ELSEVIER

Cancer Genetics and Cytogenetics 148 (2004) 7–13

Cancer Genetics and Cytogenetics

Lead article

A recurrent gain of chromosome arm 3q in primary squamous carcinoma of the vagina

Jens K. Habermann[a,b], Kristina Hellman[c], Sandra Freitag[d], Kerstin Heselmeyer-Haddad[a], Ann-Cathrin Hellström[c], Keerti Shah[e], Gert Auer[b], Thomas Ried[a,*]

[a]*Genetics Branch, Center for Cancer Research, National Cancer Institute/NIH, Building 50, Room 1408, 50 South Drive, Bethesda, MD 20892-8010, USA*
[b]*Cancer Center Karolinska, Karolinska Institute, Stockholm, Sweden*
[c]*Department of Gynecologic Oncology, Radiumhemmet, Karolinska Hospital, Stockholm, Sweden*
[d]*Institute for Medical Informatics and Statistics, University Hospital Schleswig-Holstein, Campus Kiel, Germany*
[e]*Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, USA*

Received 6 May 2003; accepted 30 May 2003

Abstract Primary carcinomas of the vagina are rare tumors, accounting for 2%–3% of all gynecologic malignancies. Only a few karyotypes based on chromosome banding techniques have been reported. We have, therefore, used comparative genomic hybridization to establish a pattern of genomic imbalances in vaginal squamous cell carcinomas. Analysis of 16 formalin-fixed and paraffin-embedded tumors revealed that 70% of vaginal carcinomas carry relative copy number increases that map to chromosome arm 3q. Other recurring gains were observed on chromosome arms 5p and 19p. Chromosomal losses were infrequent. Most tumors were aneuploid, as measured by image cytometry on Feulgen-stained tissue sections. The cytogenetic data were related to the presence of human papillomavirus genomes, expression of laminin-5 as a marker for invasiveness, and expression levels of markers for proliferative activity and mutated *TP53*. All relevant clinical data were recorded. The results suggest that vaginal carcinomas are defined by a specific distribution of chromosomal aneuploidies and that the pattern of genomic imbalances is strikingly similar to that observed in squamous cell carcinomas of the uterine cervix. Age at diagnosis ($P = 0.031$), tumor size ($P = 0.025$), and increased laminin-5 expression ($P = 0.006$) have a significant influence on the survival time.

1. Introduction

Primary carcinomas of the vagina (VAC) are rare, accounting for 2%–3% of all gynecologic malignancies [1]. Similar to cervical carcinomas, vaginal carcinomas are associated with the presence of human papillomavirus (HPV), but only in ~50% of the cases [2,3]. Several other factors are thought to be associated with the development of VAC, such as diethylstilbestrol (DES) exposure in utero, which has been associated with clear cell adenocarcinoma in the vagina [4]. Other factors include a history of cervical or vaginal dysplasia, previous gynecologic and nongynecologic neoplasias, early hysterectomy, vaginal trauma from pessaries and prolapse, smoking, low socioeconomic status, and previous pelvic irradiation [5–9]. It is established that vaginal and cervical carcinomas are etiologically related. This is based on the fact that, first, VACs seem to occur synchronously as well as metachronously in patients with cervical dysplasia or carcinomas; second, that the vagina and the cervix are lined with the same squamous cell epithelium; third, that both tissues are related embryologically; and, fourth, that both organs are exposed to the same carcinogenic agents [10–12]. The theory of multicentric primary gynecologic neoplasms (especially cervical, vaginal, and vulvar carcinomas) further supports this hypothesis [10–12]. One important difference between vaginal and cervical carcinomas is that they occur in different age groups. Vaginal carcinoma is most common in postmenopausal women in the 60–80-year-old age group. Only ~15% of the patients are less than 50 years old [13]. Patients with cervical carcinoma are mostly premenopausal, with only about one third of the patients being more than 60 years old. In analogy to cervical carcinomas, VAC is thought to develop through stages of

* Corresponding author. Tel.: (301) 402-2008; fax: (301) 402-1204.
E-mail address: riedt@mail.nih.gov (T. Ried).

0165-4608/04/$ – see front matter
doi:10.1016/S0165-4608(03)00245-0

preinvasive dysplastic lesions, termed vaginal intraepithelial neoplasia, or VAIN [14]; however, this linear progression is less well established than with cervical carcinomas, because VACs are rare and only 50% of VACs show HPV infection. As in other malignant diseases, early detection greatly affects survival rates. It is therefore a perceived goal to analyze and understand the genetic mechanisms leading to vaginal tumorigenesis.

Comparative genomic hybridization (CGH) has become an exceedingly important tool to analyze chromosomal gains and losses in tumor genomes [15]. This applies in particular to the often highly rearranged karyotypes in epithelial cancers. To date, virtually all major human carcinomas have been widely studied [16]. This has resulted in comprehensive maps of chromosomal imbalances. Of note, it is now established that the distribution of such imbalances provides a conserved tumor-specific genetic imprint [17]. For instance, almost all cervical carcinomas contain extra copies of chromosome arm 3q [18], whereas aneuploid breast carcinomas invariably reveal chromosomal gains that map to chromosomes 1, 8, and 17, either alone or in combination [19,20]. Not only is the distribution specific for different tumor entities, it also appears that some changes precede the emergence of others [17]. The distribution of such chromosomal aneuploidies points to the location of cancer-causing genes. For instance, reduced copy numbers for 17p and gains of chromosome arm 17q most likely target the tumor-suppressor gene *TP53* accompanied by extra copies of the oncogene *ERBB2* and other potential oncogenes on chromosome 17q in breast cancers. The identification and mapping of chromosomal aneuploidies therefore contribute to the positional cloning of cancer-associated genes. Primary invasive vaginal carcinomas are among the very few cancer types for which such maps of genomic imbalances have not been generated yet. We have therefore collected 16 formalin-fixed and paraffin-embedded cases of this rare tumor for a molecular cytogenetic study using CGH. Other relevant genetic and biologic parameters, such as expression levels of p53 protein and markers for proliferative activity, as well as the nuclear DNA content, were measured on consecutive tissue sections. The presence of HPV genomes was analyzed by polymerase chain reaction in the DNA used for CGH.

2. Materials and methods

2.1. Tumor samples

Clinical material was collected from biopsies taken before radiation therapy and surgery. The tumors were diagnosed on hematoxylin–eosin-stained tissue sections at the Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden. The clinical data are summarized in Tables 1 and 2. Ten sections were prepared from each tumor and were used for histologic diagnosis, immunohistochemistry (thickness: 4 μm), DNA ploidy measurements (8 μm), and microdissection and DNA extraction (50 μm). A second hematoxylin–eosin-stained section was prepared subsequent to the sections for CGH analysis, and the histologic diagnosis was confirmed in all cases. All data were obtained from the dissected areas.

2.2. DNA cytometry

Image cytometry was performed on Feulgen-stained histologic sections. The staining procedure, internal standardization, and tumor cell selection were based on methods described previously [21]. All DNA values were expressed in relation to the corresponding staining controls, which were given the value 2c to denote normal diploid DNA content. The specimens were divided into two main groups: 1) diploid cases with a distinct peak in the normal 2c region and no cells exceeding 5c and 2) aneuploid cases with a main peak around the 4c region and varying numbers of cells (>5%) exceeding 5c.

2.3. Immunohistochemistry

All slides were deparaffinized with xylene, rehydrated, and microwaved at 500 W for 2×5 minutes in 10 mmol/L citrate buffer, pH 6.0. Intrinsic peroxidase activity was blocked with 3% hydrogen peroxide in methanol, followed by incubation with horse serum (1:20 dilution) in 0.1 mol/L phosphate-buffered saline, pH 6.0. The levels of protein expression were revealed by overnight incubation with the respective antibodies (see below) diluted in 1% (weight/volume) bovine serum albumin and visualized with a standard avidin–biotin–peroxidase complex technique (Vector Laboratories, Burlingame, CA). The following antibodies, with the respective dilutions and suppliers indicated in parentheses, were used: Mib1 (1:150; Immunotech SA, Marseille, France), cyclin A (1:100; Novocastra Laboratories Ltd., Newcastle-upon-Tyne, UK), DO1 (1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), WAF1 (1:15; Calbiochem, San Diego, CA), and laminin 5 γ2 chain (1:200, containing amino acids 1017–1178; Department of Medical Biochemistry and Biophysics, Karolinska Institute Stockholm, Sweden). All slides were coded and scored as reported previously [22]. The patterns of laminin-5 expression were evaluated as a possible marker for invasive disease and metastasis.

2.4. HPV genotyping

The HPV genomes in the purified DNA samples were identified with the line-blot assay using PGMY primers. In this assay, amplified products are tested against 27 HPV type-specific probes, which are immobilized on a filter strip [23]. The 27 probes include high risk HPVs, such as HPV 16, 18, 31, and 45. Beta-globin amplification was used as a positive control for evaluation of the adequacy of tumor DNA.

2.5. CGH

Formalin-fixed and paraffin-embedded tumor samples were provided in 50-µm-thick tissue sections. The tissue was incubated in xylene (3 × 5 minutes), followed by washes in 95% ethanol. According to the subsequent hematoxylin–eosin-stained sections, the deparaffinized tissue sections were microdissected to obtain representative tissue containing at least 80% of cancer cells. The microdissected cells were placed into centrifuge tubes containing 95% ethanol. After centrifugation, the samples were dried, resuspended in 1 mL sodium isothiocyanate (1 mol/L), and incubated overnight at 37°C. DNA was prepared using high salt extraction and phenol purification and labeled by nick-translation using biotin-11-dUTP (Boehringer Mannheim, Indianapolis, IN). Genomic DNA was labeled with digoxigenin-12-dUTP (Boehringer Mannheim). Hybridization was performed on karyotypically normal metaphase chromosomes using an excess of Cot-1 DNA (GIBCO BRL, Gaithersburg, MD). The biotin-labeled sequences were visualized with avidin–fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA) and the digoxigenin-labeled sequences were detected with a mouse-derived antibody against digoxigenin followed by a secondary rhodamine-conjugated antimouse antibody (Sigma-Aldrich, Milwaukee, WI). Quantitative fluorescence imaging and CGH analysis were performed using Leica Q-CGH software (Leica Imaging Systems, Cambridge, UK).

2.6. Statistical analyses

To detect the relationships among cytogenetic imbalances, expression levels of immunohistochemical markers, HPV status, and clinical course, the data were dichotomized and analyzed by Fisher's exact test at a level of $\alpha = 0.05$ using exclusively SPSS version 10.0 (SPSS, Inc., Chicago, IL). Kaplan–Meier survival curves were estimated and compared by a log rank test.

3. Results

Here we present a comprehensive molecular cytogenetic and phenotypic characterization of 16 cases of vaginal carcinoma. All parameters were related to tumor stage and grade, clinical features, and patient survival (Tables 1 and 2).

3.1. Genomic instability

The FIGO stage, tumor grade, histology, clinical features, and immunohistochemical evaluation are summarized in Tables 1 and 2. Except for one case (case 9), all cancers revealed a highly aneuploid distribution of the nuclear DNA content. This pattern was independent of tumor stage. All tumors showed chromosomal imbalances as identified with CGH. The most common DNA gains were mapped to chromosome arms 3q and 19p (69% and 50% of all cases, respectively), 5p (50%), 6q and 19q (44%), 1q and 17p (38%), 1p, 7p, 14, 16q, 18p, and 22 (31%), and 2p, 9q, 12, and 18q (25%). Chromosomal losses were only sporadically observed and mapped to chromosome arms 13q (identified 2×), 8p (1×), and 9p (1×). The average number of copy alterations (ANCA), calculated as the number of copy number changes divided by the number of cases, amounted to 7.5 for the autosomes. A summary of genomic imbalances is presented in Fig. 1. A comparative map of genomic imbalances of vaginal carcinomas and cervical carcinomas, normalized for number of cases, is presented in Fig. 2.

3.2. HPV genotyping

The presence of HPV genomes was analyzed with a line-blot assay in the same DNA that was used for CGH. A total

Table 1
Vaginal carcinoma histopathology, ploidy, and HPV status and immunohistochemistry

Case no.	Age at diagnosis (years)	Tumor stage	Histology	Tumor size (cm)	Tumor grading	Gross appearance	Tumor location	Ploidy	HPV	La-5	p53	WAF1	Mib1	CycA
1	87	IVa	SC ca	>8	Moderate	Ulcerating	Entire vagina	Aneuploid	No	High	Low	High	High	High
2	78	IVa	SC ca	4–8	Moderate	Ulcerating	Lower two-thirds	Aneuploid	No	High	Low	High	High	High
3	73	III	SC ca	>8	Moderate	Ulcerating	Entire vagina	Aneuploid	No	Low	Low	High	High	High
4	83	III	SC ca	>8	Moderate	Ulcerating	Entire vagina	Aneuploid	HPV 52	Low	Low	High	High	High
5	86	IIb	SC ca	4–8	Moderate	Ulcerating	Upper third	Aneuploid	ND	Low	Low	High	High	High
6	77	III	SC ca	>8	Poor	Ulcerating	Entire vagina	Aneuploid	ND	Low	Low	High	High	High
7	81	IIa	SC ca	4–8	Moderate	Exophytic	Upper two-thirds	Aneuploid	No	High	Low	High	Low	High
8	51	I	SC ca	<4	Moderate	Exophytic	Upper third	Aneuploid	HPV 16	Low	Low	High	Low	High
9	61	I	SC ca	<4	Well	Endophytic	Upper third	Diploid	ND	Low	Low	High	Low	Low
10	65	IVa	SmC ca	4–8	Poor	ND	Lower two-thirds	Aneuploid	ND	High	Low	High	High	High
11	84	I	SC ca	4–8	Well	Exophytic	Upper third	Aneuploid	ND	Low	Low	High	Low	Low
12	51	IIa	SC ca	<4	Well	ND	Upper third	Aneuploid	ND	Low	Low	High	Low	High
13	49	I	SC ca	<4	Poor	Exophytic	Upper third	Aneuploid	No	Low	Low	High	High	High
14	78	I	Ad ca	<4	ND	Exophytic	Upper third	Aneuploid	No	Low	Low	High	High	High
15	83	IIa	SC ca	4–8	Well	Ulcerating	Lower third	Aneuploid	ND	High	High	High	High	High
16	71	IVa	SC ca	>8	Moderate	ND	Entire vagina	Aneuploid	ND	High	High	Low	Low	High

Abbreviations: Ad ca, adenocarcinoma; ca, carcinoma; CycA, cyclin A; HPV, human papillomavirus; La-5, laminin 5; ND, not determined; SC ca, squamous cell carcinoma; SmC ca, small cell carcinoma.

Table 2
Vaginal carcinoma clinical features and patient survival

Case no.	Metastasis	Prior hysterectomy	Prior CIS	Prior gyn malignancy	Other malignancy	VAIN	Symptoms at diagnosis	Prior pelvic radiation	Relapse	Status at last follow up
1	No	No	No	No	No	No	Bleeding	No	No	Died from VAC
2	Yes	No	No	No	Yes	No	Bleeding	No	No	Died from VAC
3	No	No	No	No	Yes	No	Bleeding	No	Yes	Died from VAC
4	No	No	No	No	No	No	Bleeding	No	Yes	Died from VAC
5	No	No	No	Cervical ca	No	No	Bleeding	Yes	No	Died from other disease
6	No	Yes	No	No	No	No	Bleeding	No	No	Died from VAC
7	No	Yes	Yes	No	No	No	Bleeding	No	No	Died from VAC
8	No	Yes	Yes	No	No	No	Bleeding	No	Yes	Alive with disease
9	No	Yes	Yes	No	No	Yes	Dysplasia	No	Yes	Died from VAC
10	Yes	No	No	Cervical ca	No	No	Bleeding	Yes	No	Died from VAC
11	No	No	No	No	No	No	Bleeding	No	Yes	Died from VAC
12	No	Yes	No	Ovarian ca	No	No	Bleeding	Yes	Yes	Died from VAC
13	No	No	Yes	No	No	No	Bleeding	No	No	Alive and disease-free
14	No	No	No	No	No	No	Bleeding	No	No	Died from other disease
15	No	No	No	No	No	No	Pain and bleeding	No	No	Died from VAC
16	No	No	No	No	No	No	Urinary tract symptoms	No	No	Died from other disease

Abbreviations: CIS, carcinoma in situ; gyn, gynecologic; VAC, carcinoma of the vagina; VAIN, vaginal intraepithelial neoplasia.

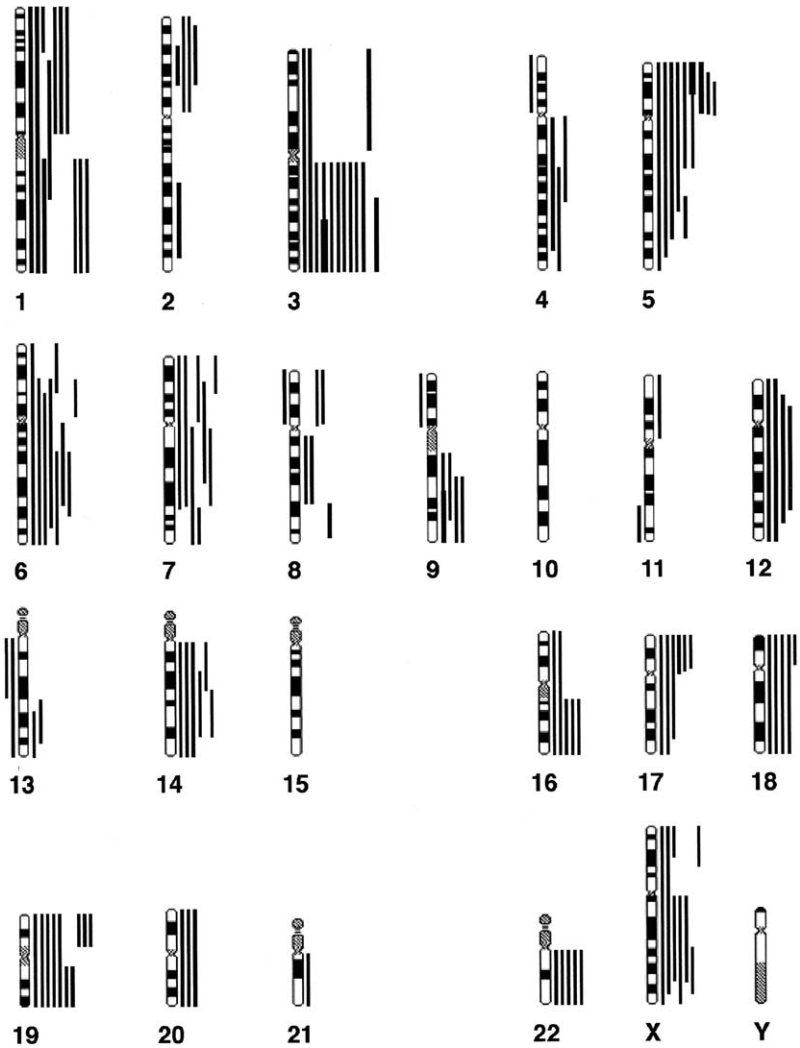

Fig. 1. Summary of genomic imbalances in 16 carcinoma-of-the-vagina specimens defined with CGH. Bars on the left side of the chromosome ideogram denote a loss of sequence in the tumor genome, bars on the right side indicate a gain. Bold squares or bars indicate high-level copy number increases (amplifications).

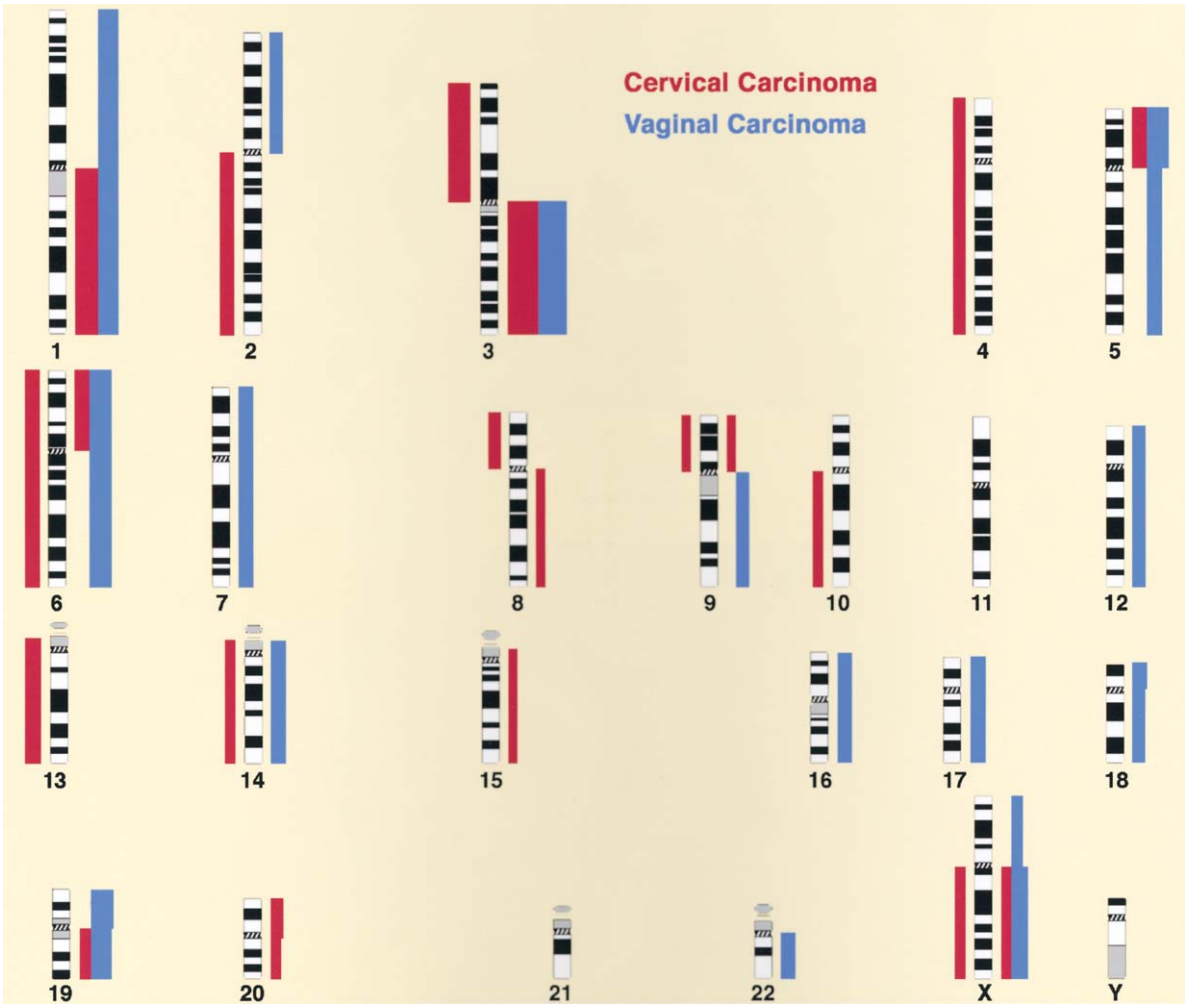

Fig. 2. Comparison of genomic imbalances in cervical carcinoma and vaginal carcinoma. Bars on the left side of the chromosome ideogram denote a loss of sequence in the tumor genome, bars on the right side indicate a gain. The number of alterations per chromosome is normalized to 10 cases for each tumor type. Only ratios greater than 2 have been considered. The group of cervical carcinomas summarized here contains 19 tumors at stage III and IV (together 63%) and 11 tumors at stage IIb. The group of vaginal carcinomas consists of 7 tumors at stage III and IV (together 44%), 4 tumors at stage II, and 5 tumors at stage I.

of eight samples could be analyzed successfully, with a primer for the β-globin gene as a control. Two tumor samples yielded positive test results: case 4 was positive for HPV 52, case 8 for HPV 16. HPV 16 belongs to the group of high-risk HPVs, whose association with cervical cancers is well established (Table 1).

3.3. Immunohistochemistry

Proliferative activity was analyzed using antibodies against cyclin A and Mib1 (Ki67). The majority of the tumors showed increased expression levels (i.e., increased activity in the majority of the cells): increased cyclin A expression could be observed in 63% of the samples and increased Mib1 expression in 88%. Increased expression of the tumor-suppressor gene *TP53* was observed in only two samples (cases 15 and 16). In case 16, WAF1 expression levels were low; this, when accompanied by the presence of increased p53 protein, is indicative of mutational inactivation of *TP53*. In general, the expression levels of WAF1 were increased (94% of the samples). Laminin-5 expression was elevated in 69% of the samples.

3.4. Statistical evaluation

To identify predictors of the clinical course, we compared subgroups of patients with respect to survival time, ANCA value, presence of HPV infection, proliferative activity, cell cycle regulation, and invasive potential. A low ANCA value (0.13) was found in both of the tumor samples that tested positive for HPV. Samples negative for HPV showed ANCA values that averaged 0.56 ($P = 0.031$). Another correlation could be observed between a larger tumor size and both an increased age at diagnosis ($P = 0.013$) and the prevalence of poorly differentiated tumors ($P = 0.034$). It also became evident that patients who were older than 70 years at time of diagnosis ($P = 0.031$), or who had a tumor size greater than 4 cm ($P = 0.025$), or who had an increased laminin-5 expression (more than 30% positive stained cells; $P = 0.006$) had a significantly shorter survival time (Fig. 3).

We did not, however, observe an influence of the histology and location of the tumor, the duration of the disease, the history of previous malignancies, or hysterectomy on the survival times. Notably, the four patients with a history of cervical intraepithelial dysplasia showed longer survival times than did all other patients combined ($P = 0.045$). These patients presented with lower tumor stages (three cases at stage I and one at stage IIa) and in three of the cases a tumor size less than 4 cm.

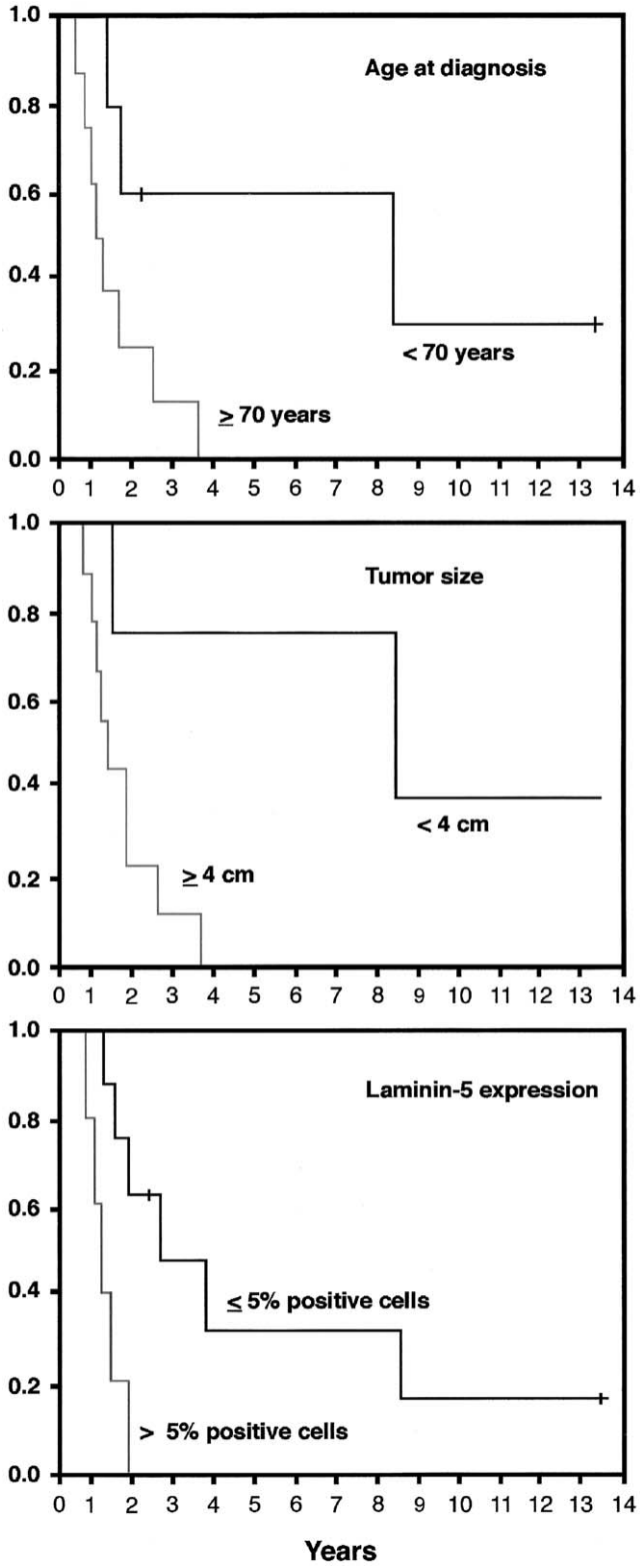

Fig. 3. Kaplan–Meier curves for age at diagnosis with vaginal carcinoma, tumor size, and laminin-5 expression. *x*-Axis: years of survival.

4. Discussion

We present what is to our knowledge the first comprehensive molecular cytogenetic analysis of primary invasive vaginal carcinomas. The chromosomal mapping of genomic imbalances using CGH revealed a recurring pattern of chromosomal aneuploidies. Therefore, one of the defining features of human carcinomas (namely, the acquisition of chromosomal gains and losses that are strongly selected for) is present also in this group of rare gynecologic tumors. When the pattern of genomic imbalances is compared with squamous cell carcinomas of the uterine cervix, a striking resemblance becomes evident. The most frequent copy number changes in vaginal carcinomas were mapped to chromosome arms 3q and 5p. This distribution almost mirrors the one in advanced cervical carcinomas, where copy number increases on 3q, 19p, and 5p occur in some 70%, 50%, and 35% of the cases, respectively [18,24,25]. This observation strengthens the hypothesis that cervical and vaginal carcinomas share many features related to their etiology, because gains of 3q and 5p are also commonly observed in cervical carcinomas [26].

In our series, the prevalence of HPV infection was lower than reported in the literature [2]. Only two of eight cases that were successfully analyzed showed the presence of HPV genomes, which is not consistent with previous reports that claim an infection rate of ~50% (e.g., [2]). We explain this discrepancy by the small number of cases in our study, and hence a greater error rate, or by methodologic difficulties in analyzing formalin-fixed DNA samples. We conclude, however, that compromising the function of the *TP53* and retinoblastoma tumor-suppressor (*RB1*) genes in the squamous cell epithelium of the vagina requires genomic aberrations as secondary events similar to those in the cervical epithelium. This is supported by our observation that *TP53* is inactivated only in a few cases. In earlier studies, we had observed a similar pattern of negative immunoreactivity for p53 in cervical carcinomas and concluded that the p53 inactivation via the E6 oncoprotein of HPV circumvents the need for acquisition of *TP53*-inactivating mutations [18]. In contrast to findings in vaginal carcinoma, HPV has been shown to be present in almost all cases of cervical carcinoma [3]. In primary carcinoma of the fallopian tube, most cases had gain on chromosome arm 3q but all cases were negative for HPV; most fallopian tube carcinomas, however, showed strong *p53* immunoreactivity. This suggests *TP53* inactivation by a gene mutation event [27]. We conclude that the gain of chromosome arm 3q is independent of the presence of HPV. Copy number increases on chromosome arm 3q are also frequently found in primary carcinomas of the vulva [28].

The CGH profiles for the two HPV-positive cases differ from the HPV-negative cases, with fewer genomic imbalances evident. The small number of cases, however, prevents us from concluding that the presence or absence of HPV has a direct effect on the pattern of chromosomal imbalances. The majority of VACs revealed a highly aneuploid distribution of the nuclear DNA content. There was only one case with a diploid distribution. In this particular case, the cancer was discovered at an early stage because the patient underwent regular examinations due to a previous diagnosis of VAIN. Consequently, the diploid pattern might be a feature of early-detected disease.

The finding that the pattern of genomic imbalances in VAC bears a close resemblance to those found in advanced cases of cervical carcinoma, together with the finding that almost all vaginal malignancies revealed a highly aneuploid distribution of the nuclear DNA content and showed high proliferative activity, might indicate that VACs are aggressive tumors, or, alternatively, that VAC is detected at rather late stages of disease progression.

Acknowledgments

The authors express their gratitude to Buddy Chen for editing the manuscript, Joseph Cheng for IT support, and Ulla Aspenblad, Inga Maurin, and Birgitta Sundelin for excellent technical assistance with immunohistochemistry and DNA cytometry.

References

[1] DeVita VT Jr, Hellman S, Rosenberg SA. Cancer: principles and practice of oncology. Volume 1. 6th edition. Philadelphia: Lippincott, Williams & Wilkins, 2001.

[2] Daling JR, Sherman KJ. Relationship between human papillomavirus infection and tumours of anogenital sites other than the cervix. IARC Sci Publ 1992;119:223–41.

[3] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–9.

[4] Herbst AL, Anderson D. Clear cell adenocarcinoma of the vagina and cervix secondary to intrauterine exposure to diethylstilbestrol. Semin Surg Oncol 1990;6:343–6.

[5] Brinton LA, Nasca PC, Mallin K, Schairer C, Rosenthal J, Rothenberg R, Yordan E Jr, Richart RM. Case-control study of in situ and invasive carcinoma of the vagina. Gynecol Oncol 1990;38:49–54.

[6] Goodman A. Primary vaginal cancer. Surg Oncol Clin N Am 1998; 7:347–61.

[7] Ptackova B. Primary carcinoma of the vagina. Neoplasma 1968;15: 309–22.

[8] Pukkala E, Weiderpass E. Time trends in socio-economic differences in incidence rates of cancers of the breast and female genital organs (Finland, 1971–1995). Int J Cancer 1999;81:56–61.

[9] Merino MJ. Vaginal cancer: the role of infectious and environmental factors. Am J Obstet Gynecol 1991;165:1255–62.

[10] Choo YC, Anderson DG. Neoplasms of the vagina following cervical carcinoma. Gynecol Oncol 1982;14:125–32.

[11] Rose PG, Herterick EE, Boutselis JG, Moeshberger M, Sachs L. Multiple primary gynecologic neoplasms. Am J Obstet Gynecol 1987; 157:261–7.

[12] Henson D, Tarone R. An epidemiologic study of cancer of the cervix, vagina, and vulva based on the Third National Cancer Survey in the United States. Am J Obstet Gynecol 1977;129:525–32.

[13] Benedet JL, Murphy KJ, Fairey RN, Boyes DA. Primary invasive carcinoma of the vagina. Obstet Gynecol 1983;62:715–9.

[14] Sillman FH, Fruchter RG, Chen YS, Camilien L, Sedlis A, McTigue E. Vaginal intraepithelial neoplasia: risk factors for persistence, recurrence, and invasion and its management. Am J Obstet Gynecol 1997; 176:93–9.

[15] Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818–21.

[16] Knuutila S, Björkqvist AM, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, Tapper J, Pere H, El-Rifai W, Hemmer S, Wasenius VM, Vidgren V, Zhu Y. DNA copy number amplifications in human neoplasms: review of comparative genomic hybridization studies. Am J Pathol 1998;152:1107–23.

[17] Ried T, Heselmeyer-Haddad K, Blegen H, Schrock E, Auer G. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. Genes Chromosomes Cancer 1999;25:195–204.

[18] Heselmeyer K, Schrock E, du Manoir S, Blegen H, Shah K, Steinbeck R, Auer G, Ried T. 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.

[19] Ried T, Just KE, Holtgreve-Grez H, du Manoir S, Speicher MR, Schrock E, Latham C, Blegen H, Zetterberg A, Cremer T, Auer G. Comparative genomic hybridization of formalin-fixed, paraffin-embedded breast tumors reveals different patterns of chromosomal gains and losses in fibroadenomas and diploid and aneuploid carcinomas. Cancer Res 1995;55:5415–23.

[20] Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J, Kallioniemi OP. Molecular cytogenetics of primary breast cancer by CGH. Genes Chromosomes Cancer 1998;21:177–84.

[21] Auer GU, Heselmeyer KM, Steinbeck RG, Munck-Wikland E, Zetterberg A. The relationship between aneuploidy and p53 overexpression during genesis of colorectal adenocarcinomas. Virchows Arch 1994;424:343–7.

[22] Habermann J, Lenander C, Roblick UJ, Kruger S, Ludwig D, Alaiya A, Freitag S, Dumbgen L, Bruch HP, Stange E, Salo S, Tryggvason K, Auer G, Schimmelpenning H. Ulcerative colitis and colorectal carcinoma: DNA-profile, laminin-5 γ2 chain and cyclin A expression as early markers for risk assessment. Scand J Gastroenterol 2001;36:751–8.

[23] Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357–61.

[24] Heselmeyer K, Macville M, Schrock E, Blegen H, Hellstrom AC, Shah K, Auer G, Ried T. Advanced-stage cervical carcinomas are defined by a recurrent pattern of chromosomal aberrations revealing high genetic instability and a consistent gain of chromosome arm 3q. Genes Chromosomes Cancer 1997;19:233–40.

[25] Kirchhoff M, Rose H, Petersen BL, Maahr J, Gerdes T, Lundsteen C, Bryndorf T, Kryger-Baggesen N, Christensen L, Engelholm SA, Philip J. 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.

[26] Murad TM, Durant JR, Maddox WA, Dowling EA. The pathologic behavior of primary vaginal carcinoma and its relationship to cervical cancer. Cancer 1975;35:787–94.

[27] Heselmeyer K, Hellstrom AC, Blegen H, Schrock E, Silfversward C, Shah K, Auer G, Ried T. Primary carcinoma of the fallopian tube: comparative genomic hybridization reveals high genetic instability and a specific, recurring pattern of chromosomal aberrations. Int J Gynecol Pathol 1998;17:245–54.

[28] Jee KJ, Kim YT, Kim KR, Kim HS, Yan A, Knuutila S. 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.