Mutation of *PIK3CA*: Possible risk factor for cervical carcinogenesis in older women

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Received April 3, 2008; Accepted June 20, 2008

DOI: 10.3892/ijo_00000164

Abstract. *PIK3CA* encodes the p110α catalytic subunit of PI 3-kinase, which regulates signaling pathways important for neoplasia, cell proliferation and apoptosis. Somatic mutations in this gene have been detected in several solid human tumors. We investigated these mutations in cervical carcinoma and its precursors, and their association with HPV infection and patient clinical data. The mutations were analyzed using post-PCR direct genomic DNA sequencing. Samples included 9 cervical cancer cell lines, 184 invasive cervical carcinomas, and 30 cervical neoplasias. Missense mutations of *PIK3CA* were identified in 15/184 (8.15%) invasive cervical carcinomas. One novel mutation G1638C (Q546H) was found. Three mutations were identified in the cervical cancer lines. No mutations were found in the precursors. The difference in mutation frequency between invasive and pre-invavasive lesions was not significant (p=0.1372). In relation to age and HPV, the mutation rate was significantly higher in patients ≥60 years (p=0.001), while the rate of HPV infection was higher in patients ≤60 years (p=0.025). No significant correlation with other clinicopathological data was found. The results suggest that *PIK3CA* mutations are a late event and uncommon in the progression of malignant tumors, but it appears that they facilitate carcinogenesis in older women.

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Key words: cervical carcinoma, *PIK3CA*, mutation, cervical intraepithelial neoplasia, human papillomaviruses

Introduction

Each year, gynecological cancers account for 5.1 million new cancer cases among women worldwide, including ~493,000 cases of cervical cancer that result in 273,000 deaths annually. Thus, cervical cancer is apparently the second most common cancer affecting women worldwide. It mainly affects women in their late 30s and older, with a high incidence rate of 9.4 per 100,000 on average in countries with screening programs. In the USA, the incidence rate in women aged 50 and older is ~14.6-15.4 per 100,000 (1). The etiological role of human papillomavirus (HPV) in cervical cancer has been supported by vast epidemiological evidence and experimental studies (2,3). Several population-based studies of women of all age groups showed that the prevalence of high-risk HPVs is highest among women ≤40 years of age, but lower among women in older age groups (40 vs. 6% in Manchester, 25 vs. 11% in Costa Rica, 20 vs. 8% in Chile) (4-6). Prevalence differed depending on the population studied (7). Therefore, it is possible that the etiology of cervical cancer among older women is caused by genetic mutations acting in synergy with a lower immune response, rather than by infection with high-risk HPV. Biologically, high-risk HPV oncoproteins E6 and E7 can abrogate the pRb and p53 tumor suppressor pathways, and cooperate to immortalize human genital keratinocytes (8).

However, cervical cancer develops in a small proportion of women who have been infected with HPVs, and it commonly arises decades after the initial HPV exposure. These facts suggest that other factors, such as genetic aberrations may contribute to malignant transformation and tumor progression.

The phosphatidylinositol 3-kinase (PI3K)-AKT signaling pathway is involved in the regulation of diverse biological processes, including cell proliferation, survival and cycle progression (9,10). The abnormal activation of this pathway is frequently observed in various types of cancer, leading to aberrant cell cycle progression, altered adhesion and motility,

Table I. Primer sequences and PCR conditions used for *PIK3CA* mutation screening.

Exon	Sequence (5'-3')	Annealing temperature (˚C)	Product length (bp)
1	F1 CTCCACGACCATCATCAGG R1 AAGCCGAAGGTCACAAAGTC	50	278
	F2 CATCAACTTCTTCAAGATGA R2 TAAGCTTTATGGTTATTTGC	52	225
9	F ATCATCTGTGAATCCAGA R TTAGCACTTACCTGTGAC	52	205
20	F1 TGACATTTGAGCAAAGACC R1 GCT CAG TTT TAT CTA AGG CTA	50	236
	F2 ATT GCA TAC ATT CGA AAG A R2 GTGTGGAATCCAGAGTGA	50	195

inhibition of apoptosis, and induction of angiogenesis (11), indicating the importance of the PI3K pathway in carcinogenesis. It has been reported that genetic alterations involving various members along this signaling pathway may lead to its activation. One of them is the *PIK3CA* gene, located at 3q26.3, which encodes the catalytic subunit p110α of class IA phosphatidylinositol 3-kinases (PI3Ks). Gene amplifications, deletions, and recently, missense mutations in the *PIK3CA* gene have been found in many human cancer types (12-16). Mutated p110α proteins proved to be oncogenic when studied *in vitro* and *in vivo* (17,18).

It has been reported that cancer types of the liver, colon, and breast, harbor the most *PIK3CA* mutations with average mutational frequencies of 36, 26, and 25%, respectively, while those of the lung, brain, gastric tract and ovaries are associated with lower frequencies (2-9%) (19). However, in thyroid cancer, *PIK3CA* gene amplification is more frequent than gene mutations, especially in papillary (5%) and in follicular (24%) types of cancer (20). Clinically, patients with *PIK3CA* mutations showed a significantly decreased survival rate, as seen with breast cancer (21).

PIK3CA gene amplification was previously investigated in cervical cancer (22,23). High rates of amplification in the *PIK3CA* gene have been seen in cervical tumors (23), but few studies existed on the genetic mutations of *PIK3CA* in cervical tumors and their precursors. In order to investigate whether *PIK3CA* mutation is commonly involved in the carcinogenesis of cervical cancer, and whether this mutation correlates to clinical parameters and HPV infections, we conducted an analysis of genetic alterations particularly in the *PIK3CA* gene in 9 cervical cancer lines, 184 cervical cancers, and 30 cases of pre-invasive neoplasia of the cervix using direct genomic DNA sequencing.

Materials and methods

Cervical cancer cell lines. Nine cervical cancer cell lines (SiHa, HeLa, C-33A, ME180, SW756, CaSki, MS751, C-4 I and HT-3) were purchased from the American Type Culture Collection (ATCC), and were cultured in growth medium recommended by the ATCC. Reagents for cell culture were purchased from Invitrogen (Lidingö, Sweden).

Tissue and clinical samples. A total of 255 cervical samples were collected from two hospitals, Umeå University Hospital and Karolinska University Hospital Huddinge, Sweden. The samples were identified through the National Cancer Registry linked to the pathological registries of their respective hospitals. Pre-invasive samples were identified through their pathological registries. Cervical cancer staging was determined according to the FIGO system. The diagnoses and samples were kept in the archives of the respective hospitals, and clinical data and samples were obtained with the approval from the local ethics boards.

Samples from 24 patients with cervical intraepithelial neoplasia (CIN), including 7 CIN1, 10 CIN2, 7 CIN3 (4 of which were carcinoma *in-situ*, CIS), and 94 samples of squamous cell carcinoma of the cervix were collected from Umeå. An additional 24 samples of squamous cell carcinoma, 107 of adenocarcinoma and 6 of adenocarcinoma *in-situ* (AIS) of the cervix were collected from Huddinge. These samples represented three cohorts (3,24,25) and were all in paraffin-embedded tissue blocks. All histological diagnoses were reviewed by at least one pathologist. Twenty samples were subsequently excluded due to the inadequate quality of the paraffin sections, leaving a total of 235 samples for mutation analysis.

DNA extraction from paraffin sections. Three to four 5 μm tissue sections were obtained from each paraffin-embedded tissue block as described earlier (24). Knives were replaced and an empty paraffin block was sectioned in between tissue blocks to avoid cross-contamination. Each tumor used in this study was determined to contain at least 70% tumor cells as assessed by histopathological examination. Genomic DNA was extracted using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany), following the manufacturer's instructions for paraffin-embedded tissue and culture cell lines.

Mutational and sequencing analysis. Genomic DNA was evaluated for the presence of *PIK3CA* mutations using the PCR amplification of target sequences, followed by direct sequencing. Primers were designed as in the previous study (13) (Table I). PCR was carried out in 50 μl of reaction mixture containing 20-100 ng of genomic DNA, 0.2 μMol/l of each primer, 200 μMol/l deoxynucleotide triphosphates,

1.5-2.0 mMol/l $MgCl_2$, 1X Taq Gold buffer and 0.2 units of Taq Gold DNA polymerase. After an initial denaturation step at 95˚C for 10 min, 40 cycles of denaturation (45 sec at 94˚C), annealing (45 sec at appropriate temperature), and extension (45 sec at 72˚C) were performed. This was followed by a final extension step of 72˚C for 5 min. PCR products were run on 2% agarose gels to verify the adequacy of the PCR reaction. The PCR products were then purified using the EXOSAP-IT method (USB Europe GmbH, Staufen, Germany). Direct sequencing was performed with an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA) using the BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. Any samples suspected of genetic alteration were subsequently sequenced in the reverse direction using the reverse PCR primers. The positive samples were confirmed by repeated PCR and sequencing using the same primers and conditions.

HPV detection and typing. In brief, the Umeå samples were treated as previously reported (3,24). The samples obtained from Huddinge were treated as reported (25). DNA extracted from the CaSki cervical cancer cell line which contained the HPV 16 genome was used as a positive control.

HPV was detected by a two-step nested PCR with MY09/11 as outer primers, which generated PCR products as templates for the inner primers, $GP5^+/6^+$. The 50 μl PCR reaction volume contained 1X AmpliTaq PCR buffer, 10 μM of each primer, 2 mM $MgCl_2$, 0.2 mM of each dATP, dCTP, dGTP, dTTP (Roche Diagnostics), 5 μl of 2% BSA and 1.5 units of Taq DNA polymerase (Promega, Madison, WI, USA). The PCR reaction mixture was first pre-incubated at 96˚C for 1 min to denature the genomic DNA. This was followed by 40 cycles of denaturation for 30 sec at 94˚C and annealing at 50˚C for MY09/11 or 40˚C for $GP5^+/6^+$ for 30 sec, and an extension at 72˚C for 45 sec. A final extension step was carried out at 72˚C for 5 min. Positive controls included DNA extracted from the CaSki cervical cancer cell line containing the HPV 16 genome. CaSki cells were used as positive controls and DNA free water was included in each PCR run to exclude eventual risk of contamination. HPV PCR products were purified with Qiaquick PCR purification kit (Qiagen, GmbH, Germany). Purified PCR products generated by HPV primers were typed by direct sequencing using ABI 310 (Applied Biosystems). The quality of extracted DNA samples was assessed by PCR using S14 as a control gene (primers: forward, 5'-TCGAAAGGGGAAGGAAAAGA-3' and reverse, 5'-CAGTGACATGGACAAAAGTG-3') (3).

Statistical analysis. The Chi-square test or Fisher's exact test were used to evaluate the possible association between *PIK3CA* mutations, HPV infections and clinicopathological characteristics. The t-test was used to compare the mean age of patients in the different groups. Kaplan-Meier analysis and the log-rank test were used to evaluate differences in survival among patient groups. $P<0.05$ was defined as being statistically significant.

Results

PIK3CA mutation identified in cervical cancer lines. Recent large-scale analyses of *PIK3CA* mutations in different tumor types revealed that ≥85% of the mutations were clustered within the p85 binding domain (exon 1), the helical domain (exon 9), and the kinase domain (exon 20) (12). Thus only exons 1, 9, and 20 of the *PIK3CA* gene were sequenced in these specimens.

We investigated the genomic DNA of 9 cervical cancer cell lines by direct sequencing of PCR products. Among the 9 cervical cancer cell lines, 3 (33.3%) mutations were detected. Two of the three mutations were the same and identified in exon 9 (G1633A, E545K) in CaSki and ME180, one of the most commonly reported hotspot mutations. The HPV negative cervical cancer cell line C-33A harbored the G263A (R88Q) mutation in exon 1, which has been reported in colon cancer. No mutation of exon 20 was detected in any of the 9 cervical cancer cell lines.

PIK3CA mutation in cervical cancer types and pre-invasive cervical neoplasia. To verify whether these findings can be found in clinical samples, we examined a total of 235 samples from cervical lesions, including invasive cancers and pre-invasive neoplasia (CIN and CIS). Among them, 214 cervical samples (comprising of 84 samples of invasive squamous cell carcinomas, 100 of invasive adenocarcinomas and 30 of pre-invasive neoplasia) were successfully sequenced for all three exons in the *PIK3CA* gene. The remaining 21 samples were excluded from this study due to failure of amplification in the PCR products or because the sequences obtained were suboptimal for evaluation. Missense mutations of *PIK3CA* were identified in 5 of the 84 (5.95%) squamous cell cancers and 10 of the 100 (10%) adenocarcinomas, resulting in an overall mutation rate of 8.15% in invasive cervical cancer specimens. No mutation was detected in the 30 samples of pre-invasive neoplastic lesions, but when compared with invasive cancer the difference was not statistically significant ($p=0.1372$). In total, 10 of the 15 mutations found in our material have been previously reported as hotspot mutations (exon 20:H1047R; exon 9:E542K and E545K) which had also been found in other tumors. Of these 10, 73.3% (11/15) were clustered within the helical domain (exon 9), especially the G1633A (E545K) mutation, which accounts for 46.7% of all mutations found in invasive cancer specimens. However, we found a novel mutation, G1638C (Q546H) identified in exon 9 in an adenocarcinoma (Fig. 1, Table II), which has not been reported before. Paraffin sections representing only normal cervical tissue from the same patients were also analyzed, and no mutations were detected. Thus the results of our study suggest that these *PIK3CA* mutations are somatic in origin rather than germline. The specific nucleotide change, the corresponding amino substitution, and the clinical data for each patient are summarized in Table II.

PIK3CA mutation and clinicopathological features in invasive cervical cancer types. Of the 184 invasive carcinomas, 104 were in stage I, 22 in stage II, 17 in stage III, and 1 was in stage IV. Forty samples were uncharacteristic in terms of their pathological stage. Regarding histological differentiation, 49 invasive carcinomas were well differentiated, 88 moderately differentiated, and 40 poorly differentiated tumors, leaving 7 samples with an ambiguous histological grade. No correlations were observed between the presence of the *PIK3CA* mutation and the tumor stage, histological grade, or subtype

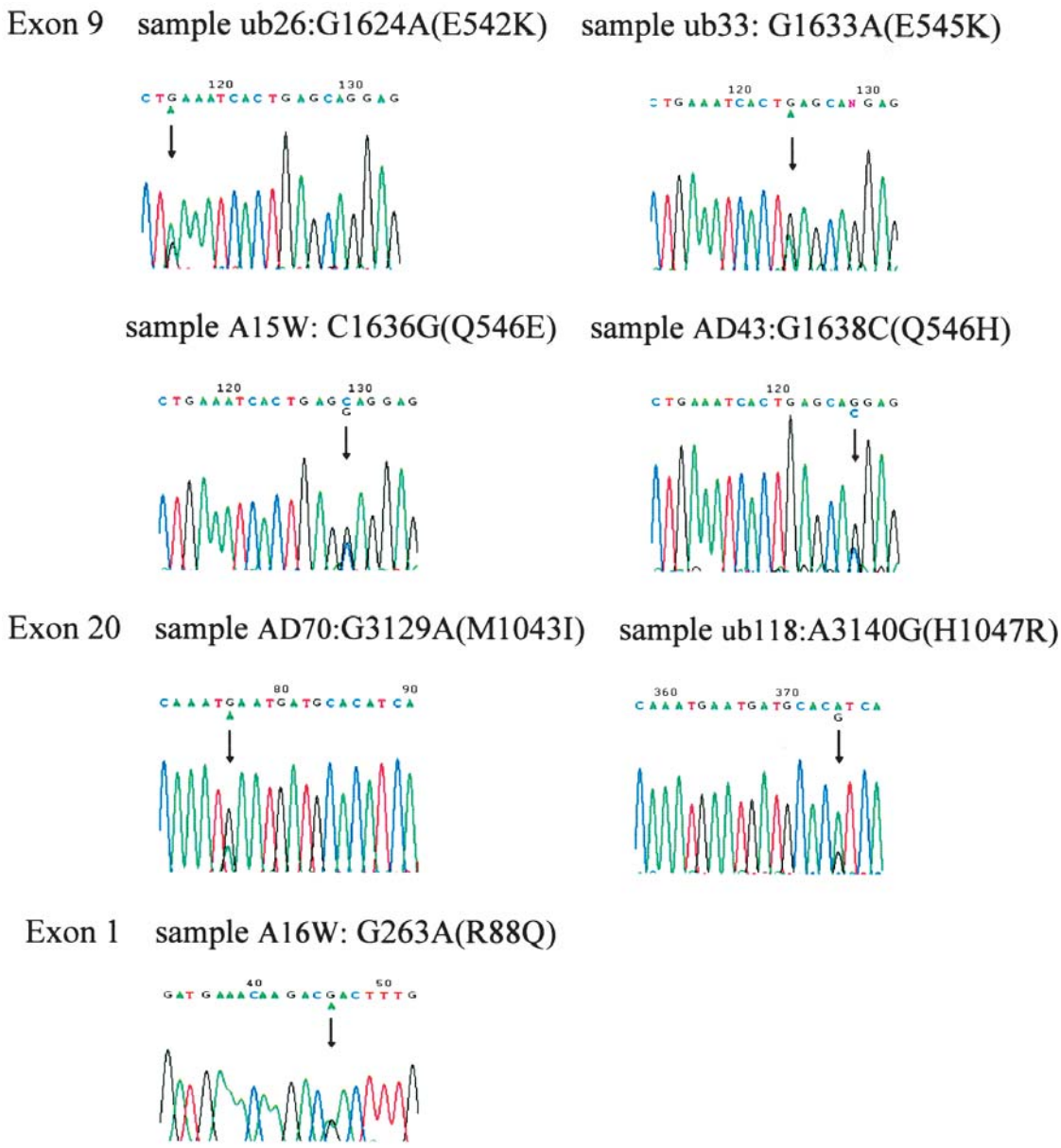

Figure 1. Mutations of *PIK3CA* found in cervical cancer. Arrows indicate the location of missense mutations. The altered nucleotide and the amino acid substitution are indicated above each sequencing trace.

of cervical cancers. Results of the clinicopathological features of the *PIK3CA* mutation in these cervical cancer types are listed in Table III.

A total of 150 patients had survival data available, among which 14 mutations were found. Their mean follow-up time was 89.03±56.28 months (range 3-270). The prognostic significance of *PIK3CA* was evaluated using the Kaplan-Meier analysis. The results showed no significant difference in survival time between patients who harbored the *PIK3CA* mutation and those who did not (p=0.8256) (Fig. 2). Similarly there was no significant difference in the analysis of a subgroup of older patients (>60 years). However, when correlating the *PIK3CA* mutation rates to the age of cancer onset in patients with invasive cervical cancer, a significant difference was found. The mean age (mean ± SD) of patients with mutations was 61.13±12.93 years and that of patients without was 49.03±13.9 years (p=0.0014). When stratified according to age, the mutation rate was significantly higher among patients ≥60 years (18.87 vs. 3.88%, p=0.001) (Table III).

Correlation between HPV infection and PIK3CA mutation. HPV has an established etiological role in cervical carcinoma. We aimed to investigate whether there is any correlation between HPV infection and the *PIK3CA* mutation. HPV analysis was previously performed on most of our samples (3,25), except for 24 samples on which HPV analysis was performed in our laboratory using the method described by Wallin *et al* (3). HPV data (clearly positive or negative) were available for 179 samples, but not for 5 samples. The baseline prevalence of HPV infection in our squamous cell carcinoma samples was 84.15% and in adenocarcinoma 68.37%. The rate of HPV infection was found to be higher among patients <60 years, compared with those ≥60 years

Table II. Clinical data of cervical cancer patients with *PIK3CA* mutation.

Patient	Age	Histotype	Stage	Differentiation	Exon	Substitution	Amino acid	HPV types
A16W	68	AD	IIB	Poor	1	G263A	R88Q	HPV-
Ub26	67	SQ	IIIA	Poor	9	G1624A	E542K	HPV16
AD69	78	AD	NA	Poor	9	G1624A	E542K	HPV16
Ub49	46	SQ	NA	NA	9	G1633A	E545K	HPV16
Ub33	75	SQ	IIIB	Moderate	9	G1633A	E545K	HPV16
1 02	69	SQ	NA	Poor	9	G1633A	E545K	HPV 33
AD19	34	AD	IB	Moderate	9	G1633A	E545K	HPV16
AD39	64	AD	IB	Well	9	G1633A	E545K	HPV16
AD54	45	AD	IB	Moderate	9	G1633A	E545K	HPV16
A19G	50	AD	IB	Well	9	G1633A	E545K	HPV16
A15W	63	AD	IIB	Moderate	9	C1636G	Q546E	HPV-
AD43	69	AD	NA	Moderate	9	G1638C[a]	Q546H	HPV16
Ub118	74	AD	IB	Moderate	20	A3140G	H1047R	HPV16
AD70	70	AD	IB	Moderate	20	G3129A	M1043I	HPV16
Ub84	49	SQ	IB	Well	20	G3129A	M1043I	HPV-

Tumors with *PIK3CA* missense mutations: 18 (AD 10, SQ 5 and 3 cell lines). Percentage of mutations: AD, 10.00 (10/100); SQ, 5.95 (5/84) and cervical cancer line 33.3 (3/9). NA, not available; AD, adenocarcinoma and SQ, squamous cell carcinoma. [a]Novel mutation.

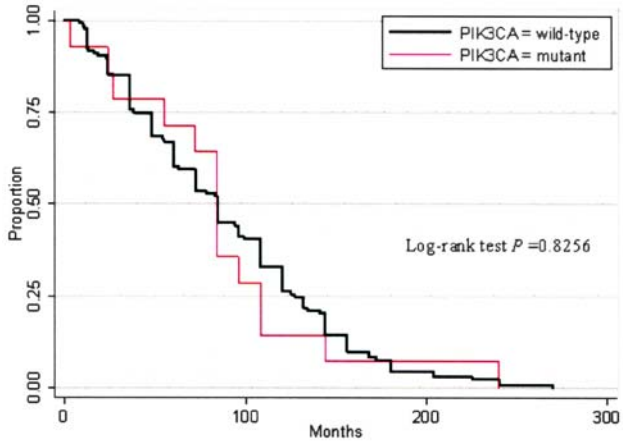

Figure 2. Kaplan-Meier survival analysis for invasive cervical patients with and without *PIK3CA* mutation.

(80.62 vs. 62.75%, p=0.012), in contrast to the age of patients with *PIK3CA* mutations (see previously). No statistically significant association was found between HPV infection and *PIK3CA* mutations in cervical cancer, indicating that the *PIK3CA* mutation and HPV may each act independently as oncogenic factors (Table IV).

Discussion

Previous studies showed that the frequency of *PIK3CA* mutation varied among different cancer types. *PIK3CA* mutation is more common in colon cancer (12), breast cancer (14,26-28), hepatocellular carcinomas (29), and endometrial carcinoma (30) with mutation rates ~20-40%. However, it is less frequent in other cancer types, such as ovarian (15), bladder (31), head and neck squamous cell cancer (16), and is rare in lung cancer (12,32). The presence of *PI3KCA* mutations is less well documented in cervical carcinoma. The rate of mutation in cervical cancer in this study is similar to that of head and neck squamous cell cancer, in which the mutation rate is 7.4% (33). The mutation rate of *PIK3CA* in this study is similar to that found in other solid tumors, though the actual mutation rate may have been underestimated since we only studied three exons.

Previous studies showed that H1047R is the most common mutation in breast, gastric and endometrial cancer (29,30). Our study showed that E545K is the most common mutation among the exons examined in cervical cancer tissue (46.7%, 7/15) and the cervical cancer cell lines (2/3). Ten of the 15 mutations in invasive cervical cancer have previously been reported as hotspot mutations (i.e., H1047R, E542K and E545K). G1638C (Q546H) is a novel mutation identified in exon 9 and has not previously been described in human cancers. One mutation, Q546E, identified in a cervical cancer sample, has been reported in 1 breast cancer (28). As Q546K and Q546L have also been found in other cancers (12,33), the residue glutamine[546] appears to be an unstable site and may be another hotspot. Of the 18 mutations identified in cervical cancer and the cervical cancer cell lines in this study, 72.2% (13/18) of mutations were clustered in the helical domain (exon 9). This strongly suggests that relative to the exon 20 mutations, exon 9 mutations dominate in cervical and colorectal cancer (12), in contrast to breast and endometrial cancer, where exon 20 mutations dominate (27,30).

No *PIK3CA* mutation was detected in any CIN and AIS lesions, while the occurrence of *PIK3CA* mutation is present in the early as well as in the late stage of invasive tumors. Thus, *PIK3CA* mutation may be associated with tumor invasiveness and the mutation may play an important role in

Table III. Correlations between PIK3CA mutations and clinicopathological characteristics of patients with cervical cancer.

	No.	61.13±12.93	49.03±13.95	0.001
Mean age (years)				
≤60	129	5	124	0.001
>60	53	10	43	
Unknown	2			
Pathological subtype				
Squamous	84	5	79	0.42
Adeno	100	10	90	
Clinical stage				
Early (I)	104	7	97	0.499
Late (II-IV)	40	4	36	
Unknown	40	4	36	
Differentiation				
Well	49	3	46	0.745
Moderate	88	8	80	
Poor	40	4	36	
Unknown	7			
HPV				
Negative	44	3	41	0.676
Positive	136	12	124	
Unknown	4		4	

Table IV. Correlations between HPV infection, *PIK3CA*, histological subtype, and patient age.

	No.	HPV positive	HPV negative	P-value
Mean age (years)		48.4	54.5	0.0146
≤60	126	101.0	25.0	0.043
>60	53	35.0	18.0	
Unknown	5			
Pathological subtype				
Squamous	82	69.0	13.0	0.0141
Adeno	98	67.0	31.0	
Unknown	4			
PIK3CA mutation				
Yes	15	12.0	3.0	0.676
No	165	124.0	41.0	

the progression from severe pre-invasive to invasive cancer. It can be speculated that mutation in the *PIK3CA* gene may give a selective advantage to certain clones of transformed cells. Since this is not a prospective study, it cannot be ascertained as to whether these *PIK3CA* mutation-negative pre-invasive neoplasias will progress or regress.

Our study showed no correlation between *PIK3CA* mutation and HPV infection. In this study there were 5 CIN3/CIS and 6 AIS samples with a high prevalence of HPV. However, no *PIK3CA* mutation was found in any of these samples. The incidence of HPV among samples with and without *PIK3CA* is similar among invasive carcinomas, compared with pre-invasive neoplasia. This suggests that *PIK3CA* mutation rather than HPV is associated with invasion. We also found 3 samples with *PIK3CA* mutations among HPV-negative samples, which further suggests that these two mechanisms are independent. A few studies have reported on the *PIK3CA* mutation status in precancerous

lesions of other cancer types. Hayes *et al* reported 2 mutations in 29 (7%) specimens of uterine complex atypical hyperplasia, which is the precursor lesion of endometrial carcinoma (34), and this mutation rate is much lower than that found among endometrial carcinomas (39%) analyzed in the study. They also concluded that *PIK3CA* mutation may promote invasion in endometrial tumorigenesis. Approximately 20% of our samples without any detectable HPV were negative for *PIK3CA* mutations, suggesting that other mechanisms of carcinogenesis may exist beyond the scope of this study.

Samuels *et al* analyzed sequences of all coding exons of *PIK3CA* in 234 colorectal cancers and revealed mutations in a total of 74 tumors (32%). However, they found no evidence of *PIK3CA* gene amplification in 96 colorectal cancers, suggesting that mutation but not amplification is a common mechanism of PI3K activation in this tumor type (12). Another study on breast cancer drew a similar conclusion (14). In contrast with these cancer types, low level amplification of *PIK3CA* was common in cervical cancer (23). Earlier studies showed that the 3q26 chromosome region was amplified by CGH studies (22), and proposed that *PIK3CA* may act as an oncogene in cervical cancer. A recent study showed high-level amplification rates (70%) in 40 cervical neoplasia specimens using quantitative real-time PCR (23). However, few studies have evaluated the mutation status of the *PIK3CA* gene in cervical cancer. Our study reported a *PIK3CA* incidence of mutation of 8.15% in invasive cervical cancer. Even though the mutation rate of *PIK3CA* was not high, we can verify that gene amplification is not the only mechanism that activates the PI3K pathway in cervical cancer.

A large-scale *PIK3CA* mutational analysis of 292 primary breast tumors shows a significant correlation with *PIK3CA* mutations to lymph node metastasis, hormone receptor and ERBB2 positivity, and an inverse relationship with functional PTEN (27). Some studies have recognized an association between *PIK3CA* mutations and the histological subtype (28,35). The mutation rates of invasive squamous cancer and adenocarcinoma in our material were 5.95% and 10%, respectively. Though the adenocarcinoma mutation rate was higher, the difference was not statistically significant. Nor did we find any correlation between *PIK3CA* mutations and tumor stage or histological grade. We found a significant difference between the age of patients with and without mutations. Furthermore, HPV infections are more prevalent among younger patients while *PIK3CA* mutations are more commonly found in older patients diagnosed with invasive cervical cancer. This has been postulated in the past, but seldom reported. This phenomenon may reflect differing mechanisms of carcinogenesis between older and younger patients with cervical cancer. *PIK3CA* mutation may be more of a risk factor for older women than younger women.

In conclusion, this study focuses on the rate of *PIK3CA* mutation in cervical cancers of the two histological types (squamous and adenocarcinomas). Concerning the cancer incidence rate in Sweden, our sample sizes are representative of the two tumor types. Cervical squamous cell carcinomas are strongly associated with high-risk HPV infections. However, HPV DNA has not been identified in the same proportion in adenocarcinoma of the cervix. Factors other than sexually-transmitted HPV are therefore potentially responsible. *PIK3CA* mutations have been suggested to be an oncogene in gynecological cancers. However, oncogenic HPV and *PIK3CA* mutations may be involved in the progression to cervical carcinomas, but may act independently of each other. In this study, *PIK3CA* mutations are more frequently found in the tumors of older than younger women and may therefore be an additional risk factor for older women. The lower immune response in older women may also mean that their immunosurveillance of either HPV infection or cellular transformation is less robust and therefore predisposes them to carcinogenesis. It can be assumed that the two factors may occur simultaneously and that the biological process is complicated by the variation of the human genome, the influence of genetic and environmental factors in different geographical regions, as well as by an uneven distribution of the varieties of HPV worldwide.

Acknowledgments

We are grateful to Joakim Dillner, Xiangning Zhang and Weng-Onn Lui for scientific comments and discussions. This study was supported by the Swedish Cancer Society, Stockholm County Council, the Swedish Medical Research Council, the Swedish Cancer Foundation and a grant (30400475) from the National Natural Science Foundation of P.R. China.

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