefly, 4 μm sections of formalin-fixed, paraffin-embedded tissues were deparaffinized using Bond Dewax Solution (Vision BioSystems), and an antigen retrieval procedure was conducted using Bond ER Solution (Vision BioSystems) for 30 minutes at 100℃. Endogenous peroxidases were quenched in a 5-minute incubation of the tissues with hydrogen peroxide. The sections were incubated for 15 minutes at ambient temperate with primary polyclonal antibodies for UCP2 (1 : 25, ProteinTech, Chicago, IL, USA) and monoclonal p53 (1 : 2,000, DO-7, Novocastra, Newcastle, UK) using a biotin-free polymeric horseradish peroxidase-linker antibody conjugate system in a Bond-max automatic slide stainer (Vision BioSystems). Nuclei were counterstained with hematoxylin.

Evaluation of immunohistochemical staining

UCP2 expression, as determined by immunohistochemical staining, appeared as fine granular and diffuse cytoplasmic st-

aining. Immunohistochemical staining for UCP2 was evaluated based on intensity and proportion. Scattered macrophages were the positive controls for UCP2.[12] Intensity and proportion scores were as follows: 0 (negative), 1 (focal weak), 2 (diffuse weak), and 3 (diffuse strong).[12] We regarded strong intensity as indicative of high expression. p53 expression showed nuclear staining. p53 expression was categorized as low expression (< 10% of tumor cells) and high expression (≥ 10% of tumor cells). Using a receiver operating characteristic curve analysis, we considered 10% as a cut-off value, because the best cutoff score that yielded the highest p53 expression area under the curve was 10%. All slides were independently evaluated by two investigators blinded to both the patient's identities and the clinical outcomes.

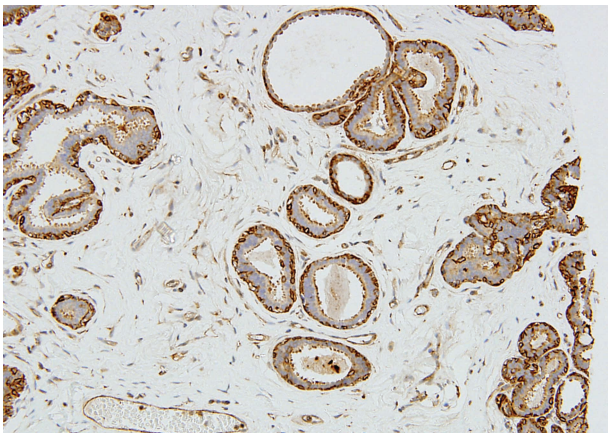

Fig. 1. Normal breast glandular epithelium is negative or weakly focal positive for uncoupling protein 2 expression.

Statistical analysis

The Pearson's chi-squared test was used to evaluate the association between UCP2 and p53 expression and several clinicopathological variables. The Kaplan-Meier method was utilized to determine the probability of survival, and the data were analyzed via the log-rank test. Overall survival was defined as survival from the date of surgery to the date of death due to cancer. A p-value of < 0.05 was considered significant.

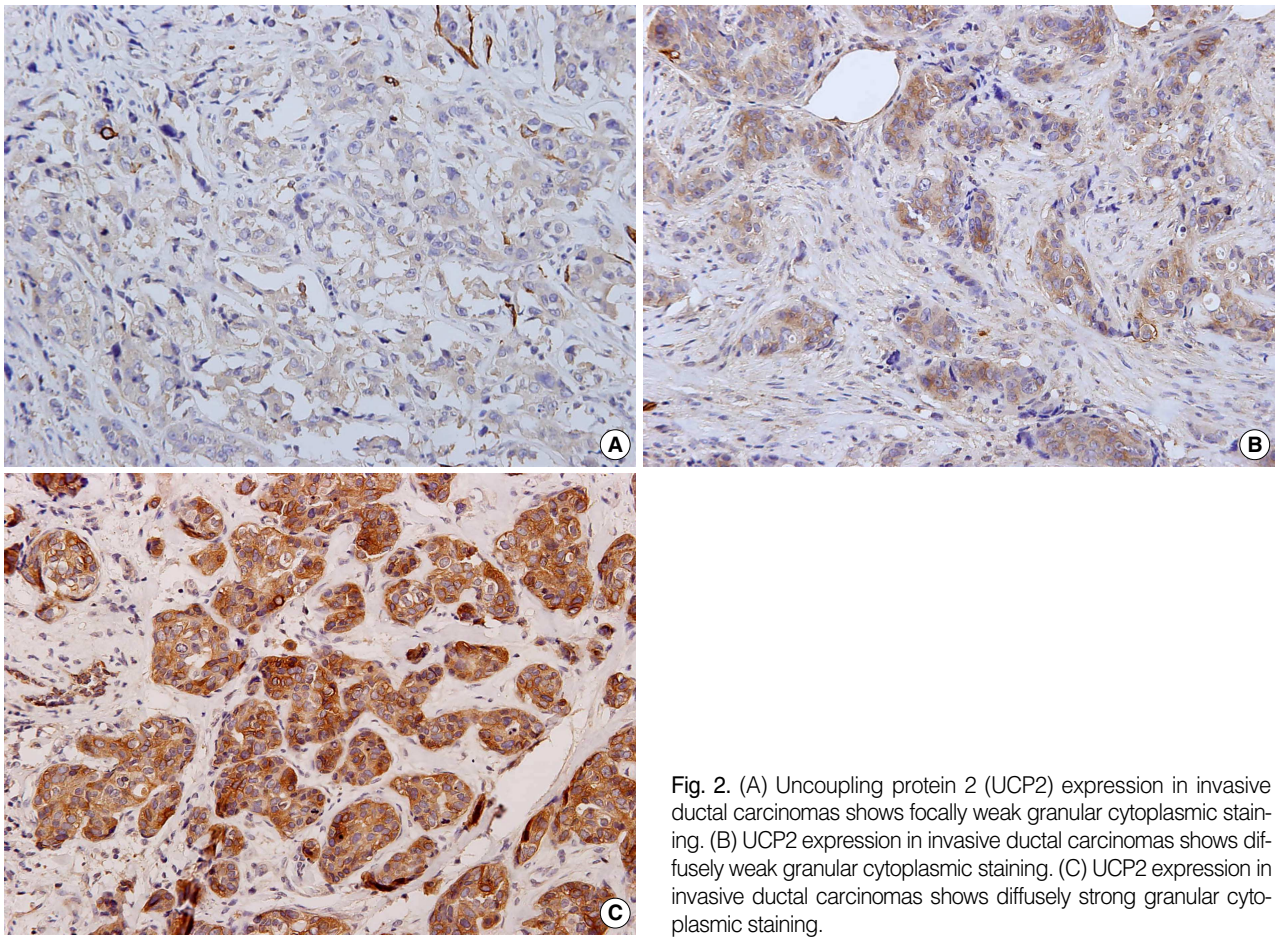

Fig. 2. (A) Uncoupling protein 2 (UCP2) expression in invasive ductal carcinomas shows focally weak granular cytoplasmic staining. (B) UCP2 expression in invasive ductal carcinomas shows diffusely weak granular cytoplasmic staining. (C) UCP2 expression in invasive ductal carcinomas shows diffusely strong granular cytoplasmic staining.

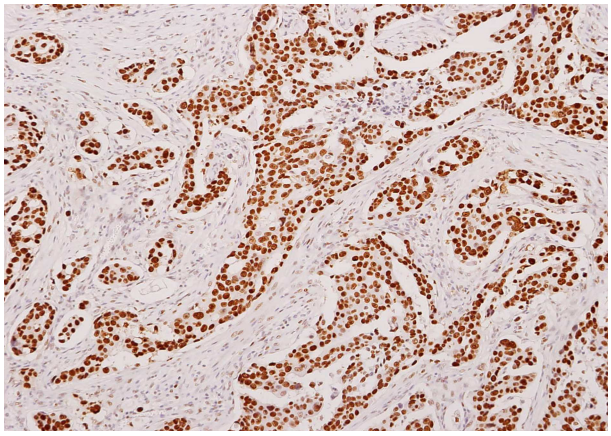

Fig. 3. p53 expression in invasive ductal carcinomas shows strong nuclear staining.

RESULTS

The 46 cases of normal breast glandular epithelium evidenced negative or weak focally positive UCP2 expression (Fig. 1). Myoepithelial cells and scattered macrophages showed strong UCP2 expression. UCP2 expression in invasive ductal carcinoma cells was negative (n = 8, 7.5%), focally weak (n = 21, 19.6%), diffusely weak (n = 25, 23.4%), and diffusely strong (n = 53, 49.5%) (Fig. 2). Negative, focally weak, and diffusely weak expressions were classified as low expression and diffusely strong as high expression. UCP2 expression in invasive ductal carcinoma cells was classified as "low expression" in 54 cases (50.5%), and "high expression" in 53 cases (49.5%). p53 ex-

Table 2. Correlation between uncoupling protein 2 (UCP2), p53 expression, and clinicopathological variables in 107 invasive ductal carcinomas

Variables	UCP2 expression			p53 expression		
	Low	High	p-value	Low	High	p-value
Age (yr)						
< 49	29 (27.1)	22 (20.5)	0.142	33 (30.8)	18 (16.8)	0.522
≥ 49	25 (23.4)	31 (29.0)		37 (34.6)	19 (17.8)	
Histological grade						
Grade I	16 (15.0)	10 (9.3)	0.038*	22 (20.6)	4 (3.7)	0.001*
Grade II	30 (28.0)	24 (22.4)		38 (35.5)	16 (15.0)	
Grade III	8 (7.5)	19 (17.8)		10 (9.3)	17 (15.9)	
Tubule formation						
> 10% of the tumor	18 (16.8)	10 (9.3)	0.089	19 (17.8)	9 (8.4)	0.752
≤ 10% of the tumor	36 (33.6)	43 (40.2)		51 (47.7)	28 (26.2)	
Nuclear pleomorphism						
Minimal to moderate	38 (35.5)	33 (30.8)	0.375	53 (49.5)	18 (16.8)	0.005*
Marked	16 (15.0)	20 (18.7)		17 (15.6)	19 (17.8)	
Mitotic count						
≤ 10/HPFs	47 (43.9)	38 (35.5)	0.050*	62 (57.9)	23 (21.5)	0.001*
> 11/10HPFs	7 (6.5)	15 (14.0)		8 (7.5)	14 (13.1)	
Lymph node metastasis						
Absent	26 (24.3)	26 (24.3)	0.925	37 (34.6)	15 (14.0)	0.225
Present	28 (26.2)	27 (25.2)		33 (30.8)	22 (20.6)	
Estrogen receptor						
Positive	40 (37.4)	33 (30.8)	0.190	52 (48.6)	21 (19.6)	0.064
Negative	14 (13.1)	20 (18.7)		18 (16.8)	16 (15.0)	
Progesterone receptor						
Positive	50 (46.7)	46 (43.0)	0.323	63 (58.9)	33 (30.8)	0.895
Negative	4 (3.7)	7 (6.5)		7 (6.5)	4 (3.7)	
Distant metastasis						
Absent	49 (45.8)	45 (42.1)	0.356	64 (59.8)	30 (28.0)	0.119
Present	5 (4.7)	8 (7.5)		6 (5.6)	7 (6.5)	
Tumor size (cm)						
< 3	18 (16.8)	25 (23.4)	0.103	30 (28.0)	13 (12.1)	0.286
≥ 3	36 (33.6)	28 (26.2)		40 (37.4)	24 (22.4)	
TNM Stage						
Ia-IIa	29 (27.1)	28 (26.2)	0.928	42 (39.3)	15 (14.0)	0.055
IIb-IV	25 (23.4)	25 (23.4)		28 (26.2)	22 (20.6)	

Values are presented as number (%).
*Significantly different by the chi-squared test.
HPF, high power field; TNM, tumor, node and metastasis.

Table 3. Correlation of uncoupling protein 2 (UCP2) and p53 expression in 107 invasive ductal carcinomas

		UCP2 expression		
		High	Low	p-value
p53 expression	High	23 (21.5)	14 (13.1)	0.045*
	Low	30 (28.0)	40 (37.4)	

Values are presented as number (%).
*Significantly different by the chi-squared test.

pression in invasive ductal carcinoma was classified as "low expression" in 70 cases (65.4%), and "high expression" in 37 cases (34.6%) (Fig. 3). As shown in Table 2, UCP2 expression was correlated significantly with histological grade ($p = 0.038$), and mitotic count ($p = 0.050$). p53 expression was correlated significantly with histological grade ($p = 0.001$), nuclear pleomorphism ($p = 0.005$), and mitotic count ($p = 0.001$). UCP2 expression was correlated significantly with p53 expression in invasive ductal carcinoma of the breast ($p = 0.045$) (Table 3). Using the Kaplan-Meier method, lymph node metastasis ($p = 0.0017$), estrogen receptor status ($p = 0.0173$), distant metastasis ($p < 0.00001$), and TNM stage ($p < 0.00001$) were identified as significant prognostic factors. However, UCP2 expression ($p = 0.8308$) and p53 expression ($p = 0.3292$) showed no significant difference for the overall survival rate in patients with invasive ductal carcinoma (Table 4).

Table 4. Analysis of 13 clinicopathological variables for overall survival rate in 107 invasive ductal carcinomas

Variables	Overall survival rate (p-value)
Age	0.2165
Tumor size (> 3 cm vs ≤ 3 cm)	0.9527
Lymph node metastasis	0.0017*
Histological grade (grade I, II vs grade III)	0.1107
Tubule formation (> 10% vs ≤ 10%)	0.7436
Nuclear pleomorphism	0.3591
Mitosis	0.1932
Estrogen receptor	0.0173*
Progesterone receptor	0.4206
Distant metastasis	< 0.00001*
TNM stage	< 0.00001*
UCP2 expression	0.8308
p53 expression	0.3292

*Significantly different by Kaplan-Meier test.
TNM, tumor, node and metastasis; UCP2, uncoupling protein 2.

DISCUSSION

UCP2 is a negative regulator of ROS and acts as a sensor of mitochondrial oxidative stress.[9] A variety of malignant tumors, including thyroid tumors, lymphomas, and colon cancer overexpress UCP2.[12-14] Recently, UCP2 overexpression was revealed in some breast cancer cell lines.[15] Increased UCP2 expression has also been observed in human colon cancer cells, and has been correlated with the degree of neoplastic change.[12] It has been suggested that UCP2 acts as an adaptive mechanism to reduce oxidative stress in colonic tumor tissues.[12] Collins *et al.*[16] observed that UCP2 overexpression in HepG2 human hepatoma cells lowers intracellular ROS levels and attenuates apoptosis induced by a variety of challenges. To date, UCP2 expression in human breast cancer tissue has not been investigated.

In the present study, UCP2 expression in invasive ductal carcinoma of the breast was stronger than in normal breast glandular epithelium. In Horimoto's colon cancer study, UCP2 overexpression was suggested to protect a wide array of cell types from apoptosis and that the cytoprotective effect of UCP2 is likely based on a reduction in mitochondrial ROS generation. Tumor cells may use UCP2 as a metabolic adaptation to avoid ROS-mediated apoptosis.[12] Our results also support that UCP2 overexpression is an adaptive response limiting ROS in breast cancer cells.[15]

Second, UCP2 overexpression was correlated significantly with higher histological grade and mitotic counts in invasive ductal carcinoma. Histological grade and mitotic counts are reflective of the aggressiveness of a malignancy. These findings suggest that more aggressive invasive ductal carcinoma cells express more UCP2, which is believed to be an adaptive mechanism for reducing ROS production.[12] Changes in cancer cell metabolism are frequently associated with more aggressive tumor growth and drug resistance, thereby resulting in a worse prognosis.[17] One of the purposes of metabolic switches in cancer cells is to reduce ROS generation.[12] Thus, UCP2, as a negative ROS regulator, might be involved in changes in cancer cell metabolism associated with tumor aggressiveness. As a result, oxidative stress in breast cancer may contribute to induce a more malignant transformation.

Derdak *et al*.[3] observed that UCP2-overexpressing colon cancer cells perform an active role in promoting cancer cell survival through antiapoptotic effects of p53. We determined whether UCP2 expression in invasive ductal carcinoma was correlated with p53 expression, which is associated with apoptosis. As a result, a positive correlation between UCP2 and p53 expression was observed in invasive ductal carcinoma. Based on these findings, we suggest that the function of UCP2 in invasive ductal carcinoma may be derived from a modulation of

p53 function in accordance with previous results.[3] However, further studies into the relationship between UCP2-overexpressed breast cancer and antiapoptotic effects will be required.

Human colon cancer cells that overexpress UCP2 inhibit ROS accumulation and apoptosis after exposure to chemotherapeutic agents.[3] It has also been suggested that a link may exist between UCP2 and the molecular mechanisms of chemoresistance, and that UCP2 may be a molecular target of great usefulness in novel treatment strategies.

In conclusion, UCP2 expression was stronger in invasive ductal carcinoma cells than in normal breast glandular epithelium. Additionally, UCP2 expression in invasive ductal carcinoma increased proportionally with histological grade and mitotic count. Our results assume that UCP2 overexpression may be an adaptive response, which limits ROS in breast cancer cells, and that oxidative stress contributes to induce a more malignant transformation in invasive ductal carcinoma of the breast.

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