Arch Gynecol Obstet (2005) 273: 104–106
DOI 10.1007/s00404-005-0039-5

ORIGINAL ARTICLE

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CD44-v6 concentrations in carcinoma of the uterine cervix: lack of prognostic significance

Received: 21 February 2005 / Accepted: 25 April 2005 / Published online: 11 July 2005

Abstract Several kinds of cellular adhesion molecules, like different splicing variants of CD 44, have gained important as prognostic or markers for metastatic disease. Fresh frozen samples from 64 cervical carcinoma (CX) were stored in liquid nitrogen and examined using ELISA-technique, testing the prognostic impact. Normal cervical tissue served as control. CD 44-v6 concentration, was significant elevated in tumor tissue, when compared to the controls ($P = 0.04$). There was no correlation to tumor stage ($P = 0.61$), lymphovascular space involvement ($P = 0.075$) or pelvic lymph node involvement ($P = 0.81$). The CD 44-v6 concentration was not informative regarding recurrence-free and overall survival. Contrary to immunohistochemistry, the quantification of CD 44-v6 using ELISA-technique does not provide any further information.

Keywords Cervical carcinoma · Uterine · CD 44v6 · Prognosis · ELISA

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Introduction

CD 44 is a transmembrane glycoprotein which is widely expressed in hematopoietic and mesodermal cells. CD 44-isoforms or splicing variants (CD 44-v) are the result of an alternative splicing of different exons and can be modified by post-translational glycosilation [8]. Multiple functions are attributed to the various isoforms, such as lymphocytic homing, hematopoiesis, wound healing, embryonic development and apoptosis [8]. The splicing variants also play a role in tumor cell differentiation, invasion and have gained attention as markers of metastasis [6].

An altered expression of several splicing variants has also been described in cervical carcinogenesis [4]. In high-grade CIN and microinvasive cervical carcinomas, an overexpression of CD 44-v4 and -v6 was seen [3–5].

The expression of CD 44-v6 has been reported to be related to poor prognostic outcome in cervical cancer [13] and may be a predictor of lymphatic spread [2]. This study evaluates the prognostic relevance of the quantitative analysis of CD 44-v6, using the ELISA-technique in surgically treated cervical cancer.

Materials and methods

The study included 64 cervical cancer patients who were treated surgically by radical hysterectomy (Piver type III) and systematic radical pelvine lymphadenectomy. None of the patients received any neoadjuvant treatment.

For assessment of the histologic parameters, hysterectomy specimens were handled as previously described [9]. For typing and grading the WHO-classification for tumors [14] and for staging the pTNM-classification was used. Fresh tumor tissue samples were shock frozen and stored until use in liquid nitrogen. Before storing in

liquid nitrogen, the samples were examined by frozen section to verify tumor tissue.

For quantitative analysis using ELISA-technique, the frozen tissue samples (about 1 cm^3) were resuspended in Tris-buffer (10 mmol/l Tris/HCl, 1.5 mmol/l EDTA, 5.0 mmol/l Na_2MoO_4, pH 4) and homogenized with an Ultra-Turrax in ice-water for 4×50 s, with 4 min cooling between the steps. 0.1 ml 10% Tritox-X-100-solution was added to 0.9 ml of the homogenate and centrifuged at 4°C over night. Usually, the tissue supernatant was assayed 1:100 with sample dilution buffer of the CD 44-v6 ELISA-kit (Bender, MedSystems, Ingelheim, Germany).

Twentyfive samples from tumor-free cervical tissue served as controls.

Statistical analyses were carried out using the Chi^2-test and Fisher's exact test to compare CD 44-v6 values in normal and tumorous cervical tissue.

Differences in survival were analyzed using the log-rank test. The Cox-model was used to adjust for prognostic factors. *P*-values of less than 0.05 were considered as statistically significant. Statistical analysis was performed using SPSS for windows (release 9.0.0).

Results

The patients characteristics are summarized in Table 1.

The CD 44-v6 concentration in the tissue of cervical cancer specimens was significantly elevated compared to normal cervical tissue.

However, there was no difference between cases with and without pelvic lymph node metastases, with and without lymphovascular space involvement, with histopathological tumor stage or between patients with and without recurrent disease (Table 2). Additionally, there was no difference in the CD44-v6 concentration if the pT1b- and pT2a-tumors (early stage disease) were grouped together and compared with those showing parametrial involvement, representing advanced stage tumors (31.6 ng/ml versus 44.4 ng/ml; $P = 0.23$), as similar was in cases with and without recurrent disease ($P = 0.74$) as well as in correlation to the overall survival.

Table 1 Clinico-pathologic characteristics

	n	Percentage
Tumor stage		
pT1b1	21	32.8
pT1b2	6	9.3
pT2a	3	4.7
pT2b	34	53.2
Lymphovascular space involvement		
No (L0)	19	29.7
Yes (L1)	45	70.3
Pelvine lymph node status		
pN0	34	53.1
pN1	30	46.9
Recurrent disease		
No	50	78.1
Yes	14	21.8

Table 2 Quantitative analysis of CD 44-v6 by ELISA-technique

	CD 44-v6 concentration (ng/ml)	*P*-value
Normal cervical tissue	26.2	
Cervical cancer tissue	39.2	$P = 0.04$
Tumor stage		
pT1b1	40.1	
pT1b2	28.9	
pT2a	25.8	
pT2b	44.4	$P = 0.61$
Lymphovascular space involvement (LVSI)		
No (L0)	30.0	
Yes (L1)	41.5	$P = 0.075$
Pelvine lymph status		
pN0	39.2	
pN1	39.6	$P = 0.81$
Recurrent disease		
No	40.8	
Yes	32.2	$P = 0.23$

Discussion

Using immunohistochemistry, a gradually increased CD 44-v6 expression was seen when low- and high-grade CIN-lesions and microinvasive carcinomas were compared [3–5]. The present study, using quantitative analysis using ELISA-technique, showed a significant higher concentration of CD 44-v6 in cervical carcinomas when it was compared to normal cervical tissue, supporting the data on immunohistochemistry regarding the role of CD 44-v6 in cervical carcinogenesis.

The regulation of the expression of CD 44-standard and the alternative splicing in carcinomas of the uterine cervix and other neoplasms is mediated by a complex network of different factors such as c-erbB-2, Egr-1 1nd AP-1 and is connected to the Ras-associated MAP kinase pathway [11, 15], factors which may be alterated by the infection of cells with high-risk types of HPV. Recently, Recio and Merlino [12] demonstrated that the hepatocyte growth factor/scatter factor (HGF/SF) upregulates the expression of CD 44-v6 which may stimulate the metastatic distribution of cervical carcinomas by lymphatic spread.

As reported in an earlier study of our group, the detection of CD 44-v6 is associated with lymphatic space involvement and pelvic lymph node involvement [2] and correlated with the number of positive nodes.

Ayhan et al. [1] reported that the CD 44-v6 overexpression in FIGO IB cases was related to reduced disease-free (not statistically significant) and overall survival ($P = 0.03$). In another analysis [13], the expression of CD 44-v6 was correlated with poor 5-year-overall survival. In patients with advanced stage tumors (FIGO IIB to IVB), treated with radiation therapy,

positive CD 44-v6 immunolabeling was correlated with a decreased 5-year survival rate [10], tumor associated death as well as poor therapeutic response [7].

To the best of our knowledge, the present study reports the first time the quantitative analysis of CD 44-v6 in cervical carcinoma by ELISA. Unfortunately, quantitative analysis did not add further information, additional to previous immunohistochemical studies.

References

1. Ayhan A, Baykal C, Al A, Ayhan A (2001) Altered CD 44 variant 6 expression in FIGO IB cervical carcinoma. Gynecol Oncol 83:569–574
2. Biesold C, Köhler U, Horn LC et al (1995) CD 44 exon v6 as a predictor of lymphatic metastases in cervical carcinoma—an immunocytochemical study of 94 cases. Arch Gynecol Obstet 256:147–153
3. Callagy G, O'Grady A, Butler D et al (2000) Expression of CD 44 in uterine squamous neoplasia: a predictor for microinvasion ? Gynecol Oncol 76:73–79
4. Dall P, Hekele A, Ikenberg H et al (1996) Increasing incidence of CD 44-v7/8 epitope expression during uterine cervical carcinogenesis. Int J Cancer (Pred Oncol) 69:79–85
5. Faleiro-Rodrigues C, Lopes C (2004) E-Cadherin, CD44 and CD44v6 in squamous intraepithelial lesions and invasive carcinomas of the uterine cervix: an immunohistochemical study. Pathobiology 71:329–336
6. Günthert U (1996) CD 44 in malignant disorders. Curr Top Microbiol Immunol 213:271–285
7. Harima Y, Togashi A, Horikoshi K et al (2004) Prediction of outcome of advanced cervical cancer to thermoradiotherapy according to expression profiles of 35 genes selected by cDNA microarray analysis. Int J Radiat Oncol Biol Phys 60:237–248
8. Haynes BF, Liao HX, Patton KL (1991) The transmembrane hyaluronate receptor (CD 44): multiple functions, multiple forms. Cancer Cells 3:347–350
9. Horn LC, Fischer U, Bilek K (2001) Pathologic-anatomic description and basic morphological information in the management of dysplasias and carcinomas of the cervix uteri. Zentralbl Gynakol 123:255–265
10. Kainz C, Kohlberger P, Tempfer C et al (1995) Prognostic value of CD 44 splice variants in human stage III cervical cancer. Eur J Cancer 31A:1706–1709
11. Maltzmann JS, Carmen JA, Monroe JG (1996) Transcriptional regulation of the Icam-1 gene in antigen receptor- and phorbole ester-stimulated B lymphocytes: role for the transcription factor EGR 1. J Exp Med 183:1747–1759
12. Recio JA, Merlino G (2003) Hepatocyte growth factor/scatter factor induces feedback up-regulation of CD 44-v6 in melanoma cells through Egr-1. Cancer Res 63:1576–1582
13. Speiser P, Wanner C, Tempfer C et al (1997) CD 44 is an independent prognostic factor in early-stage cervical cancer. Int J Cancer 74:185–188
14. Wells M, Östör AG, Crum CP, Franceschi S, Tommasino M, Nesland JM, Goodman AK, Sankaranayanan R, Hanselaar AG, Albores-Saavedra J (2003) Epithelial tumors of the uterine cervix. In: Tavassoli FA, Devilee P (eds) Pathology and genetics of tumours of the breast and female genital organs. World Health Organization Classifocation of Tumours. IARC Press, Lyon, pp 259–279
15. Wobus M, Kuns R, Wolf C, Horn LC et al (2001) CD 44 mediates constitutive type I receptor signaling in cervical carcinoma cells. Gynecol Oncol 83:227–234