Int J Gynecol Cancer 2005, **15,** 120–126

Gain of chromosome 3q is an early and consistent genetic aberration in carcinomas of the vulva

P. STOLTZFUS*, K. HESELMEYER-HADDAD†, J. CASTRO*, N. WHITE†, C. SILFVERSWÄRD*, K. SJÖVALL‡, N. EINHORN*, K. TRYGGVASON§, G. AUER*, T. RIED† & B. NORDSTRÖM*
**Department of Oncology and Pathology, Stockholm, Sweden; †Center for Cancer Research, NCI, Bethesda, MD; ‡Department of Obstetrics and Gynecology, Karolinska Hospital and §Karolinska Insitutet, Stockholm, Sweden*

Abstract. Stoltzfus P, Heselmeyer-Haddad K, Castro J, White N, Silfverswärd C, Sjövall K, Einhorn N, Tryggvason K, Auer G, Ried T, Nordström B. Gain of chromosome 3q is an early and consistent genetic aberration in carcinomas of the vulva. *Int J Gynecol Cancer* 2005;**15:**120–126.

The aim was to determine whether specific gains of chromosome 3q and laminin-5γ2-chain expression can improve early detection of invasive capacity in precancerous and squamous cell carcinoma of the vulva (VSCC). Six VSCC and three precancerous lesions were studied. Multicolor fluorescence *in situ* hybridization (FISH) probe sets were applied to nuclei suspensions prepared from archival material using the Hedley method. The probe panel consists of the centromers of chromosome 7, chromosome 3, and the *TERC* gene residing on the long arm of chromosome 3. Laminin-5γ2-chain immunohistochemical analysis was performed on corresponding specimens and was expressed only in the VSCC. The genome-specific FISH analysis revealed 3q amplification in 43% of the nuclei analyzed for the VSCC and 22% of the nuclei for the precancerous lesions. Low-level 3q amplifications were found in precancerous lesions with an average fold increase of 1.15 for 3q. The invasive lesions showed higher average fold increases for 3q, averaging 1.32. Laminin-5γ2-chain protein was expressed only in VSCC, whereas 3q gains were observed both in precancerous lesions and in VSCC, indicating that gain of chromosome 3q is an early and consistent event during carcinogenesis of VSCC.

KEYWORDS: chromosome 3q, laminin-5γ2 chain, VIN, vulvar carcinoma.

Invasive vulva carcinoma accounts for approximately 4% of gynecological cancers[1], and the majority (>90%) are squamous cell carcinomas (SCCs). Two types of SCC of the vulva (VSCC) can be discerned: (a) one type which usually occurs as unifocal lesions in elderly women and (b) the other type of VSCC is observed in younger women arising from persisting multifocal precancerous lesions which might progress to invasive cancer in the lower anogenital tract. The second type is usually connected to human papillomavirus (HPV) infection[2–4], and the incidence is increasing[5,6]. There is an association between the development of vulvar intraepitelial neoplasia (VIN) and cervical intraepitelial neoplasia (CIN)[7].

Early detection is critical for a successful treatment of malignant disease. It can, however, be difficult to differentiate between precancerous and early VSCC using only routine histopathology[8]. Molecular markers would therefore be helpful in stratifying those lesions with a high propensity for progression to invasive carcinoma.

The analysis of VSCC by comparative genomic hybridization (CGH) has revealed a similar distribution of

Address correspondence and reprint requests to: Britta Nordström, MD, PhD, Radiumhemmet, Karolinska Hospital, 171 76 Stockholm, Sweden. Email: brita.nordstrom@karolinska.se

chromosomal imbalances as in cervical SCC, suggesting a common genetic background and similar pathogenic pathways in these two tumor entities[9]. In cervical carcinomas, gain of 3q indicates transition of precancerous cervical lesions to invasive carcinoma[10]. Specific gains of chromosome arm 3q are also found in other SCCs, including tumors of the vagina[11], anus[12], head and neck[13], esophagus[14], lung[15], and ovary[16,17].

Possible candidate genes on chromosome arm 3q include *TERC*, which encodes the RNA component of telemerase[17], and *PIK3CA*, a determinant of cell growth and apoptosis[18]. Genomic copy number increases of 3q are also more frequent in tonsillar cancer and carcinomas of the anogenital region that are HPV positive, suggesting that 3q gain is an important event in oncogenesis of HPV-related tumors[12,19–21].

Increased expression of γ2 chain of the laminin-5 serves as a sensitive marker for tumor invasiveness[22]. The family of laminin proteins are extracellular components of the basement membrane[23]. The γ2 chain of the laminin-5 plays a central function in cell attachment and migration, and overexpression of laminin-5γ2 chain is present in different carcinomas[24], including SCC of the cervix[25], vagina[26], anogenital tract[27], and colorectum[28].

In this study, fluorescence *in situ* hybridization (FISH) was used with multicolored probe sets for the detection of genomic imbalances of chromosome arm 3q and immunohistochemistry with a polyclonal antibody directed against the laminin-5γ2 chain to explore the value of molecular markers for the early diagnosis of VSCC and for the prediction of the progressive potential of premalignant lesions.

Patients and methods

Patients

Nine patients with preinvasive and invasive VSCC were treated and followed during 1983–2003 at the Radiumhemmet and the Department of Obstetrics and Gynecology at the Karolinska Hospital. The clinical records of these patients were evaluated. The histopathological diagnosis was reviewed by two of the authors (CS and BN). The patients included in the study consisted of six patients with invasive VSCC, and two patients had VIN3 lesions. One patient with a vaginal intraepithelial neoplasia (VAIN3) was included as well. Four patients with VSCC presented with unifocal lesions in vulva, and two had multifocal disease. Three of the patients with VSCC had lymph node metastasis. Two of the patients with VIN/VAIN3 lesions had multifocal lesions in the vulva, cervix, or vulva. One patient had a unifocal VIN3 lesion. The three patients with VIN/VAIN3 lesions did not progress to invasive carcinoma (Table 1). The observation time for the patients in the study was 1–20 years (Table 1). One patient was lost to follow-up after 20 years (patient no. 4), and three patients have died from disease (patients no. 6, 7, and 9) (Table 1).

Table 1. Patient characteristics

Patient number	Age at first biopsy (years)	Follow-up (years)	Chronological histopathological changes
1	64	19	1984 CIN3 1984–2002 repeated mult VAIN3
2	40	6	1987 mult VIN3
3	41	1	1998 uni VIN3
4	32	20	1983 CIN3 1989 VSCC 1992–93 mult precancerous lesions 1993 mult VSCC 1994 VIN3 1995 vulva condyloma 2003 no disease
5	74	12	1991 uni VSCC
6	50	4 (DOD)	1991 uni VSCC, LN met
7	76	1 (DOD)	1990 uni VSCC
8	77	9	1991 mult VSCC, LN met 2000 VIN3 2003 no disease
9	72	1 (DOD)	1990 uni VSCC, LN met

CIN3, cervical intraepithelial neoplasia 3; DOD, dead of disease; LN met, regional lymph node metastasis; mult, multifocal lesions; uni, unifocal lesions; VAIN3, vaginal intraepithelial neoplasia 3; VIN3, vulvar intraepithelial neoplasia 3; VSCC, vulvar squamous cell carcinoma.

Methods

A modified Hedley method[29,30] was used for isolating nuclei from formalin-fixed, paraffin-embedded tissues for multicolor FISH analysis on a single-layer preparation. One 50-μm section from each of the formalin-fixed, paraffin-embedded tissues was deparaffinized in Xylene, rehydrated in ethanol series and dH_2O, and disintegrated in 500 μl of 0.1% Protease/×1 phosphate-buffered saline (PBS) (Protease: Type XXIV, Bacterial, P 8038, Sigma, St Louis, MO; Dulbecco's ×1 PBS, Life Technologies, Rockville, MD) at 45°C for 45–60 min. The reaction was stopped by adding ×1 PBS at room temperature. The samples were filtered through a 30-μm nylon membrane (CN 051, DAKO, Glostrup, Denmark), centrifuged, and resuspended in ×1 PBS. Cytospin slides were prepared by the Shanndon Cytospin® and fixed in an ethanol series. Multicolored FISH probes were applied to the single-layer nuclei preparations.

Multicolor FISH

Multicolor FISH analysis was performed on each case with probes specific to centromere 7 (CEP7, labeled with Spectrum Aqua, SA), CEP3 (labeled with Spectrum Green, SG), and a BAC contig that contains the human telemerase gene (*TERC*) on chromosome band 3q26 (labeled with Spectrum Orange, SO). The centromeric probes were used as internal controls for signal enumeration. The probes were provided by Vysis/Abbott Laboratories, Inc. (Downers Grove, IL). The performance of the probe panel was evaluated on methanol/acetic acid-fixed peripheral lymphocyte cultures derived from karyotypically normal individuals. The Cytospin slides were incubated overnight in a 1 M sodium thiocyanate solution at 37°C. The slides were then washed in ×1 PBS, digested in RNAse and pepsin, and fixed in an ethanol series. Slides were denatured in 70% formamide/saline-sodium citrate (SSC) for 5–7 min at 80°C. After overnight hybridization at 37°C, the coverslips were gently removed and slides were dipped in 50% formamide/SSC at 45°C and washed three times in the same solution for 10 min each. Followed by a 10-min incubation in ×2 SSC at 45°C, slides were washed in 1% Nonidet P (NP)-40/×2 SSC at 45°C for 5 min. The slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), and antifade was added (details of the experimental procedures can be retrieved from http://riedlab.nci.nih.gov/).

Signal enumeration

FISH and image analyses were performed using a Leica DM-RXA fluorescence microscope (Leica, Wetzlar, Germany) equipped with custom optical filters for DAPI, SA, SG, and SO (Chroma Technologies, Rockingham, VT) and ×40 Plan Apo (NA 1.25) objective. Images were taken in areas of optimal cell density with minimal cellular clumps using the ORCA ER (IEEE1394 I/F) digital camera (Hamamatsu, Bridgewater, NJ). Leica Q-FLUORO was used to acquire multifocus images for each of the DAPI, SA, SG, and SO optical filters. Ten to 16 images were acquired, and signal enumeration was performed on these digital images for approximately 100–500 nuclei for each case. The counted signals were listed and evaluated in an Excel-based customized software.

Laminin-5γ2-chain immunohistochemistry

Preparation and characterization of polyclonal antibodies raised in rabbit against a glutathione-*S*-transferase fusion protein containing the C-terminus of the laminin-5γ2 chain (containing amino acid residues 1017–1178) were performed as described previously[31]. Immunohistochemistry was performed using the standard horseradish peroxidase avidin-biotin-complex technique (Vector, Elite Standard Kit, catalog PK-6100, Vector Laboratories, Inc., Burlingame, CA) described earlier[27,31].

Laminin-5γ2-chain expression was estimated and visually scored from 0 to 3+ by two investigators to whom the clinical or the histopathological data of the patients were unknown. A lesion was scored as laminin-5 positive when >1% of the cells showed distinct cytoplasmatic, non-nuclear immunopositivity. The estimation and categorization of the immunostaining were performed as described earlier[27].

Results

Interphase FISH with a three-color probe was used to explore the value of genetic markers for the diagnosis of VSCC. The probe set was devised based on extensive CGH analyses of cervical, vaginal, and vulvar carcinomas. The probe panel includes centromere enumeration probes specific to chromosomes 3 and 7 and a BAC contig that contains the human telomerase gene *TERC*, which maps to chromosome band 3q26 (Fig. 1A). A modified Hedley method was used to obtain interphase nuclei from archival tissue sections. Multicolor FISH probes were applied to the single-layer nuclei preparations. Three precancerous lesions and six invasive VSCC were analyzed by interphase FISH and by laminin-5γ2-chain immunohistochemistry on adjacent tissue sections.

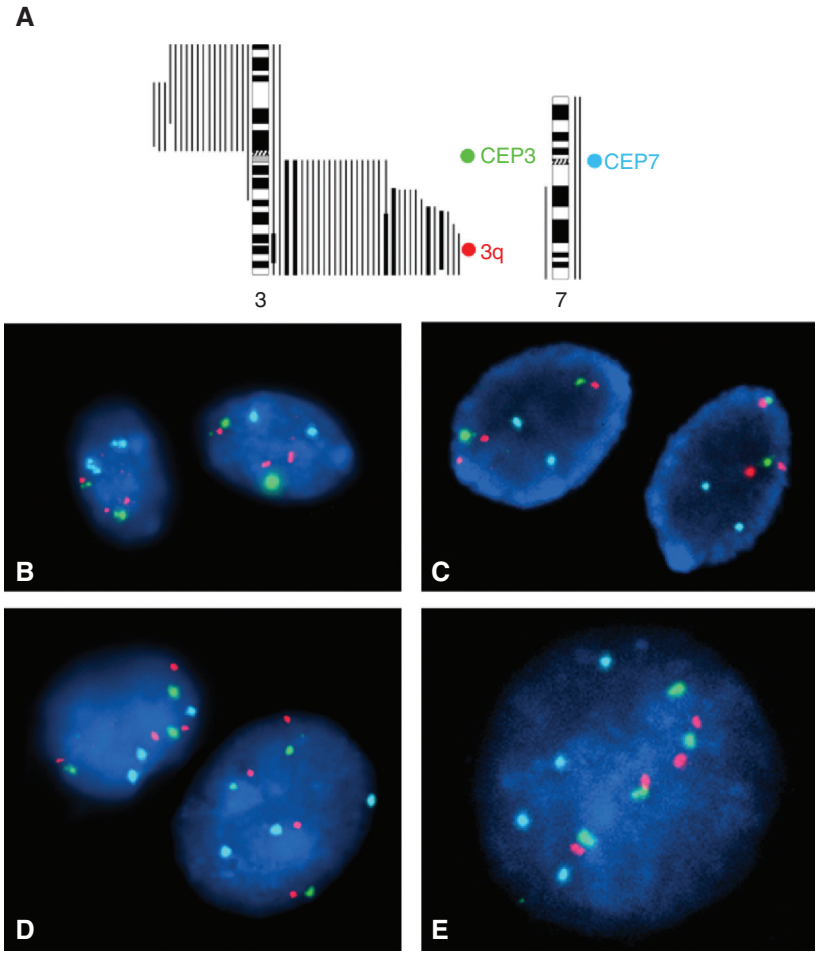

Fig. 1. A) Triple-color probe set for the detection of chromosomal aneuploidies in vulvar squamous cell carcinoma (VSCC) including probes specific to CEP3 (green), CEP7 (blue), and chromosome 3q *TERC* gene-specific probe (red)[32]. B and C) Nuclei from patient no. 5 and 6 respectively demonstrating copy gains of chromosome 3q in diploid cells. Isochromosome formation indicated by two copies of 3q signal (red) adjacent to the CEP3 signal (green). D) Nuclei from patient no. 8 revealing triploid CEP7 (blue) and CEP3 (green) signals with a four-copy gain of 3q (red). E) Nucleus from patient no. 2 showing tetraploid signals for all three probes, seen frequently in the premalignant lesions.

In the preinvasive lesions, the 3q gain was observed in two of three samples. 3q overrepresentation, however, was found in all six VSCC lesions. The average fold increase for 3q copy number was determined by the ratio of the absolute number of 3q signals to the absolute number of CEP7 signals. Low-level 3q amplifications were found in the preinvasive lesions, with an average fold increase of 1.15 for 3q. The invasive lesions showed higher average fold increases for 3q, which amounted to 1.32. A relative gain of 3q was defined by the 3q signal found in copy numbers greater than the CEP7 signal. The invasive cases revealed a relative gain of 3q in 43% of the nuclei counted, whereas 3q gains were found in 22% of the nuclei counted for the preinvasive lesions. Interestingly, one VIN3 lesion showed <1% 3q gain; however, the VAIN3 lesion has similar high gains as seen in VSCC. Hybridization of the probe panel to karyotypically normal lymphocyte preparations revealed an average of 0.012% aberrant copy numbers in the analyzed cells.

3q gain was present in multiple permutations in relation to the CEP7 and CEP3 signal; however, predominant cell clones were present in all cases. Four of six VSCC lesions showed two copies for CEP7 and CEP3, whereas the probe for 3q was present in either three or four copies in the majority of the cells. This pattern would be consistent with a gain of chromosome 3q in diploid cells (Fig. 1B and C). In another case (patient no. 8), however, the majority of cells had three copies of CEP7 and CEP3, whereas four copies of the probe set for the *TERC* gene were observed (Fig. 1D). Patient no. 9 revealed four copies for the centromere probes, while multiple permutations of the 3q gain were enumerated (primarily in five or more copies). In this case, cells with a triploid pattern and gain of 3q were also observed. Isochromosome formation was evident in four of the VSCCs. An isochromosome formation was deducted when two copies of chromosome arm 3q signals appeared adjacent to the CEP3 signals in interphase nuclei (Fig. 1B and C). Isochromosome 3q formation results in a relative loss of chromosome arm 3p.

Relative 3q gain was found in two of the three preinvasive lesions. One lesion from a patient with a

long clinical history of mutifocal cancer *in situ* of the cervix and vagina (patient no. 1) showed a 3q gain in 54% of the cells. In this case, three copies of CEP3 and 3q were found as a dominant clone, suggesting a whole chromosome 3 gain. The other two preinvasive lesions showed a dominant tetraploidy clone, ie, four copies of all three signals were enumerated (Fig. 1E). For patient no. 2, 32% of the nuclei revealed a tetraploid pattern for all three signals, and a relative 3q gain was observed in 11% of the nuclei. FISH analysis for patient no. 3 showed tetraploidy as the most common aberration among 11% of its nuclei; however, this case did not reveal a gain of 3q.

All six lesions with invasive SCC had moderate (++) to strong (+++) expression of laminin-5γ2-chain protein. Three regional lymph node metastases were included in laminin-5γ2-chain analysis and had strong (+++) expression. The staining was observed in cytoplasm of the cancer cells along the invasive front and to isolated cancer cell clusters invading the stroma. Laminin-5γ2-chain expression was not found in the stromal cells or in the basement membrane. Laminin-5γ2-chain expression was not detected in VIN3 and VAIN3 lesions (Table 2).

Discussion

Vulva carcinoma is a rare disease, and clinical material is difficult to obtain. In our study, a collection of archival precancerous and SCC lesions from the genital tract with long clinical follow-up were investigated. To identify objective genetic markers that could assist in the diagnosis and prognostication of this disease, we applied multicolor FISH with a custom-designed probe set to the archived tumor material. Our data revealed 3q as a consistent gain in all VSCC and present in a lower percentage of precancerous lesions. Our results are in agreement with the results from a similar analysis of routine cytological samples of different degrees of cervical dysplasia: although 76% of CIN3 lesions carried extra copy numbers of 3q, only 63% of CIN2 lesions and much lower percentages of CIN1 and atypical squamous cells of undetermined significance lesions were positive for this genetic marker[32]. These results may indicate that pathogenesis of vulvar and cervical carcinomas follow similar pathways.

All samples were also analyzed for the expression pattern of laminin-5γ2. Laminin-5γ2 serves as an independent marker for tumor invasiveness. In our study, the 3q gain preceded increased expression of laminin-5γ2, suggesting that genomic copy number increases of 3q and that the *TERC* gene is an early event during carcinogenesis of VSCC.

The HPV is frequently present in carcinomas of the lower anogenital tract including cervical, vulvar, and anal SCC. The expression of the E6 protein of HPV-16 in genital keratinocytes activates the expression of the telomerase gene, *hTERT*, which maps to chromosome arm 5p[33]. CGH analysis of VSCC by Allen *et al*.[20] found 3q amplification in 50% of HPV-positive tumors, whereas no amplification was found in HPV-negative tumors. Jee *et al*.[9] found the 3q gain in 40% of vulva carcinomas analyzed by CGH; however, the HPV status was not assessed. In our analysis, 3q was amplified in all SCC and in two thirds of the precancerous lesions. There is no information about the HPV status of the cases included in our study, and this constitutes an incitement for further research.

The frequency of 3q amplification was detected in only 40–50% of vulva carcinomas by CGH[9,20], whereas 100% of our VSCC showed 3q amplification. This discrepancy may be explained by the resolution of the techniques employed. CGH is a powerful technique for whole-genome analysis, however, at the cost of relatively low resolution. It is therefore possible that small amplicons resulting in low-level genomic copy number increases of *TERC* would remain undetected by CGH. Interphase FISH has the sensitivity to detect single-gene amplifications in individual nuclei. Our study revealed low-level 3q amplifications, consistent with previous findings. Sugita *et al*.[17] has found low-level 3q amplification in cervical, ovarian, and squamous cell lung cancers, with amplification levels not higher than 3.3 copies per cell in cervical carcinoma using Southern blot analysis.

In many instances, the hybridization pattern observed in interphase nuclei was consistent with isochromosome 3q formation. Isochromosome formation was found in four of our cases, suggesting a loss of 3p. Again, loss of 3p is consistent with published patterns of genomic imbalance in vulvar carcinomas[9]. Isochromosome 3q formation was suggested to be related to tumor invasiveness in cervical carcinomas[34].

The notable variation of 3q amplification found in the lesions, particularly among the premalignant cases, may be attributed to the degree of dilution of aberrant cells with adjacent normal cells. This would be a reflection of the sampling error rather than of the insensitivity of the diagnostic test. Sample 1 showed 54% of cells with 3q gain, whereas sample 2 showed 11% of cells with 3q gain. Our previous data have established that such a degree of aneuploidy never occurs in normal epithelial cells[32,35]. The increase of

Table 2. Histopathological changes in relation to chromosome 3q gain and laminin-5γ2-chain expression in anogenital lesions of all patients

Patient number	Lesion	Cells counted	Number (%) of nondiploid* cells	Number (%) of cells with relative 3q gain† by FISH	Ratio of 3q to CEP7 in all cells	3q isochromosome formation	Laminin-5γ2-chain expression
1	VAIN3, mult	167	97 (58)	91 (54)	1.4	No	0
2	VIN3, mult	150	69 (46)	17 (11)	1.06	No	0
3	VIN3, uni	519	99 (19)	15 (0.03)	1.0	No	0
4	VSCC, mult	126	85 (67)	83 (66)	1.34	No	++
5	VSCC, uni	216	107 (50)	97 (45)	1.25	Yes	++
6	VSCC, uni LN met	301	190 (63)	184 (61)	1.38	Yes	+++ LN met +++
7	VSCC, uni	225	147 (65)	137 (61)	1.58	Yes	++
8	VSCC, mult LN met	192	151 (79)	103 (54)	1.25	Yes	+++ LN met +++
9	VSCC, uni LNmet	159	103 (65)	50 (31)	1.15	No	+++ LN met +++

CIN3, cervical intraepithelial neoplasia 3; FISH, fluorescence *in situ* hybridization; LN met, regional lymph node metastasis; mult, multifocal lesions; uni, unifocal lesions; VAIN3, vaginal intraepithelial neoplasia 3; VIN3, vulvar intraepithelial neoplasia 3; VSCC, vulvar squamous cell carcinoma.

*Nondiploid refers to any pattern other than 2-2-2 for CEP7-CEP3–3q.

†Relative 3q gain is defined by the 3q signal found in copy numbers greater than the CEP7 signal.

3q signal numbers in case 3 is not significant, however, is consistent with previous observations in cervical dysplastic lesions[32]. Not all such lesions show an increase in 3q, which could be indicative of a=low propensity for progression, sampling errors, or alternative pathways for tumorigenesis.

Laminin-5γ2-chain expression has been used as a marker of invasiveness in cervical cancer[25] and lower anogenital lesions[27] and provides prognostic information in cancer of the vagina[26]. Laminin-5γ2-chain analysis was included in the study to provide additional information that permits identification of the invasive capacity of individual tumor samples. In the study of lower anogenital lesions[27], laminin-5γ2 chain was expressed both in precancerous lesions and in invasive carcinoma. In the group of women with multifocal changes, all of them who had VIN3 changes and positive expression of laminin-5γ2 chain later developed invasive cancer. In contrast, in this study, laminin-5γ2 chain was present in all VSCCs but not in the precancerous lesions. These patients with no laminin-5γ2-chain expression in the precancerous lesions did not progress to invasive cancer during follow-up of 1–19 years. The divergent results in the two studies may indicate different pathways involved in permitting tumor invasion.

In conclusion, our data demonstrate specific chromosomal and genetic aberrations in VSCC and precancerous lesions of the lower genital tract by using multicolored FISH in archival material. This specific genetic analysis revealed the amplification of 3q in 100% of the VSCC and in two thirds of the precancerous lesions. Laminin-5γ2-chain expression, however, was detected only in the invasive lesions. The results indicate that gain of chromosome 3q precedes the acquisition of invasive capacity in vulvar lesions and, thus, is an early and consistent anomaly in vulvar carcinogenesis. The detection of genomic amplification of *TERC* in diagnostic samples could be an independent test for the diagnosis of vulvar carcinomas and precursor lesions.

Acknowledgments

This work was supported by the Cancer Society of Stockholm and accepted by the local ethics committee (KI forskningsetikkommitté Nord) at the Karolinska Hospital, 2000-05-08, Dnr 00-182.

References

1 Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *CA Cancer J Clin* 1999;**49:**8–31.

2 Andersen WA, Franquemont DW, Williams J, Taylor PT, Crum CP. Vulvar squamous cell carcinoma and papillomaviruses: two separate entities? *Am J Obstet Gynecol* 1991;**165:**329–35.

3 Hording U, Kringsholm B, Andreasson B *et al.* Human papillomavirus in vulvar squamous-cell carcinoma and in normal vulvar tissues: a search for a possible impact of HPV on vulvar cancer prognosis. *Int J Cancer* 1993;**55:**394–6.

4 Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstet Gynecol* 1996;**87:**59–64.

5 Joura EA, Losch A, Haider-Angeler MG, Breitenecker G, Leodolter S. Trends in vulvar neoplasia. Increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. *J Reprod Med* 2000;**45:**613–5.

6 Jones RW. Vulval intraepithelial neoplasia: current perspectives. *Eur J Gynaecol Oncol* 2001;**22:**393–402.

7 Hording U, Daugaard S, Junge J, Lundvall F. Human papillomaviruses and multifocal genital neoplasia. *Int J Gynecol Pathol* 1996;**15:**230–4.

8 Ried T. Interphase cytogenetics and its role in molecular diagnostics of solid tumors. *Am J Pathol* 1998;**152:**325–7.

9 Jee KJ, Kim YT, Kim KR *et al.* Loss in 3p and 4p and gain of 3q are concomitant aberrations in squamous cell carcinoma of the vulva. *Mod Pathol* 2001;**14:**377–81.

10 Heselmeyer K, Schrock E, du Manoir S *et al.* Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. *Proc Natl Acad Sci USA* 1996;**93:**479–84.

11 Habermann JK, Hellman K, Freitag S *et al.* A recurrent gain of chromosome arm 3q in primary squamous carcinoma of the vagina. *Cancer Genet Cytogenet* 2004;**148:**7–13.

12 Heselmeyer K, du Manoir S, Blegen H *et al.* A recurrent pattern of chromosomal aberrations and immunophenotypic appearance defines anal squamous cell carcinomas. *Br J Cancer* 1997;**76:**1271–8.

13 Ell SR, Ashman JE, Stafford ND, Greenman J. Global genetic differences in squamous cell carcinoma of the head and neck. *Laryngoscope* 2002;**112:**1094–9.

14 Noguchi T, Kimura Y, Takeno S *et al.* Chromosomal imbalance in esophageal squamous cell carcinoma: 3q gain correlates with tumor progression but not prognostic significance. *Oncol Rep* 2003;**10:**1393–400.

15 Chujo M, Noguchi T, Miura T *et al.* Comparative genomic hybridization analysis detected frequent overrepresentation of chromosome 3q in squamous cell carcinoma of the lung. *Lung Cancer* 2002;**38:**23–9.

16 Sham J, Tang T, Fang Y *et al.* Recurrent chromosome alterations in primary ovarian carcinoma in Chinese women. *Cancer Genet Cytogenet* 2002;**133:**39–44.

17 Sugita M, Tanaka N, Davidson S *et al.* Molecular definition of a small amplification domain within 3q26 in tumors of cervix, ovary, and lung. *Cancer Genet Cytogenet* 2000;**117:**9–18.

18 Ma YY, Wei SJ, Lin YC *et al.* PIK3CA as an oncogene in cervical cancer. *Oncogene* 2000;**19:**2739–44.

19 Dahlgren L, Mellin H, Wangsa D *et al.* Comparative genomic hybridization analysis of tonsillar cancer reveals a different pattern of genomic imbalances in human papillomavirus-positive and -negative tumors. *Int J Cancer* 2003;**107:**244–9.

20 Allen DG, Hutchins AM, Hammet F *et al.* Genetic aberrations detected by comparative genomic hybridisation in vulvar cancers. *Br J Cancer* 2002;**86:**924–8.

21 Haga T, Kim SH, Jensen RH, Darragh T, Palefsky JM. Detection of genetic changes in anal intraepithelial neoplasia (AIN) of HIV-positive and HIV-negative men. *J Acquir Immune Defic Syndr* 2001;**26:**256–62.

22 Pyke C, Salo S, Ralfkiaer E *et al.* Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res* 1995;**55:**4132–9.

23 Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. *Dev Dyn* 2000;**218:** 213–34.

24 Pyke C, Romer J, Kallunki P *et al.* The gamma 2 chain of kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers. *Am J Pathol* 1994;**145:** 782–91.

25 Skyldberg B, Salo S, Eriksson E *et al.* Laminin-5 as a marker of invasiveness in cervical lesions. *J Natl Cancer Inst* 1999;**91:**1882–7.

26 Hellman K, Hellstrom AC, Silfversward C *et al.* Cancer of the vagina: laminin-5gamma2 chain expression and prognosis. *Int J Gynecol Cancer* 2000;**10:**391–6.

27 Nordstrom B, Einhorn N, Silfversward C *et al.* Laminin-5 gamma 2 chain as an invasivity marker for uni- and multifocal lesions in the lower anogenital tract. *Int J Gynecol Cancer* 2002;**12:**105–9.

28 Habermann J, Lenander C, Roblick UJ *et al.* Ulcerative colitis and colorectal carcinoma: DNA-profile, laminin-5 gamma2 chain and cyclin A expression as early markers for risk assessment. *Scand J Gastroenterol* 2001;**36:** 751–8.

29 Castro J, Heiden T, Wang N, Tribukait B. Preparation of cell nuclei from fresh tissues for high-quality DNA cytometry. *Cytometry* 1993;**14:**793–804.

30 Heiden T, Wang N, Tribukait B. An improved Hedley method for preparation of paraffin-embedded tissues for flow cytometric analysis of ploidy and S-phase. *Cytometry* 1991;**12:**614–21.

31 Salo S, Haakana H, Kontusaari S *et al.* Laminin-5 promotes adhesion and migration of epithelial cells: identification of a migration-related element in the gamma2 chain gene (LAMC2) with activity in transgenic mice. *Matrix Biol* 1999;**18:**197–210.

32 Heselmeyer-Haddad K, Janz V, Castle PE *et al.* Detection of genomic amplification of the human telomerase gene (TERC) in cytologic specimens as a genetic test for the diagnosis of cervical dysplasia. *Am J Pathol* 2003;**163:** 1405–16.

33 Veldman T, Horikawa I, Barrett JC, Schlegel R. Transcriptional activation of the telomerase hTERT gene by human papillomavirus type 16 E6 oncoprotein. *J Virol* 2001;**75:**4467–72.

34 Kirchhoff M, Rose H, Petersen BL *et al.* Comparative genomic hybridization reveals a recurrent pattern of chromosomal aberrations in severe dysplasia/carcinoma in situ of the cervix and in advanced-stage cervical carcinoma. *Genes Chromosomes Cancer* 1999;**24:**144–50.

35 Heselmeyer-Haddad K, Chaudhri N, Stoltzfus P *et al.* Detection of chromosomal aneuploidies and gene copy number changes in fine needle aspirates is a specific, sensitive, and objective genetic test for the diagnosis of breast cancer. *Cancer Res* 2002;**62:**2365–9.

Accepted for publication January 20, 2004