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HSP70 and ER Expression in Cervical Intraepithelial Neoplasia and Cervical Cancer

Heat shock protein (HSP) is thought to play important roles in the cell cycle and various process of carcinogenesis. This study was performed to evaluate the expression of heat shock protein (HSP70) and estrogen receptor (ER) and Ki-67 and to assess relationship between them in cervical squamous cell neoplasia. The materials were 50 cervical squamous cell lesions, consisted of 30 cervical intraepithelial neoplasia (CIN) (6 moderate dysplasia, 11 severe dysplasia, 13 carcinoma in situ), and 20 invasive squamous cell carcinoma (ISCC) cases. These specimens were immunohistochemically stained for HSP70, ER and Ki-67. The score of HSP70 was significantly higher in ISCC than CIN. Expression rate of the ER was not significantly higher in CIN than in ISCC. Ki-67 labelling index was significantly higher in the ISCC and high HSP70 positive staining group. These results suggested that HSP70 may play an important role in tumor cell proliferation and is related with ISCC than CIN, but ER may be not related with tumor cell proliferation and differentiation. HSP70 may be a useful prognostic factor in cervical dysplasia and cancer.

Key Words : *Heat-Shock protein 70; Estrogen receptors, Ki-67 antigen; Cervix neoplasms*

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INTRODUCTION

The heat shock proteins (HSPs) in cells are produced by various environmental factors, pathological conditions, and physiological stresses (1). Most HSPs have been grouped into families of different molecular weights. Members of different families are characterized not only by their size but also by a number of common physiological functions. These are mostly based on the ability of HPSs to form complexes with other proteins, thus altering their functional status (1). In a variety of animal models, HSPs of the 70-, 90-, 100-kDa classes elicit tumor-specific immunity to tumor challenges and the enhanced expression of HSP genes of the 90- and 70-kDa families has been observed after oncogene transformation of cell line (2, 3). HSP70 has been thought to play important roles in the cell cycle and various processes of carcinogenesis (4). Experiments reported so far primarily focused on in vitro or animal studies, and little was known about HSP70 expression in human malignant diseases. Recently, there were several reports about HSP70 expression in malignant tumors, such as breast cancer (5, 6), pancreatic cancer (7), lung cancer (8) and colon cancer (9).

In the normal cervix, basal cells of the squamous epithelium, metaplastic cells, and endocervical glandular cells were estrogen receptor (ER) positive and respond actively to sex steroid (10). In contrast, neoplastic cells of CIN and invasive carcinoma were ER negative (11) and cervical cancer was conventionally not considered to be steroid hormone responsive tissue. But one report has claimed that a human cervical carcinoma cell line could be made to proliferate by the addition of estradiol (12). In breast cancer, several studies of estrogen priming have been conducted in an attempt to recruit cells in the proliferative phase of the cell cycle to enhance sensitivity of tumors to chemotherapy (13, 14).

The determination of tumor cell proliferation is one of the more widely used tools for assessing prognosis and the proliferative activity of tumors has been extensively investigated with different approaches, among which Ki-67 monoclonal antibody is one of the most widely studied proliferation-associated markers and recognizes a labile epitope on a nuclear antigen in cycling cells, and the antigen is expressed in all active parts of the cell cycle (15).

In this study, we examined the expressions of the HSP70, ER and Ki-67 labelling index (LI) to investigate

the relationships among HSP70, sex steroid receptor status and the cell proliferation kinetics in cervical dysplasia and cancer.

MATERIALS AND METHODS

Cervical cancer and dysplasia tissues were obtained by punch biopsy, cone biopsy and radical hysterectomy at the Kyungju Hospital of Dongguk University between January 1994 and August 1996. The histological review of the histology slides was done, based on the WHO classification (16).

Histologic evaluation

The cervical lesions were histologically classified into 6 moderate dysplasia, 11 severe dysplasia, 13 carcinoma in situ, and 11 nonkeratinizing invasive squamous cell carcinomas and 9 keratinizing invasive squamous cell carcinoma. In addition, 6 benign cervical lesions were studied as benign controls.

Immunohistochemical study

For immunohistochemical staining, 5 μm thick sections of each specimen were mounted on positively charged slides and deparaffinized in xylene, and rehydrated through a decreasing concentration of ethanol. The antigen was retrieved in a pressure cooker with 0.01 M citrate buffer (pH 6.0) for 3-5 minutes. Following incubation with normal horse serum, the sections were incubated with the 1:500 dilution of anti-HSP70 polyclonal antibody (A500, Dako, Denmark), 1:50 dilution of anti-ER monoclonal antibody (Dako, Denmark) and 1:100 dilution of Ki-67 monoclonal antibody (Immunotech, Marseille, France). Bound antibody was detected by incubation of section for 30 minutes with biotinylated horse antimouse antibody (1:200 in TBS: Dako, Denmark) followed by streptavidin-horseradish peroxidase complex (1:100 in TBS: Dako, Denmark). Prior to the addition of each antibody, the slides were washed extensively in TBS. Color was developed with 3-amino-9-ethylcarbazole, and sections were counterstained with hematoxylin before mounting.

For evaluation of the expression of HSP70, the scores corresponding to the sum of both (a) staining intensity (0=negative; 1=weak; 2=intermediate; 3=strong) and (b) percentage of positive cells (0=0% positive cells; 1=1-30% positive cells; 2=31-60% positive cells; 3=61-100% positive cells) were established. The sum of a+b was defined as score of HSP70. The score 0 was classified as negative staining and the score 1-2 as low positive staining and the score 3-6 as high positive staining. The expression of ER was classified the score 0 as negative staining and the score 1-6 as positive staining. The scoring of Ki-67 stained nuclei was done by counting 1,000 tumor cells from 10 fields on each slide (at $\times$400 magnification) and the results were expressed as a percentage of the Ki-67 positive cells (Ki-67 LI).

Statistical analysis

For statistical analysis, the chi-squared test, paired t-test and Fisher's exact test were used to examine the relationship among expression of HSP70, ER and Ki-67 LI. P values less than 0.05 were considered as significant.

RESULTS

Expression of the HSP70 and score of HSP70 in CIN and cervical cancer

Immunohistochemical expression of HSP70 was localized in the cytoplasm of tumor cells (Fig. 1A) and the positivity was heterogenous among individual specimens.

The expression of HSP70 was observed in 33 out of the 50 (66%) CIN and ISCC, and the high positive staining (score 3-6) was observed in 13 out of the 50 (26%) cervical lesions. The expression of the HSP70 was significantly higher in ISCC than in CIN ($p=0.003$) and the score of HSP70 was also higher in ISCC ($p=0.012$). The score of HSP70 was significantly higher in the keratinizing type than in the nonkeratinizing type ($p=0.012$) (Table 1).

Table 1. Expression of HSP70 and score of HSP70 in CIN and cervical cancer

Diagnosis	No. of total	*HSP70 positive (%)	†Score of HSP70 (%)	
			0~2	3~6
CIN	30	15 (50)	26 (87)	4 (13)
MD	6	4 (67)	5 (83)	1 (17)
SD	11	7 (64)	9 (82)	2 (18)
CIS	13	4 (31)	12 (92)	1 (8)
ISCC	20	18 (90)	11 (55)	9 (45)
KIC	9	9 (100)	2 (22)	7 (88)†
NKIC	11	9 (82)	9 (82)	2 (18)†

CIN, cervical intraepithelial neoplasia; MD, moderate dysplasia; SD, severe dysplasia; CIS, carcinoma in situ; ISCC, invasive squamous cell carcinoma; KIC, keratinizing invasive squamous cell carcinoma; NKIC, nonkeratinizing invasive squamous cell carcinoma.

*$p=0.003$ and †$p=0.012$: statistical significance was determined by χ^2 test.

†$p=0.012$: statistical significance was determined by Fisher's exact test.

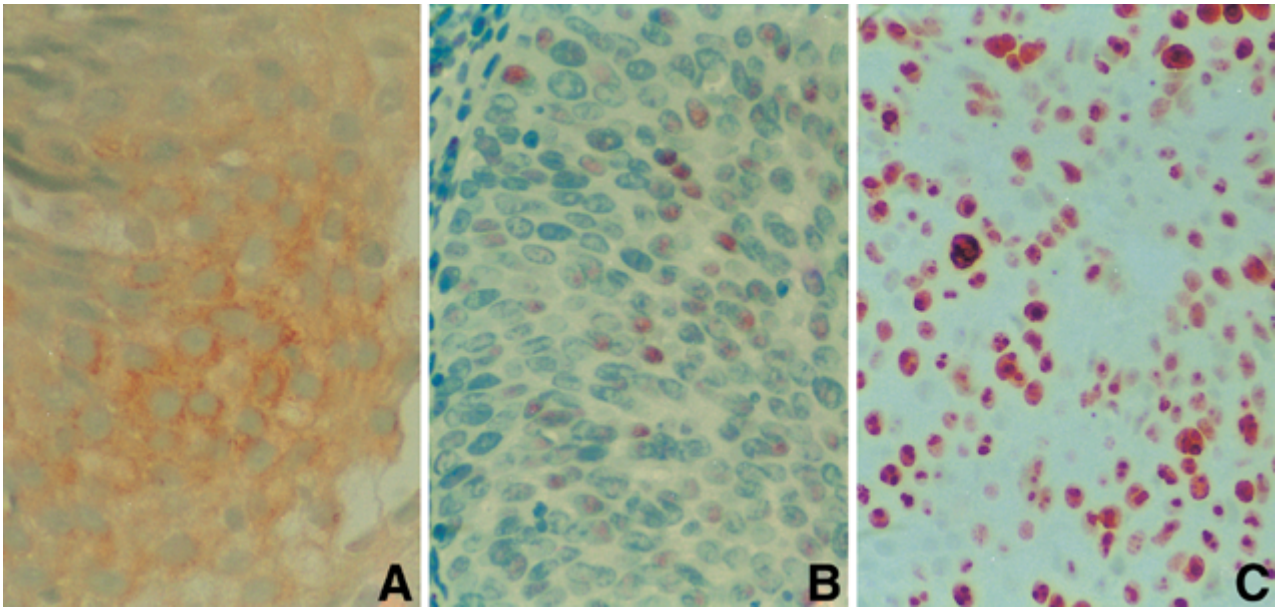

Fig. 1. Immunohistochemical staining of invasive squamous cell carcinoma of the cervix for HSP70 (A), ER (B), and Ki-67 (C), showing cytoplasmic (A) and nuclear (B, C) immunoreactivity.

Table 2. ER expression in CIN and cervical cancer

Diagnosis	No. of total	No. of positive (%)	p value
CIN*	30	5 (17)	
MD	6	2 (33)	NS
SD	11	2 (18)	
CIS	13	1 (8)	
ISCC*	20	2 (10)	NS
KIC	9	1 (11)	
NKIC	11	1 (9)	

CIN, cervical intraepithelial neoplasia; MD, moderate dysplasia; SD, severe dysplasia; CIS, carcinoma in situ; ISCC, invasive squamous cell carcinoma; KIC, keratinizing invasive squamous cell carcinoma; NKIC, nonkeratinizing invasive squamous cell carcinoma.
*$p>0.05$: statistical significance was determined by χ^2 test.

Table 3. Expression of the Ki-67 in CIN and cervical cancer

Diagnosis	LI Mean ± SD (%)
CIN (n=30)	47.9 ± 11.2
MD (n=6)	47.8 ± 15.3
SD (n=11)	45.0 ± 8.2
CIS (n=13)	50.4 ± 11.7
ISCC (n=20)	63.3 ± 18.7
KIC (n=9)	71.3 ± 15.5
NKIC (n=11)	56.6 ± 19.1

LI, labelling index; CIN, cervical intraepithelial neoplasia; MD, moderate dysplasia; SD, severe dysplasia; CIS, carcinoma in situ; ISCC, invasive squamous cell carcinoma; KIC, keratinizing invasive squamous cell carcinoma; NKIC, nonkeratinizing invasive squamous cell carcinoma.
$p=0.001$: statistical significance was determined by paired T test.

Expression of the ER in CIN and cervical cancer

The expression of ER was localized in the nucleus of tumor cells (Fig. 1B) and was observed in 7 out of 50 (14%) cervical lesions. Expression rate of the ER was higher in CIN than ISCC but there was no significant correlations ($p>0.05$) (Table 2).

Ki-67 labelling index in CIN and cervical cancer

The expression of Ki-67 was localized in the nucleus of tumor cells (Fig. 1C). Ki-67 labelling index was significantly higher in ISCC than in CIN ($p=0.001$) (Table 3).

Relationship between HSP70 expression, Ki-67 labelling index and ER in CIN and cervical cancer

With regard to the correlation between Ki-67 labelling index and score of HSP70 expression, Ki-67 labelling index was significantly higher in the high positive

Table 4. Relationship between HSP70 expression and Ki-67 labelling index in CIN and cervical cancer

	*HSP70−	*HSP70+	†Score of HSP70	
			0~2	3~6
Ki-67 LI Mean±SD (%)	51±11.0	55.8±18.6	49.4±12.7	67.3±18.8

HSP70, heat shock protein 70; Ki-67 LI, Ki-67 labelling index.
*$p>0.05$ and †$p=0.0004$: statistical significance was determined by paired T test.

Table 5. Relationship between ER expression and Ki-67 labelling index in CIN and cervical cancer

	ER−	ER+
Ki-67 LI		
Mean	54.3	52.6
Median	53	50
Range	31-92	40-72
SD	17.1	11.8

ER, estrogen receptor; Ki-67 LI, Ki-67 labelling index.
$p>0.05$: statistical significance was determined by paired T test.

Table 6. Relationship between the HSP70 and ER expression in CIN and cervical cancer

	HSP70 (+)(%)	HSP70 (−)(%)	Total
ER (+)	4 (8)	3 (6)	7
ER (−)	28 (56)	15 (30)	43
Total	13	18	50

HSP70, heat shock protein 70; ER, estrogen receptor.
$p>0.05$: statistical significance was determined by Fisher's exact test.

staining group than in the negative and low positive staining group ($p=0.0004$), but there was no significant association between HSP70 positive cases and HSP70 negative cases (Table 4). With regard to the relationship between ER expression and Ki-67 labelling index ($p>0.05$), and between ER and HSP70 ($p>0.05$), there were no correlations (Table 5, 6).

DISCUSSION

HSPs are involved in cell cycle regulation, control of DNA damage, and the metabolism of gene products and play important roles in carcinogenesis (17). and HSP expression is upregulated in tumor cells and therefore, HSP expression is a likely marker of the malignant tumors (18). Furthermore, HSP70 is implicated in the degree of tumor differentiation, the rate of tumor proliferation. Several authors reported that the distribution and intensity of HSP70 expression was highest in the epithelial compartment of oral squamous cell carcinoma than in the dysplastic oral epithelium and the distribution of HSP70 expression in well differentiated ISCC was more diffuse than in poorly differentiated ISCC (18) and in endometrial carcinoma, the HSP70 expression was correlated with a poorly differentiated state (19). In our study, the expression of HSP70 was significantly higher in ISCC than in CIN and the score of HSP70 was also higher in ISCC. With respect to the type of ISCC, score of HSP70 was significantly higher in keratinizing type than in nonkeratinizing type. These suggested that HSP70 is associated with malignant potential and correlated inversely with the differentiation state in cervical carcinoma.

Recent studies have shown that the intracellular localization of HSP70 was expressed at higher level in breast cancerous tissues associated with a high PCNA LI than in non-cancerous tissues (20) and this suggested that HSP70 is required for tumor cells to proliferate (21). In the present study, with regard to the correlation between Ki-67 labelling index and score of HSP70 expression, Ki-67 labelling index was significantly higher in the HSP70 high positive staining group than in low positive staining group.

HSP70 has been demonstrated to bind the sex steroid receptors, although its exact role in the receptor-activation pathway remains to be determined. Several authors (22, 23) reported that the ER was correlated with the expression of HSP70 in human breast cancer. But other workers (19) reported that the expression of HSP70 was correlated with the absence of sex steroid receptors in endometrial carcinomas. In our study, there is no correlation between the ER and the expression of HSP70 in CIN and ISCC.

The presence of estrogen receptor was reported in all the samples of normal cervical tissue examined in premenopausal woman (24). Most studies of ER status in cervical dysplasia and carcinoma have employed the biochemical method. Among these series, there was a large variability for positivity of ER (10, 11, 30). Vargas et al. (25) and Hahnel et al. (26) reported low or undetectable levels of ER in cervical cancer using immunohistochemical method and ligand-binding assay, respectively. Other workers using ligand-binding assay have reported somewhat higher levels (27-29). In 1988 Henry et al. (30) reported the immunohistochemical distribution of ER in dysplastic cervical epithelium and found that there was a decreasing intensity of staining with increasing severity of the dysplastic process. Different results were described by Nonogaki et al. (11) and they reported that basal cells of the normal squamous epithelium, metaplastic cells, and endocervical glandular cells were ER positive, but neoplastic cells of CIN and ISCC were ER negative. In our study, in which an immunohistochemical technique was used, ER was positive in 14%, and there was no significant differences between CIN and ISCC in ER positivity. The differences between studies may be the result of differences in method of tissue collection, differences in storage conditions, differences in assay techniques and perhaps differences in patient populations.

In the relationship between the ER status and cell proliferation-associated antigen, Ki-67, Konishi et al. (31) reported that reduced ER expression is associated with the proliferation of neoplastic cervical squamous cells

which are induced by HPV infection. In cases of breast carcinoma, there was a large variability. Molino et al. (32) and Skalova and Michal (33) reported that a significant association was found beween Ki-67 values and estrogen receptor. However, Moriki et al. (34) and Bilous et al. (35) reported that the Ki-67 proliferation index correlated inversely with estrogen receptor status in breast cancer. Some reports suggested that hormone-receptor pathway and proliferative activity are not related in meningioma (36) and ovarian cancer (37). They confirmed that the steroid hormone receptors in meningioma and ovary are not estrogen regulated as in other sex steroid dependent tissues, such as the breast and the endometrium. The present study also demonstrated no significant differences between ER status and Ki-67 LI and this suggests that the cancer of the cervix is considered to be less responsive to steroid hormone, although the normal cervix is known to respond actively to sex steroids (10).

In conclusion, these result suggested that HSP70 may play an important role in tumor cell proliferation and is related with ISCC than CIN, but ER may not be related with tumor cell proliferation and differentiation. There are no correlation between HSP70 and ER, and between ER and Ki-67. HSP70 may be a useful method for the evaluation of malignancy and a useful prognostic factor in cervical dysplasia and cancer.

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