Mapping common deleted regions on 5p15 in cervical carcinoma and their occurrence in precancerous lesions

Hugo Arias-Pulido1,3,4, Gopeshwar Narayan1, Hernan Vargas3, Mahesh Mansukhani1 and Vundavalli VVS Murty*1,2

Address: 1Department of Pathology, College of Physicians & Surgeons of Columbia University, 630 West 168th Street, New York, 10032, USA, 2Institute for Cancer Genetics, College of Physicians & Surgeons of Columbia University, 630 West 168th Street, New York, 10032, USA, 3Department of Tumor Molecular Biology, Instituto Nacional de Cancerología, Cl. 1 No 9-85, Bogotá, Colombia and 4Department of Molecular Genetics and Microbiology, BRF 123, School of Medicine, 915 Camino de Salud, NE., Albuquerque, NM, 87131, USA

E-mail: Hugo Arias-Pulido - harias59@yahoo.com; Gopeshwar Narayan - gn110@columbia.edu; Hernan Vargas - hvargas76@yahoo.es; Mahesh Mansukhani - mm322@columbia.edu; Vundavalli VVS Murty* - vvm2@columbia.edu

*Corresponding author

Published: 1 October 2002
Received: 26 September 2002
Accepted: 1 October 2002

This article is available from: http://www.molecular-cancer.com/content/1/1/3
© 2002 Arias-Pulido et al; licensee BioMed Central Ltd. This article is published in Open Access: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article’s original URL.

Keywords: Cervical carcinoma, cervical intraepithelial neoplasia, chromosome 5p, loss of heterozygosity, deletion mapping, human papilloma virus

Abstract

Background: Previous studies have shown that the short arm of chromosome 5 (5p) exhibit frequent genetic changes in invasive cervical carcinoma (CC), and that these changes arise early during the carcinogenesis, in precancerous lesions. These data therefore suggest that loss of candidate tumor suppressor genes located on 5p is associated with the development of CC. However, the precise location of 5p deletions is not known.

Results: We performed a detailed deletion mapping of 5p in 60 cases of invasive CC. We found that 60% of the tumors exhibit a 5p loss of heterozygosity (LOH). The patterns of LOH allowed us to identify two minimal regions of deletions, one at 5p15.3 spanning a 5.5 cM genetic distance and a second site of 7 cM at 5p15.2-15.3. In addition, we also identified 5p deletions in 16% lesions of high-grade cervical intraepithelial neoplasia (CIN). 5p LOH was found in 63% of HPV 16 positive tumors, while only 33% tumors with other HPV-types had 5p LOH. The differences in frequency of 5p LOH between tumors harboring HPV16 in combination with other HPV types and tumors harboring HPV16 DNA alone were significantly higher, suggesting a synergistic effect of high-risk types in causing genomic instability.

Conclusion: These findings implicate the presence of tumor suppressor gene(s) on 5p relevant to CC tumorigenesis.

Background

Converging points of evidence implicate infection by high-risk human papilloma virus (HPV) types as a critical etiologic factor in cervical tumorigenesis [1]. Epidemiological and experimental data, however, show that only a small fraction of HPV-infected cervical intraepithelial neoplastic (CIN) lesions progress to invasive cervical carcinoma (CC) [2]. These findings suggest somatic genetic
mutations play a critical role in the initiation and progression of CC. Delineation of these genetic changes is crucial to understanding the molecular basis of CC. A number of genetic changes have been identified so far in invasive CC. However, the precise mechanism of each of these genetic alterations in the multi-step pathway of cervical tumor development is largely unknown. The development of CC is preceded by distinct morphological changes from normal epithelium to carcinoma through low-grade and high-grade squamous intraepithelial lesions (SILs). The genetic basis of this progression is also poorly understood.

Cytogenetic and molecular genetic analyses have identified the short arm of chromosome 5, 5p, as frequently affected by duplications and deletions in a high proportion of CCs [3–6]. These findings suggest the presence of positively regulated amplified oncogenes and negatively regulated tumor suppressor genes (TSGs) on this chromosomal arm. Comparative genomic hybridization (CGH), a molecular cytogenetic method that identifies chromosomal gains and losses, and loss of heterozygosity (LOH) studies have also demonstrated recurrent deletions on the 5p in precancerous lesions [7–10]. Although, these studies have identified 5p as a commonly affected region, the precise site of deletion that harbor putative TSG is unknown because of the lack of systematic and detailed deletion mapping studies.

To further characterize the 5p region in CC, we performed a high-resolution deletion mapping in invasive cancer and found that 60% of patients exhibit LOH. We identified two sites of minimal deletions at 5p15.3 and 5p15.2-15.3 spanning 5.5 cM and 7 cM genetic distance, respectively. We have also shown that the 5p15 region deletions occur in 16% of high-grade CIN lesions. Here, we have identified two sites of candidate tumor suppressor genes and confirmed the previous reports that the 5p deletions occur in a small proportion of precancerous lesions.

**Figure 1**
Identification of minimal deleted regions by high-resolution LOH mapping on 5p in invasive CC. Patterns of LOH. Thirty-six tumors with 5p LOH are shown. A G-banded ideogram of chromosome 5 is shown on top. Thick horizontal line shown below the ideogram represents the corresponding chromosomal region (scaled only approximately). Small vertical lines show STRP markers on the thick horizontal line and their corresponding genetic map distances. The percentage of LOH is shown in horizontal box below each STRP marker. The patterns of LOH are shown below. A horizontal line represents each tumor; Marker region with LOH is shown by light shaded lines, and dark shaded lines show the retention of heterozygosity. Tumor numbers are shown on left of horizontal lines. The thick light shaded line denoted on left with # represents 17 tumors that showed LOH of the entire 5p. Thick black rectangles at the bottom indicate region of minimal deletion.
Results

Identification of two regions of minimal deletions at 5p15.2-p15.3

Analysis of LOH in 60 invasive CCs using 20 STRP markers mapped to 5p showed deletions in 36 of 60 (60%) tumors in at least one marker (Table 1). Of the 36 tumors with LOH, 17 (47.2%) had LOH at all the informative markers suggesting genetic monosomy of 5p (Fig. 1). The patterns of LOH in the remaining 19 tumors that exhibited regional losses on 5p were used to map the common deleted regions (Fig. 1). This has identified two minimal deletions at 5p15.3 and 5p15.2-15.3 (Fig. 1 & Fig. 2). All the tumors that defined the minimal deletions showed more than 60% reduction in signal intensity in the deleted alleles.

The distal 5p15.3 deletion spans the telomeric region encompassing D5S392, D5S678, and D5S417 markers in 32 tumors. Although two tumors (T-13 and T-49) showed deletions of terminal marker D5S392 alone at 0 cm genetic distance, which is the distal most marker on 5p, we considered the deletion boundaries defined by the proximal marker D5S417 in tumors T-6 and T-105 (Fig. 1 & Fig. 2). The second and proximal region of deletion spans 6 markers (D5S406, GATA82H02, D5S1455, D5S2054, D5S635 and D5S676), and was deleted in 28 tumors. The deletion boundaries were defined proximally by the tumors T-59 and T-66, while the distal boundary was identified by the tumors T-59 and T-114 (Fig. 1 & Fig. 2). All tumors that showed 5p deletions fell within the two defined regions of deletions, while 25 tumors exhibited deletions at both regions. Thus, this analysis identified two discrete and closely mapped sites of minimal deletions at 5p15.3 and 5p15.2-15.3, which were restricted to 5.5 cm and 7 cm genetic distance, respectively.

Gene expression analysis of candidate tumor suppressor genes in CC cell lines

In an effort to identify the genes inactivated in the recurrent 5p deletions in CC, we identified candidate genes with tumor suppressor function. Three genes were identified at the terminal 5p15.3 deletion. These include, PDCD6, a protein that plays a role in T cell receptor-, FAS-, and glucocorticoid-induced cell death [11]; TERT, a gene that encodes a reverse transcriptase component of telomerase, dysfunction of which promotes chromosomal instability [12]; and TRIP13, a thyroid hormone receptor interacting protein that binds with the human papillomavirus type 16 (HPV16) E1 protein [13]. We have identified POLS gene that encodes a DNA polymerase sigma, and plays a role in DNA replication and sister chromatid cohesion at the proximal minimal deleted region [14]. We performed a semi-quantitative RT-PCR analysis of all four genes (PDCD6, TERT, TRIP13, and POLS) in eight CC cell lines and did not find evidence for lack of or down regulated expression compared to normal cervix (Fig. 3).

5p deletions in relation to HPV

5p deletions did not show any significant differences with age at diagnosis and stage of the tumor (data not shown). Fifty-eight (96.7%) of the 60 tumors studied were positive for HPV DNA. The remaining two tumors were HPV-neg-

Table 1: Frequency of LOH on 5p in cervical carcinoma

| Chromosome Band | Locus | Genetic map position (in cM) | No. studied/ Informative | LOH (%) |
|-----------------|-------|-----------------------------|-------------------------|---------|
| 5p15.3          | D5S392| 0.0                         | 60/39                   | 21 (53.8) |
|                 | D5S678| 0.0                         | 59/27                   | 11 (40.7) |
|                 | D5S417| 5.5                         | 59/28                   | 15 (53.6) |
|                 | D5S405| 8.2                         | 58/29                   | 11 (37.9) |
|                 | D5S2088| 8.2                        | 59/48                   | 20 (41.7) |
|                 | D5S406| 10.7                        | 60/39                   | 17 (43.6) |
|                 | GATA82H02| 11.0                       | 60/42                   | 21 (50.0) |
| 5p15.2          | D5S1455| 13.0                       | 60/38                   | 20 (52.6) |
|                 | D5S2054| 13.2                       | 60/37                   | 17 (45.9) |
|                 | D5S635| 13.8                       | 60/40                   | 18 (45.0) |
|                 | D5S676| 15.6                       | 57/29                   | 11 (37.9) |
|                 | D5S1957| 17.7                       | 59/42                   | 17 (40.5) |
|                 | D5S208| 18.0                       | 60/40                   | 13 (32.5) |
|                 | D5S630| 18.6                       | 60/46                   | 17 (37.0) |
| 5p14            | D5S432| 21.4                       | 58/42                   | 18 (38.1) |
|                 | D5S117| 22.0                       | 58/37                   | 13 (35.1) |
|                 | D5S416| 27.9                       | 59/46                   | 18 (39.1) |
|                 | D5S486| 31.0                       | 57/40                   | 13 (32.5) |
| 5p13.2          | D5S419| 39.5                       | 60/48                   | 18 (37.5) |
| 5p11-12         | D5S426| 51.6                       | 60/41                   | 13 (31.7) |
ative. HPV 16 alone or in combination with other HPV types was found in 46 tumors, while 12 tumors had other HPV types including HPV 18. 5p LOH was found in 29 of 46 (63%) HPV 16 positive tumors, while only 4 of 12 (33%) tumors with other HPV-types had 5p LOH. The two tumors that did not harbor HPV infection also had LOH at 5p. These differences were found to be statistically not significant (Chi square 4.93; p = 0.10). However, when the analysis was restricted to HPV16 harboring tumors, it was found that the patients harboring HPV16 DNA alone had 5p LOH in 20 of 36 (55.6%) tumors, while the patients infected with HPV16 in combination with other HPV types exhibited 9 of 10 (90%) LOH. This difference was found to be statistically significant (Chi square, 8.75; p = 0.05).

5p deletions occur at low frequency in precancerous lesions
To understand the role of 5p deletions in cervical cancer progression, we evaluated LOH in 26 microdissected CIN specimens (25 high-grade CINs and one low-grade CIN) using four STRP markers (Table 2). Four of the 25 informative tumors had LOH at one or more markers. One low-grade CIN did not show LOH. Thus, deletions at 5p identified in high-grade CINs suggest that these genetic alterations occur prior to development of invasive carcinoma.

Discussion
Extensive genetic studies have documented frequent alterations affecting various chromosomal regions in CC. LOH and CGH analyses have consistently shown frequent loss of genomic material in a number of chromosomal regions...
suggested the presence of potential tumor suppressor genes at these sites [4,6,15,16]. Of these, chromosome 5p deletions have been noted to be very frequent in invasive CC [5–7]. However, the locations of exact regions of LOH are not known because of a lack of systematic and high-resolution deletion mapping of the 5p region in CC. We have previously identified that markers mapped to 5p15 regions are more frequently deleted [4,7]. To further characterize the 5p deletions in CC, we performed a high-density LOH mapping in the present study. We found high frequency of LOH and identified two minimal regions of deletions at 5p15.3 and 5p15.2-15.3.

The 5p15.3 minimal deletion spans between 0–5.5 cM genetic distance. Consistent with this pattern, the markers DSS392 and DSS417 exhibited the highest frequency (53.8% and 53.6%, respectively) of LOH among all the tested loci. The genomic size of the 5p15.3 deletion interval is approximately 3.2 Mb. A database search has identified three candidate genes, PDCD6, TERT, and TRIP13, with suggestive tumor suppressor gene function. The programmed cell death gene, PDCD6, is a member of the family of intracellular Ca2+ binding proteins and a part of the apoptotic machinery controlled by T-cell receptor (TCR), Fas, and glucocorticoid signals [11,17]. TERT encodes a reverse transcriptase required for the replication of chromosome termini and plays a role in telomere elongation. Although telomerase expression is a hallmark of cancer, the mice lacking the RNA component of telomerase (mTERC) exhibit progressive telomere shortening and chromosomal instability associated with epithelial tumors [12]. Consistent with the established role of this enzyme in cell immortalization and cancer pathogenesis [18], we did not find expression in normal cervix while it was abundantly present in all eight cell lines studied (Fig. 3). Recently, it has been shown that the hTERT amplification and over expression is common in CC [19]. TRIP13, thyroid receptor interacting protein 13, is a transcription factor that regulates expression of a variety of specific target genes including the human papillomavirus type 16 (HPV16) E1 protein [13]. Similar to TERT, the TRIP13 also showed very low expression in normal cervix and highly elevated levels of expression in CC cell lines (Fig. 3). Presence of these genes at the 5p15.3 interval suggests that they may be target TSGs of these deletions in CC. However, expression analysis of these genes did not provide evidence of down-regulation in CC cell lines.

The second site of minimal deletion at 5p15.2-15.3 between 10.7 and 17.7 cM genetic distance spans approximately 2.5 Mb genomic region. A database search has identified POLS, a topoisomerase-related function protein-4-1, gene that encodes polymerase (DNA directed) sigma within the deleted region. The yeast homolog TRF4 plays a critical role in chromosome segregation by coordinating between DNA replication and sister chromatid cohesion [14]. Analysis of expression of this gene in CC cell lines did not show evidence of down regulation. Although the genes tested so far showed no deregulated expression, it remains to be seen whether any of these genes or the others mapped to the minimal deleted regions are targets of mutations in CC. However, the present study identified two discrete sites of deletions at 5p15.3 and 5p15.2-15.3 that harbor putative TSGs important in CC development providing a basis for their identification.

5p deletions also have been reported in precursor CIN lesions with the frequencies between 14.7 and 42% [7–10]. We have previously shown, in a different geographical population, that 21% of CINs had 5p deletions. Based on the follow-up studies, we suggested that 5p genetic deletions might identify high-risk lesions for progression to invasive cancer [7]. In a more recent study it was also shown that the 5p deletions were significantly more frequent in dysplasias that were recurrent compared to non-recurrent dysplasias [9]. These data therefore suggest that 5p genetic alterations occur early in cervical carcinogenesis and they may be critical to the development of invasive cancer. Supporting previous reports, in the present study, we detected LOH at 5p15 in 16% of the high-grade CINs (Table 2).

Table 2: 5p LOH in cervical precancerous lesions

| Locus       | No. of CINs studied | No. Informative | LOH (%) |
|-------------|---------------------|-----------------|---------|
| DSS2088     | 25                  | 22              | 1 (4.5%)|
| GATA82H02   | 24                  | 17              | 3 (17.6%)|
| DSS1455     | 18                  | 9               | 1 (11.1%)|
| DSS635      | 23                  | 17              | 2 (11.8%)|

The role and mechanisms involved in high-risk HPV induced genomic instability has been of considerable speculation [reviewed in ref. [20]]. High-risk HPV E6 and E7 gene expression can independently immortalize various human cell types in vitro. The E6 gene interacts with p53 and an apoptotic protein BAK, followed by its degradation, which results in chromosomal instability and resistance to apoptosis [21,22]. The E7 protein interacts with pRB followed by its degradation [23]. The p53 and pRb proteins have been shown to play multiple roles in cell growth and in genomic integrity. There have been contradictory reports on targeted 5p deletions in relation to HPV infection [5,24]. Ku and co-workers have shown that 5p deletions occur in significantly high proportion of CC infected with HPV 16 compared to low-risk HPV types [5]. Contradictory to this, another study has shown that 5p deletions are independent of HPV16 and HPV18 infec-
tion [24]. In the present study, we did not find any significant differences in 5p LOH between patients harboring HPV16 and other HPV types. However, we found that patients harboring HPV16 in combination with other HPV types showed significant increase in 5p loss compared to patients harboring HPV16 alone. These data, therefore, suggest that multiple high-risk HPV infections may have synergistic effect in causing genomic instability. Thus, currently it is unclear the role of high-risk HPV types in causing 5p genomic instability. Further studies are needed to address this question.

Conclusions
In summary, the present study confirms our previous observations that genetic loss of 5p is a common feature of invasive CC. Here we identified two common sites of deletions that harbor candidate tumor suppressor genes on this chromosomal arm and showed that multiple infection of high-risk HPV types may cause targeted genomic instability at 5p. In addition, we have shown that the genetic deletions on 5p occur early during the development of CC in high-grade CIN lesions. Our identification of minimal deletions on 5p and LOH in CINs has several potential implications. First, it should facilitate the identification of the target genes of importance in cervical tumorigenesis. Second, it may provide critical genetic markers for identifying cervical intraepithelial neoplasias at high-risk to progression.

Methods
Tumor and normal tissues
We analyzed 60 tumor biopsies consisting of previously untreated primary invasive CCs with the corresponding peripheral blood samples, and 26 formalin-fixed and paraffin-embedded CIN specimens. The tissues were obtained from patients treated at the Instituto Nacional de Cancerologia, Colombia after appropriate informed consent and approval of the protocols by the institutional review board. The invasive tumors were clinically classified as FIGO stage IB (5 tumors), IIB (14 tumors), IIIB (37 tumors), and IV (4 tumors). Histologically, 57 tumors were classified as squamous cell carcinomas and 3 as adenocarcinomas. Twenty-five CIN specimens were classified as high-grade and one as low-grade lesions. Eight CC cell lines SiHa, SW756, C-41, Ca Ski, C-33A, HT-3, MS751 and ME-180 were obtained from the American Type Culture Collection (ATCC, Manassas, VA), and were grown in culture.

Detection of HPV types
The PGMY09/11 L1 consensus HPV L1 primers and reverse line blot hybridization system was used to detect 38 genital HPV types as described earlier [27,28]. Appropriate control experiments were set up using bulk master mix components to eliminate potential contamination. Individual assay sensitivity was analyzed by the use of serial dilutions of SiHa cell line crude cell digest, targeting 10⁴, 10³, 10², 10¹, and 10⁰ input copies of HPV-16. A no template negative control was set up in all experiments.

Analysis of gene expression
Total RNA isolated from the cell lines was reverse transcribed using random primers and the Pro-STAR first strand RT-PCR kit (Stratagene, La Jolla, CA). Semi-quantitative analysis of gene expression was performed by 26 to 28 cycles of multiplex RT-PCR with β-actin as control and gene specific primers spanning at least 2 exons. The gene primers used and their positions in cDNA were:

- **PDCD6-F** 5'-GCTTCCCTGTGAACGTTTCC-3' (108–127 bp)
- **PDCD6-R** 5'-TCTATGATCATCCCGGAG-3' (349–368 bp)
- **TERT-F** 5'-GCGTGGGTTGGGATTTTAC-3' (2647–2666 bp)
- **TERT-R** 5'-CAGGCGCTGCTTCTTACAG-3' (2778–2797 bp)
TRIP13-F 5'-CAGGACACCTGCAAAGAAG-3' (132–151 bp)

TRIP13-R 5'-TGCGAATGCAACGATGCAT-3' (321–340 bp)

POL5-F 5'-CCCTTGTCGGAAGAACGACG-3' (43–62 bp)

POL5-R 5'-AGGACCTTGTGGAACAGCGG-3' (263–282 bp)

The PCR products were run on 1.5%-6% agarose gels, visualized by ethidium bromide staining and quantitated using the Kodak Digital Image Analysis System (Kodak, New Haven, CT).

Authors' contributions

Author 1 (HAP) carried out the molecular genetic analysis of LOH on invasive cancer and performed HPV typing. Author 2 (GN) performed LOH analysis on CIN lesions and gene expression analysis. Author 3 (HV) participated in the collection of tumor material and clinical information. Author 4 (MM) participated in microdissection of CIN lesions. Author 5 (VVVS) has conceived and coordinated the study. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the Elaine B. Lesser Fund from the Herbert Irving Comprehensive Cancer Center, Columbia University to VVVSM and a grant (2101-04-021-99) from Colciencias, Colombia, to HAP.

References

1. Kubbataj MHG, Voudeke KH: Role of E6 and E7 oncoproteins in HPV-induced anogenital malignancies. Sem Viral 1996, 7:295-304.

2. Ostre AG: Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol 1993; 12:186-192.

3. Aktin NB: Significance of chromosome 5 and 17 changes in the development of carcinoma of the cervix uteri. Cytogene Cell Genet 2000; 91:44-46.

4. Mitra AB, Murry WV, Li RG, Pratap M, Luthra UK, Chaganti RS: Allelotype analysis of cervical carcinoma. Cancer Res 1994; 54:4481-4487.

5. Ku WH, Liu IL, Yen MS, Chang Chien CC, Yue CT, Ma YY, Chang SF, Ng HT, Wu CW, Shen CY: Genomic deletion and p53 inactivation in cervical carcinoma. Int J Cancer 1997; 72:270-276.

6. Mullukandov MR, Khodoliev NG, Askin NB, Burk RD, Johnson AB, Klinger HP: Genomic alterations in cervical carcinoma: losses of chromosome heterozygosity and human papilloma virus tumor status. Cancer Res 1996; 56:197-205.

7. Mitra AB, Murry VVVS, Singh V, Li RG, Pratap M, sodhani P, Luthra UK, Chaganti RSK: Genetic alterations at 3p15: A potential marker for progression of precancerous uterine cervical lesions. J Natl Cancer Inst 1995; 87:742-745.

8. Luft F, Gebert J, Schneider A, Melsheimer P, von Knebel DM: Frequent allelic imbalance of tumor suppressor gene loci in cervical dysplasia. Int J Gynecol Pathol 1999; 18:374-380.

9. Lin WM, Michalopulos EA, Dhurandar N, Cheng PC, Robinson W, Ashfaq R, Coleman RL, Muller CY: Allelic loss and microsatellite alterations of chromosome 3p14.2 are more frequent in recurrent cervical dysplasias. Clin Cancer Res 2000; 6:1410-1414.

10. Kirchhoff M, Rose H, Petersen BL, Maahr J, Gerdes T, Philip J, Lundsvein C: Comparative genomic hybridization reveals non-random chromosomal aberrations in early preinvasive cervical lesions. Cancer Genet Cytool 2001; 129:47-51.

11. Viso P, Lacana E, D'Adamio L: Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. Science 1996; 271:521-524.

12. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA: Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 2000, 406:641-645.

13. Yasugi T, Vidal M, Sakai H, Howley PM, Benson JD: Two classes of human papillomavirus type 16 E1 mutants suggest pleiotropic conformational constraints affecting E1 multimerization, E1 interaction, and interaction with cellular proteins. J Virol 1997; 71:5942-5951.

14. Wang Z, Castano IB, de las Penas A, Adams C, Christman MF: Pol kappa: A DNA polymerase required for sister chromatid cohesion. Science 2000, 289:774-777.

15. Rider JS, Kamaraosoa T, Huestein PC, Li L, Li Y, Gerhard DS: Allelo-typing of all chromosomal arms in invasive cervical cancer. Oncogene 1996, 13:2737-2741.

16. Kersemaekers AM, Herman J, Fleuren GJ, van der Vijver MJ: Loss of heterozygosity for defined regions on chromosomes 3, 11 and 17 in carcinomas of the uterine cervix. Br J Cancer 1998, 77:192-200.

17. Jung WS, Kim KS, Kim KD, Lim JS, Kim JW, Kim E: Apoptosis-linked gene 2 binds to the death domain of Fas and dissociates from Fas during Fas-mediated apoptosis in Jurkat cells. Biochem Biophys Res Commun 2001, 288:430-436.

18. Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, van de Vijver MJ: Amplification of the telomerase reverse transcriptase (hTERT) gene in cervical carcinomas. Genes Chromosomes Cancer 2002; 34:269-75.

19. Duensing S, Munger K: Human papillomaviruses and centrosome duplication errors: modeling the origins of genomic instability. Oncogene 2002, 21:6241-6248.

20. Wernesa BA, Levine AJ, Howley PM: Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1999, 284:76-79.

21. Jackson S, Harwood C, Thomas M, Banks L, Storey A: Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev 2000, 14:3065-3073.

22. Dyson N, Howley PM, Munger K, Harlow E: The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 1997, 90:785-795.

23. Zhang A, Zheng C, Hou M, Lindwall C, Wallin KL, Angstrom T, Yang X, Hellstrom AC, Blennow E, Bjorkholm M, Zetterberg A, Gruber A, Xu D: Amplification of the telomerase reverse transcriptase (hTERT) gene in cervical carcinomas. Genes Chromosomes Cancer 2002, 34:269-75.

24. Jackson S, Harwood C, Thomas M, Banks L, Storey A: Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev 2000, 14:3065-3073.

25. Dyson N, Howley PM, Munger K, Harlow E: The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma catalytic subunit gene. Science 1989, 243:934-937.

26. Mitra AB: Genetic deletion and human papillomavirus infection in cervical cancer: loss of heterozygosity sites at 3p and 5p are important genetic events. Int J Cancer 1999, 82:322-324.

27. Pullido HA, Fukrussin MH, Chatterjee A, Esplin ED, Beleño N, Martinez G, Posso H, Evans GA, Murty VVVS. Identification of a 6 cM minimal deletion at 11q23.1-2.2 and exclusion of PPP2R1B gene as a deletion target in cervical cancer. Cancer Res 2000, 60:6677-6682.

28. Chatterjee A, Pullido HA, Koul S, Beleño N, Perilla A, Posso H, Manukhanik M, Murty VVVS. Mapping the Sites of Putative Tumor Suppressor Genes at 6p25 and 6p21.3 in Cervical Carcinoma: Occurrence of Allelic Deletions in Precancerous Lesions. Cancer Res 2001, 61:2119-2123.

29. Gravitt PE, Cayton CL, Apple RJ, Wheeler CM: Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 2001, 39:3020-3027.

30. Peyton CL, Gravitt PE, Hunt WC, Haldrey LS, Zhao M, Appel RJ, Wheeler CM: Determinants of genital human papillomavirus detection in a US population. J Infect Disease 2001, 183:1554-1564.