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Overexpression of PI3K-p110α in the progression of uterine cervical neoplasia and its correlation with pAkt and DJ-1

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Summary

Objective: To investigate the expression of PI3K-p110α, pAkt, PTEN, the signaling molecules from PI3K/Akt signaling pathway, DJ-1, an oncoprotein and HSP90α, a molecular chaperone, and their correlation in uterine cervical neoplasia, in order to elucidate their role in cervical carcinogenesis. *Materials and Methods:* Using immunohistochemistry, the authors analyzed the expression of PI3K-p110α, pAkt, PTEN, DJ-1 and HSP90α, and their correlation in ten normal tissues, cervical intraepithelial neoplasia (CIN) including 30 CIN1 and 31 CIN3, and 33 cases of invasive squamous cell carcinoma (SCC). *Results:* The expression of all proteins significantly increased in CIN3 compared to CIN1, and only the expression of PI3K-p110α significantly increased in invasive SCC compared to CIN3. There was a significant positive correlation between the expression of PI3K-p110α and DJ-1, as well as PI3K-p110α and pAkt in CIN3 and invasive SCC. *Conclusion:* Overexpression of PI3K-p110α is associated with progression of uterine cervical neoplasia, and the expression of pAkt and DJ-1 is positively correlated with PI3K-p110α expression in this process.

Key words: PI3K-p110α; DJ-1; pAkt; Cervical intraepithelial neoplasia; Squamous cell carcinoma.

Introduction

Development of uterine cervical carcinoma is associated with multiple molecular events, in addition to human papillomavirus (HPV) infection [1-3]. Frequent genetic aberration on chromosome 3q has been observed in cervical dysplasia and carcinoma by comparative genomic hybridization [4]. The area of gain has been refined at 3q26.3, encoding the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K-p110α) and an oncogene in this region is called *PIK3CA* [3]. PI3K phosphorylates the membrane phospholipid phosphatidylinositol 4,5-biphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3), which triggers phosphorylation of Akt [5]. pAkt, a phosphorylated and active form of Akt, affects diverse cellular processes including survival, proliferation, protein synthesis, and glucose metabolism [6]. On the other hand, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) negatively regulates PI3K activity by dephosphorylating PIP3 [7]. Loss of PTEN or overexpression of pAkt can result in the development of many types of tumors [7, 8].

DJ-1 has been characterized as an oncogenic product that transforms NIH3T3 cells and has a stronger cooperative transforming activity with H-Ras [9]. DJ-1 acts as a negative regulator of PTEN and increases cell survival by hyperphosphorylation of Akt in mammalian cells [10]. Overexpression of DJ-1 has been shown to predict a poor prognosis in pancreatic [11] and esophageal cancers [12].

Heat shock protein 90 (HSP90) is a molecular chaperone that maintains the stability and activity of oncogenic client proteins including ERBB2, Akt, steroid hormone receptors, mutant p53, HIF-1alpha, and hTERT [13]. Thus, inhibition of HSP90 provides simultaneous repression of multiple signaling pathways that have been involved in the development of malignancy [13]. HSP90 has two isoforms, HSP90α and HSP90β, and HSP90α is stress-inducible and overexpressed in many cancer cells [14].

There has been no report concerning the expression of DJ-1, HSP90α, PI3K-p110α, pAkt, and PTEN and their correlation in cervical neoplasia, although each molecule has been examined individually [8, 15-17]. In this study, the authors analyzed the expression of these markers in normal cervix, cervical intraepithelial neoplasia (CIN), and invasive squamous cell carcinoma (SCC) of uterine cervix, and their association during the progress of cervical neoplasia.

Materials and Methods

The present authors retrieved 30 (27 punch biopsies, two conizations, and one hysterectomy) cases of CIN grade 1 (CIN1), 31 (23 conizations and eight hysterectomies) cases of CIN grade 3 (CIN3), and 33 (9 conizations and 24 hysterectomies) cases of invasive SCC from the files of the Department of Pathology, Eulji Medical Center, Eulji University School of Medicine, Seoul, Korea, between 2000 and 2004. HPV DNA data of patients were not available and the authors used p16INK4a immunostaining as an ancillary test for a marker of HPV infected CIN [18]. Ten cases

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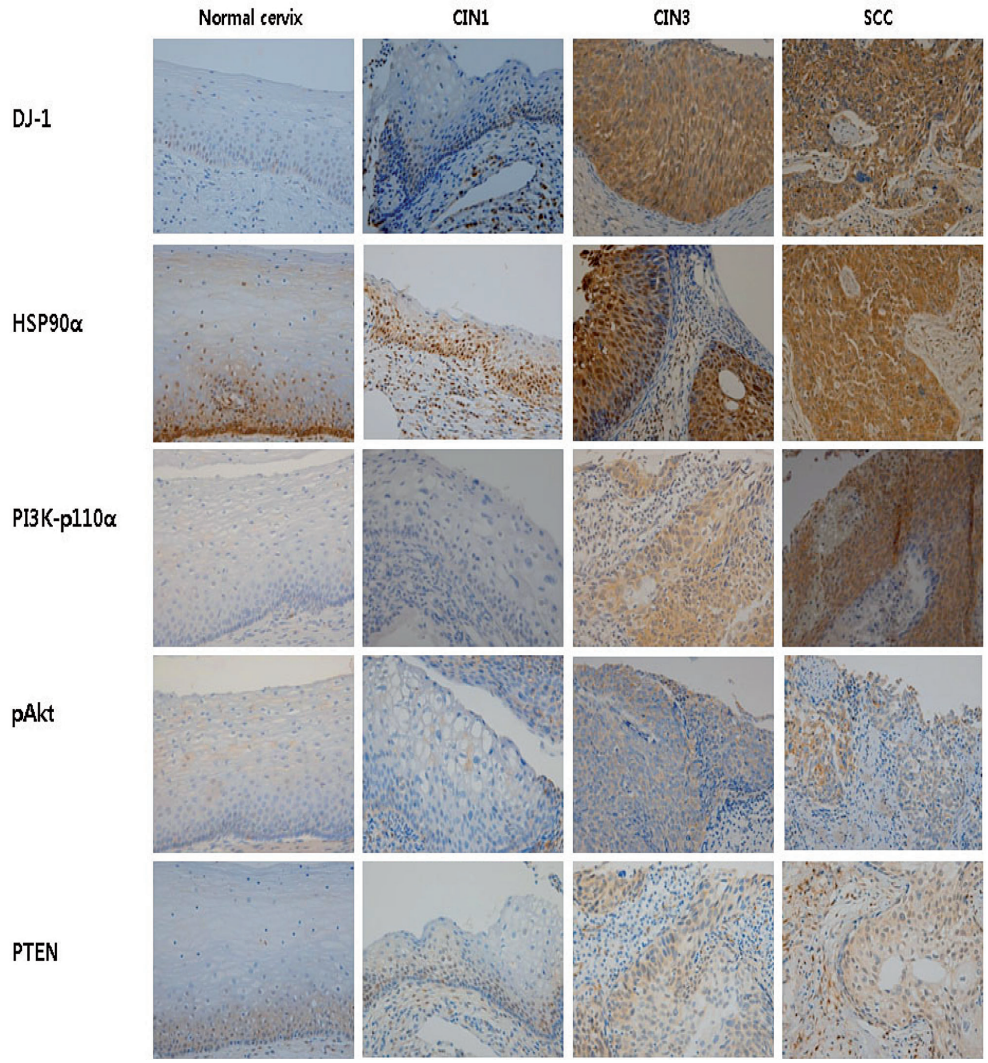

Figure 1. — Representative immunohistochemical staining of DJ-1, HSP90α, PI3K-p110α, pAkt, and PTEN in normal uterine cervix, CIN1, CIN3, and SCC.

of histologically normal and p16INK4a negative cervical mucosa from hysterectomy specimen were included. CIN2 cases were excluded because of its poor reproducibility of histopathologic diagnosis. The stage of 24 invasive SCC patients underwent hysterectomy was classified into nine cases of Stage Ia, ten Stage Ib, and five Stage IIa-IIIb according to the international Federation of Gynecology and Obstetrics (FIGO) staging system. After reviewing all hematoxylin and eosin-stained slides from each case, a representative block of each lesion was selected. Tissue microarray (TMA) was constructed using a manual microarray instrument. The tissue cores (two mm in diameter) from the targeted areas were taken from the paraffin blocks and arranged in a new recipient block for TMA.

Immunohistochemistry

The authors performed immunohistochemical staining using an autostainer. Four micron tissue sections were cut from TMA blocks and positioned on poly-L-lysine coated slides. After deparaffinization and rehydration, antigen retrieval was performed using citrate buffer (pH 6.0) at 121°C for ten minutes. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for five minutes, and the sections were incubated with specific antibodies against DJ-1 (1:1,000), HSP90α (1:10,000), pAkt (1:500), PI3K-p110α (1:500), PTEN (1:250), and p16INK4a (p16INK4a kit). Color was developed using diaminobenzidine, and the sections were counterstained with hematoxylin. Positive and negative controls for DJ-1, HSP90α, pAkt, PI3K-p110α, and PTEN were optimized as previously described [19]. Diffuse cytoplasmic staining of pAkt, PI3K-p110α, and PTEN was considered positive. In the case of DJ-1 and HSP90α, either cytoplasmic or nuclear staining was considered positive. Immunohistochemical staining results were evaluated independently by two pathologists (S.K. Choi and H. Lee). When the interpretation between two observers was different, final decision was reached by re-evaluating the slides at a conference microscope. The immunoreactivity was determined semiquantitatively by assessing the percentage of stained cells and the intensity of staining. The percentage of positive cells was divided into three groups as staining in < 5%, 5-50%, and > 50% of the neoplastic cells. The staining intensity was rated as negative, weak and strong. The overall staining score was classified as: 0, no expression (less than 5% staining with any intensity); 1, low expression (5-50% staining with any intensity or over 50% staining with weak intensity); and 2, high expression (over 50% staining with strong intensity).

Table 1. — *DJ-1, HSP90α, PI3K-p110α, pAkt, and PTEN staining score in CIN1, CIN3, and SCC.*

	Score	CIN1		CIN3		SCC		P_{1-3}	P_{3-S}
		N	%	N	%	N	%		
DJ-1	0	24	80	3	9.7	0	0	**< 0.001**	0.082
	1	6	20	19	61.3	17	51.5		
	2	0	0	9	29.0	16	48.5		
HSP90α	0	0	0	4	12.9	2	6.1	**0.002**	0.312
	1	28	93.3	17	54.8	24	72.7		
	2	2	6.7	10	32.3	7	21.2		
PI3K-p110α	0	30	100	10	32.3	2	6.1	**< 0.001**	**0.026**
	1	0	0	20	64.5	29	87.8		
	2	0	0	1	3.2	2	6.1		
pAkt	0	30	100	17	54.8	17	51.5	**< 0.001**	0.615
	1	0	0	14	45.2	15	45.5		
	2	0	0	0	0	1	3.0		
PTEN	0	29	96.7	16	51.6	19	57.6	**< 0.001**	0.632
	1	1	3.3	15	48.4	14	42.4		
	2	0	0	0	0	0	0		

CIN1: cervical intraepithelial neoplasia grade 1; CIN3: cervical intraepithelial neoplasia grade 3; SCC: squamous cell carcinoma; N: number.
P_{1-3} reflects *p*-value between CIN1 and CIN3; P_{3-S}, *p*-value between CIN3 and SCC. Variables found to be significant ($p < 0.05$) are shown in bold.

Table 2. — *Spearman correlation between markers in CIN3 and SCC.*

	HSP90α	PI3K-p110α	pAkt	PTEN
DJ-1	0.055(P=0.667)	**0.266 ($p = 0.033$)**	0.144 ($p = 0.257$)	0.232 ($p = 0.066$)
HSP90α		-0.135 ($p = 0.286$)	-0.023 ($p = 0.858$)	-0.076 ($p = 0.549$)
PI3K-p110α			**0.288 ($p = 0.021$)**	0.210 ($p = 0.097$)
pAkt				0.226 ($p = 0.073$)

CIN3: cervical intraepithelial neoplasia grade 3; SCC: squamous cell carcinoma. Variables found to be significant ($p < 0.05$) are shown in bold.

Statistical analysis

Comparative analysis of immunoexpression between CIN1, CIN3, and invasive SCC was performed using Chi square test and Fisher's exact test. Correlation between markers was analyzed by Spearman correlation test in CIN3 and invasive SCC. CIN1 was excluded in correlation test between markers because PI3K-p110α and pAkt were not expressed in CIN1. Statistical significance was set at $p < 0.05$. All analysis was performed using the SPSS ver. 14.0.

Results

Staining pattern of DJ-1, HSP90α, PI3K-p110α, pAkt, and PTEN in the normal cervix, CIN1, CIN3, and invasive SCC is shown in Figure 1. In normal cervical squamous epithelia, HSP90α and PTEN expression was observed in basal layer, whereas the expression of DJ-1, PI3K-p110α, and pAkt was negative. HSP90α staining was strong nuclear and cytoplasmic, and PTEN staining was weakly cytoplasmic in basal layer.

Table 1 summarizes the staining score of markers according to the disease severity. The expression of all markers was significantly upregulated in CIN3 compared to CIN1. However, comparing CIN3 to SCC, only the expression of PI3K-p110α significantly increased in SCC. DJ-1 was not stained in koilocytes and only weakly expressed in nuclei of the cells of basal layer in 20% of CIN1 cases. Diffuse cytoplasmic and nuclear DJ-1 expression was observed in 90.3% of CIN3 (low expression, 61.3% and high expression, 29.0%) and 100% of SCC cases (low expression, 51.5% and high expression, 48.5%). HSP90α was expressed in koilocytes as well as basal layer cells of all CIN1 cases (low expression, 93.3% and high expression, 6.7%). HSP90α staining was diffusely cytoplasmic and nuclear in 87.1% of CIN3 (low expression, 54.8% and high expression, 32.3%) and 93.9% of SCC cases (low expression, 72.7% and high expression, 21.2%). PI3K-p110α staining was negative in all CIN1 case, but mostly weakly cytoplasmic in 67.7% of CIN3 (low expression, 64.5% and high expression, 3.2%) and 93.9% of SCC cases (low expression, 87.8% and high expression, 6.1%). pAkt staining was also negative in all CIN1, but weakly cytoplasmic in 45.2% of CIN3 and 45.5% of SCC, and strong only in 3% of SCC cases. PTEN expression showed weak cytoplasmic staining pattern in 3.3% of CIN1, 48.4% of CIN3, and 42.4% of SCC cases.

The authors identified positive correlations in the expression of PI3K-p110α and DJ-1 (Spearman's correlation coefficient (cc), 0.266; $p = 0.043$) and PI3K-p110α and pAkt (Spearman's correlation coefficient (cc), 0.288; $p = 0.021$) in CIN3 and SCC (Table 2).

Discussion

In this study, the authors found a significantly increased expression of PI3K-p110α, pAkt, DJ-1, HSP90α, and PTEN in CIN3 compared to CIN1. The staining of PI3K-p110α was completely negative in CIN1 and normal cervical tissue, whereas PI3K-p110α was overexpressed in CIN3 (67.7%) and SCC (93.9%), corresponding to previous studies [3, 20]. Amplification of *PI3KCA* gene copy number was shown in CIN3 and SCC specimens by quantitative real-time polymerase chain reaction [3]. Zhang *et al*. showed overexpression of PI3K at the protein and mRNA level in cervical cancer tissues compared with non-neoplastic tissues and higher PI3K expression in HeLa cells than in immortal human keratinocyte HaCaT cells [20]. There were studies representing gain of chromosome 3q in HPV-infected tumor cells that would indicate activation of PI3K [1, 4], but there is no study on the expression of PI3K in koilocyte. In the present study, PI3K-p110α was not expressed in koilocyte and it remains to be seen whether genetic alteration of PI3K-p110α is associated with morphologic change of HPV-infected cells. Recently, mutation of *PI3KCA* exon 9 and exon 20 was found in 23% of cervical cancer patients and FIGO Stage I/II patients with *PIK3CA* mutant tumors showed worse overall survival [21]. Schwarz *et al*. showed mutational activation of *PI3KCA* in cervical cancer was associated with incomplete metabolic response, resulting in poor response to chemoradiotherapy [15]. LY294002, PI3K inhibitor inhibited the proliferation of cells and the expression of pAkt and phospho-mTOR, providing a possible therapeutic strategy of PI3K inhibitor for cervical cancer [20]. As mutational status of *PI3KCA* is associated with overall survival in cervical cancer patients and response rate of the patients treated with PI3K/Akt/mTOR inhibitor [22], it would be required to evaluate the tumoral *PIK3CA* mutational status.

Positive correlation between pAkt and PI3K-p110α in this study corresponds to previous results [3], but no significant difference in expression rate of pAkt in CIN3 (45.2%) and SCC (48.5%) was found.

DJ-1 promotes cell survival through modulating PI3K/Akt pathway [10]. In esophageal SCC patients, significant correlation is present between DJ-1 and pAkt expression, and a high level of nuclear DJ-1 is significantly associated with high distant metastatic potential and poorer survival [12]. Higher expression of DJ-1 has been associated with higher pathologic stage in esophageal and glottic SCC [12, 23], and urothelial carcinoma [19]. The present authors previously showed that DJ-1 expression was significantly higher in invasive urothelial carcinoma (T1-T3) compared to non-invasive urothelial carcinoma (Ta) of bladder [19]. DJ-1 is characterized as one of the molecular markers indicative of cervical cancer progression by proteomic analysis by Arnouk *et al*. demonstrating significant increase of DJ-1 in SCC compared to normal tissue [17]. In the present study, DJ-1 was significantly overexpressed in CIN3 (90.3%) compared to weak expression in basal layer of CIN1 (20%) with negative staining in koilocytes, supporting the assumption that molecular change of DJ-1 occur during latency period of HPV infection [17]. Although the authors showed no significant difference of DJ-1 expression between CIN3 and SCC, the result of high expression rate of DJ-1 in CIN3 (90.3%) and SCC (100%), and positive correlation between DJ-1 and PI3K-p110α suggests the involvement of DJ-1 in progression of cervical cancer through PI3K/Akt signaling pathway.

Contrary to other proteins, HSP90α was abundantly expressed in nuclei and cytoplasm of most cells through CIN to SCC tissues, including koilocytes in this study. As heat shock proteins (HSPs) are known to interact with p53, upregulation of HSP90 may be induced by destabilization of p53 by oncogenic HPV E6 expression under negative feedback control [16, 24]. Castle *et al*. demonstrated that expression of HSP40, HSP60, and HSP70 increased in proportion to the severity of CIN, while expression of HSP90 was negligible in normal cervix and CIN, but the reason for lack of HSP90 was not explained [16]. HSP90α expression in cervical neoplastic lesion may reflect its involvement in whole process from early HPV infection to invasive cancer. However, the present authors did not have data of HPV DNA testing and therefore further study including HPV genomic organization data and assessing their association with HSP90α would be needed [25]. It is well known that Akt is one of client proteins of HSP90, and HSP90 inhibitors reduce Akt expression in gynecologic cancer cells [26], however, any correlation between expression of HSP90α and pAkt was not observed in our results.

PTEN staining result in the present study was conflicting compared to previous data showing that loss of PTEN expression was associated with tumor progression in the cervical epithelium [8], and pelvic lymph node metastasis in early-stage cervical cancer [27]. However, direct mutation of *PTEN* gene has been generally absent in cervical cancer [28]. In addition, a study showing evenly distributed PTEN immunostaining in normal cervical mucosa and tumor tissue suggests little impact of PTEN on tumor suppression [3]. Combined analysis of *PTEN* mutation would be needed to define the role of PTEN in cervical carcinogenesis.

In summary, this study is the first attempt to represent simultaneous assessment of PI3K-p110α, pAkt, DJ-1, HSP90α, and PTEN in CIN and SCC of uterine cervix. The present results suggest that the overexpression of PI3K-p110α is associated with stepwise progression from CIN to SCC, and pAkt and DJ-1 may be positively correlated with PI3K-p110α, irrespective of PTEN loss in PI3K/Akt signaling pathway. Further studies including the mutational analysis of these molecules and their association with HPV infection will provide better understanding of their role in cervical carcinogenesis.

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