CD44v6 as a Prognostic Factor in Cervical Carcinoma FIGO Stage IB

J. BOUDA1, L.BOUDOVA2, O. HES2, M. HAVIR1, C. TEMPFER3, P. KOHLBERGER4, T. SVOBODA5, Z. ROKYTA1 and P. SPEISER4

1Department of Obstetrics and Gynecology, 2Department of Pathology and 5Department of Radiotherapy, Medical Faculty Hospital, Charles University, Plzen, Czech Republic; 3Department of Obstetrics and Gynecology, University of Freiburg, School of Medicine, Germany; 4Department of Obstetrics and Gynecology, University of Vienna, Medical School, Vienna, Austria

Abstract. The aim of our study was to clarify whether CD44v6 evaluation can serve as a universally applicable prognostic factor in patients with FIGO stage IB cervical carcinoma. A retrospective study was performed on 178 FIGO stage IB (142 IB N0, 36 IB N1) radically operated cervical carcinoma patients. The expression of CD44v6 was investigated by immunohistochemistry (IHC). The prognostic significance of established prognostic factors and CD44v6 expression was analyzed by univariate and multivariate analyses. To test the reproducibility and to account for interobserver variability, all specimens were evaluated independently at two institutions. Two different IHC scoring systems, several cut-off levels for CD44v6 positivity and several statistical methods for IHC results evaluation were used. In a univariate analysis, the most significant prognostic factor for overall survival (OS) was lymph node status (p<0.001) followed by tumor volume, LVSI, GOG score (p<0.01) and a deep stromal invasion (p=0.06). We found a strong correlation between CD44v6 expression and squamous cell carcinoma (SCC) (SCC vs. adenocarcinoma - p<0.001) and between CD44v6 expression and deep stromal invasion, LVSI and GOG score (p<0.05). The CD44v6 expression was not a statistically significant prognostic factor for OS in a univariate analysis (p=0.39 Vienna; p=0.54 Freiburg). In a multivariate analysis, the most significant prognostic factor for OS was lymph node status (p=0.002), followed by tumor diameter and LVSI (p<0.05). CD44v6 expression was not a statistically significant prognostic factor for OS or disease-free interval (DFI) independent of the scoring method used. In conclusion, we demonstrated that CD44v6 expression is associated with LVSI, deep stromal invasion and SCC, but has no prognostic influence on OS and DFI in a population of 178 women with FIGO stage IB cervical carcinoma.

The CD44 adhesion molecule plays an important role in the metastasis of malignant tumors of different origins. A standard form of CD44 (CD44s) and 10 variant isoforms (CD44v1-CD44v10), produced by alternative splicing of premessenger RNA, have been described. CD44s and its isoforms are involved in many physiological processes in humans, such as cell-cell and cell-extracellular matrix interactions, cell traffic, presentation of chemokines and the transmission of growth signals. CD44 also participates in the uptake and intracellular degradation of hyaluronic acid, as well as in the transmission of signals mediating hematopoiesis and apoptosis (1). CD44 also mediates lymphocyte homing, i.e. the migration of circulating lymphocytes to lymphatic tissues and their binding to lymph nodes.

Many cancer cell types, as well as their metastases, express high levels of CD44 and it was hypothesized that CD44s and its isoforms are involved in the mechanism of tumor invasion and metastasis by mediating tumor cell interaction with the endothelium and the subendothelial matrix.

In a normal cervical epithelium, the expression of CD44s and its isoforms is limited to the basal and spinal cell layers (2). During carcinogenesis, CD44 expression shows dynamic changes leading to a more complex pattern of expression also involving superficial cell layers (3-5). Such changes could provide metastatic cells with a selective advantage during carcinogenesis (3). However, the exact mechanisms and factors influencing this process are unknown. The expression of CD44 isoforms in cervical carcinoma cells and its prognostic significance for tumor behavior and overall survival (OS) have been examined in several studies which differ as to the criteria of patient selection as well as to the methods of CD44 staining and scoring (6-12). CD44v7-8

Correspondence to: Jiri Bouda, M. D., Department of Obstetrics and Gynecology, Medical Faculty Hospital, Capkovo nam. 1, 307 08 Plzen, Czech Republic. Tel: 377617213, Fax: 377617290, e-mail: boudaj@medima.cz

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and CD44v6 expression have been reported to be associated with a shortened overall survival (OS) (6, 10, 11, 13-15). CD44v6 was significantly correlated with lymphovascular space invasion (LVSI) and with an increased probability of regional lymph node metastases (7, 16). CD44v6 expression was found to be an independent prognostic factor in node-negative FIGO stage IB cervical carcinoma (6, 10, 11). In contrast, the results of other studies using different methods did not confirm these results (8, 9, 12).

The aim of this study was to investigate whether CD44v6 expression is a universally applicable independent prognostic factor for OS and disease-free interval (DFI). Therefore, we assessed CD44 expression in a population of 178 Czech patients with FIGO IB cervical carcinoma.

Patients and Methods

Patients. We examined 178 patients with cervical carcinoma consecutively treated at the Department of Obstetrics and Gynecology, Medical Faculty Hospital, Plzen, Czech Republic, between 1988 and 1995. All patients were treated by radical hysterectomy and pelvic lymphadenectomy, properly staged according to the International Federation of Obstetricians and Gynecologists (FIGO) classification of 1995. All patients were regularly followed-up at least for 5 years (range 5-13 years, median 7 years) since the operation. Ninety-two percent of patients underwent adjuvant pelvic radiotherapy. Histological slides were reviewed regarding the histological type, grading, lymph vascular space invasion (LVSI) and lymph node status by two experienced pathologists blinded to the clinical and CD44 data.

Immunohistochemistry. Immunohistochemistry (IHC) was performed at the Department of Gynecology and Obstetrics, University of Vienna Medical School, Austria. Routinely formalin-fixed and paraffin-embedded specimens were studied for the expression of the epitope encoded by the CD44 splice variant CD44v6 using the Antigen Retrieval System (Bio Genex, San Ramon, CA, USA) and the murine monoclonal antibody (MAb) (clone VFF-7, Bender Co., Vienna, Austria), as previously described (10).

The slides were evaluated independently in Vienna and Freiburg by two investigators blinded to the clinical data.

Positive control. The positive control slide was prepared from epidermal tissue, known to contain the antigen. In the positive control tissue, the MAb stained similarly.

Negative control. The negative control slide was prepared from the same tissue block as the specimen. Instead of the primary antibody, we used a normal, non-immune serum supernatant from the same source as the primary antibody.

Results

One hundred and seventy-eight patients with FIGO IB cervical carcinoma (142 IB N0, 36 IB N1) and adequate surgical staging, with a follow-up of at least 5 years, were included in the study. The median age of the patients was 40.5 years (range 24-68 years). There were 153 (86%) squamous cell carcinomas, 19 (10.7%) adenocarcinomas and 6 (3.3%) other carcinomas in the series. During the follow-up period, 34 patients (19.1%) showed disease recurrence and 31 patients (17.4%) died of the tumor.

Statistical evaluation. The correlation between established prognostic factors and CD44 expression on the one hand and OS and DFI on the other was tested by univariate analysis using three statistical methods: Mantel-Haenszel’s, Gehan’s (modified Wilcoxon’s) and Prentice’s tests. The most significant prognostic factor for OS was regional lymph node status (IB N0 vs. IB N1; p<0.001; Gehan’s test). Other statistically significant factors (p=0.01-0.05) were tumor diameter, GOG score (17) and LVSI (Figure 1). The depth of stromal invasion (both absolutely and relatively) resulted in a borderline statistical significance (p=0.06). Age, tumor type, i.e. squamous cell carcinoma (SCC) vs. adenocarcinoma (AC), and grade were not statistically significant. There were similar results for DFI (Table I).
We used two different scoring systems for CD44v6 evaluation. In Vienna, semi-quantitative scoring was performed. No staining and weak staining of 10% of tumor cells (negative and slightly positive in semi-quantitative evaluation) were considered negative (88 patients; 49%). Marked staining of 10-50% of tumor cells and strong staining of more than 50% of tumor cells (moderately and strongly positive in semi-quantitative evaluation) were considered positive (90 patients; 51%).

In Freiburg, IHC was evaluated as a sum of the area (0 – no positivity, 1– up to 1/3 of positive cells, 2– up to 2/3 of positive cells, 3 – more than 2/3 of positive cells) and the intensity of the staining (0– none, 1– weak, 2– moderate, 3– strong). In our statistical evaluation, we considered a sum of 0-3 negative (125 patients; 70%) and a sum of 4-6 positive (53 patients; 30%).

When we correlated the IHC results from Freiburg and Vienna using the Chi-square test, there was a significant correlation between these results.

Furthermore, we performed a univariate analysis of the CD44v6 correlation with clinicopathological parameters using the Chi-square test. We found the most significant correlation between CD44v6 positivity and the type of tumor (SCC vs. AC) \( p < 0.001 \) \((p < 0.001)\), followed by LVSI.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of pat. (%)</th>
<th>OS, p-value (Gehan)</th>
<th>DFI, p-value (Gehan)</th>
<th>CD44v6 (Vienna) ( \chi^2 ), p-value</th>
<th>CD44v6 (Freiburg) ( \chi^2 ), p-value</th>
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<tbody>
<tr>
<td>Age (2 groups)</td>
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<tr>
<td>1. ≤40</td>
<td>84 (47%)</td>
<td>1 vs. 2, ( p = 0.76 )</td>
<td>1 vs. 2, ( p = 0.80 )</td>
<td>( p = 0.5 )</td>
<td>( p = 0.8 )</td>
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<tr>
<td>2. &gt;40</td>
<td>94 (53%)</td>
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<tr>
<td>Lymph node status (2 groups)</td>
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<tr>
<td>1. IBNo</td>
<td>142 (80%)</td>
<td>1 vs. 2, ( p &lt; 0.001 )</td>
<td>1 vs. 2, ( p &lt; 0.001 )</td>
<td>( p = 0.6 )</td>
<td>( p = 0.9 )</td>
</tr>
<tr>
<td>2. IBN1</td>
<td>36 (20%)</td>
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<tr>
<td>Tumor diameter (3 groups)</td>
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<tr>
<td>1. &lt;20 mm</td>
<td>88 (49%)</td>
<td>1 vs. 2, ( p &lt; 0.05 )</td>
<td>1 vs. 2, ( p &lt; 0.01 )</td>
<td>( p = 0.1 )</td>
<td>( p = 0.3 )</td>
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<tr>
<td>2. 21-40 mm</td>
<td>72 (41%)</td>
<td>1 vs. 3, ( p &lt; 0.01 )</td>
<td>1 vs. 3, ( p &lt; 0.01 )</td>
<td>( p &lt; 0.05 )</td>
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<tr>
<td>3. ≥40 mm</td>
<td>18 (10%)</td>
<td>2 vs. 3, ( p = 0.5 )</td>
<td>2 vs. 3, ( p = 0.41 )</td>
<td>( p = 0.08 )</td>
<td>( p = 0.4 )</td>
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<tr>
<td>Stromal invasion I (3 groups)</td>
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<tr>
<td>1. &lt;10 mm</td>
<td>102 (57%)</td>
<td>1 vs. 2, ( p = 0.06 )</td>
<td>1 vs. 2, ( p &lt; 0.05 )</td>
<td>( p = 0.08 )</td>
<td>( p = 0.4 )</td>
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<tr>
<td>2. 11-15 mm</td>
<td>52 (29%)</td>
<td>1 vs. 3, ( p = 0.1 )</td>
<td>1 vs. 3, ( p &lt; 0.05 )</td>
<td>( p = 0.08 )</td>
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<tr>
<td>3. ≥16 mm</td>
<td>24 (14%)</td>
<td>2 vs. 3, ( p = 0.9 )</td>
<td>2 vs. 3, ( p = 0.9 )</td>
<td>( p = 0.08 )</td>
<td>( p = 0.08 )</td>
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<tr>
<td>Stromal invasion II (2 groups)</td>
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<tr>
<td>1. inner 2/3</td>
<td>95 (53%)</td>
<td>1 vs. 2, ( p = 0.06 )</td>
<td>1 vs. 2, ( p &lt; 0.05 )</td>
<td>( p = 0.08 )</td>
<td>( p &lt; 0.05 )</td>
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<tr>
<td>2. outer 3/3</td>
<td>83 (47%)</td>
<td></td>
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<td></td>
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<tr>
<td>Tumor type (3 groups)</td>
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<tr>
<td>1. SCC</td>
<td>153 (86%)</td>
<td>1 vs. 2, ( p = 0.1 )</td>
<td>1 vs. 2, ( p = 0.16 )</td>
<td>( p &lt; 0.001 )</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>2. AC</td>
<td>19 (11%)</td>
<td></td>
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<tr>
<td>3. others</td>
<td>6 (3%)</td>
<td></td>
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<tr>
<td>Tumor grade (3 groups)</td>
<td></td>
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<tr>
<td>1. well</td>
<td>20 (11%)</td>
<td>1 vs. 2, ( p = 0.7 )</td>
<td>1 vs. 2, ( p = 0.88 )</td>
<td>( p = 0.3 )</td>
<td>( p = 0.3 )</td>
</tr>
<tr>
<td>2. moderately</td>
<td>105 (59%)</td>
<td>1 vs. 3, ( p = 0.8 )</td>
<td>1 vs. 3, ( p = 0.44 )</td>
<td>( p = 0.3 )</td>
<td>( p = 0.3 )</td>
</tr>
<tr>
<td>3. poorly</td>
<td>53 (30%)</td>
<td>2 vs. 3, ( p = 0.2 )</td>
<td>2 vs. 3, ( p &lt; 0.06 )</td>
<td>( p = 0.08 )</td>
<td>( p &lt; 0.05 )</td>
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<tr>
<td>LVSI (2 groups)</td>
<td></td>
<td></td>
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<tr>
<td>1. negative</td>
<td>88 (49%)</td>
<td>1 vs. 2, ( p &lt; 0.01 )</td>
<td>1 vs. 2, ( p &lt; 0.01 )</td>
<td>( p = 0.08 )</td>
<td>( p &lt; 0.05 )</td>
</tr>
<tr>
<td>2. positive</td>
<td>90 (51%)</td>
<td></td>
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<tr>
<td>GOG score (3 groups)</td>
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<tr>
<td>1. &lt;40</td>
<td>37 (21%)</td>
<td>1 vs. 2, ( p &lt; 0.05 )</td>
<td>1 vs. 2, ( p &lt; 0.05 )</td>
<td>( p = 0.2 )</td>
<td>( p &lt; 0.05 )</td>
</tr>
<tr>
<td>2. 40-119</td>
<td>82 (46%)</td>
<td>1 vs. 3, ( p &lt; 0.01 )</td>
<td>1 vs. 3, ( p &lt; 0.01 )</td>
<td>( p = 0.2 )</td>
<td>( p &lt; 0.05 )</td>
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</table>
p=0.08 (p=0.025), and the depth of stromal invasion p=0.075 (p=0.022). There was no significant correlation between the CD44v6 expression and other clinicopathological parameters (Table I).

Assessing the impact of CD44v6 expression and OS and DFI (Figure 2), we found a slightly shorter mean survival in patients with CD44v6-positive cervical carcinomas (985 vs. 750 days in Vienna evaluation, 950 vs. 844 days in Freiburg evaluation). There was no statistically significant correlation between CD44v6 expression and OS (p=0.39 Vienna, p=0.54 Freiburg) and DFI (p=0.67 Vienna, p=0.96 Freiburg).

Finally, we performed a Cox multivariate analysis of OS and DFI, using the most significant prognostic factors of the univariate analysis (lymph node status, tumor diameter, GOG score and LVSI) and CD44v6 results both from Vienna and Freiburg. Again, the most significant factor was regional lymph node status (p=0.002), followed by the tumor diameter (p=0.03), LVSI (p=0.03) and GOG score (p=0.09), whereas CD44v6 expression was not statistically significant (p=0.78 Vienna, p=0.32 Freiburg). When we did not divide CD44v6 results into two groups, but instead considered them a continuous variable (0-3 Vienna, 0-6 Freiburg), the results were similar (lymph node status p=0.002, tumor diameter p=0.03, LVSI p=0.04, GOG score p=0.07, CD44v6 Vienna p=0.60, CD44v6 Freiburg p=0.19). The results of the multivariate analysis for DFI were similar (Table II).

**Discussion**

The most important prognostic factors in early stage cervical carcinoma are the stage and regional lymph node status. Other established prognostic factors are the depth of invasion, tumor volume and LVSI (17, 18). Combinations of the above mentioned factors are used in different scoring systems (17, 19). However, guidelines for treatment strategies including adjuvant therapy differ and, therefore, there has been an effort to identify additional prognostic factors to further individualize treatment algorithms.

In cervical carcinoma, strong correlations between CD44v6 expression and LVSI and regional lymph node metastases have been reported (7, 16). Poor prognosis in patients with CD44v6 tumor positivity (both early and late stage cervical carcinoma) was documented (14, 15) and CD44v6 was described as an independent prognostic factor in early stage cervical carcinoma (6, 10, 11).

On the other hand, conflicting data were reported by Saegusa et al. (9), who found a correlation of CD44v6 expression and the histological type of tumor, but no correlation between CD44v6 expression and clinicopathological factors or survival. Tokumo et al. (12) reported similar results. In a study by Gaducci et al. (8), pretreatment serum levels of the glycoproteins CD44s, CD44v5 and CD44v6 were not related to FIGO stage and histological type and were correlated neither with the common prognostic variables nor with the clinical outcome.

Although the majority of authors investigating other types of malignancies documented the aggressive behavior of CD44v6-positive tumors and a prognostic value of CD44v6 evaluation, contradictory data were also published with respect to these malignancies (20-27).

The basic parameters of our patient population – the median age, the carcinoma type distribution and the results of 5-year OS – are similar to previously published data (17, 18, 28). Only patients with at least 5 years of follow-up were included in our study. In accordance with other studies, the most significant prognostic factor of OS and DFI was regional lymph node status, followed by tumor diameter, LVSI, GOG score and deep stromal invasion. Interestingly, patients with AC had a better prognosis in our patients series than patients with SCC, although this difference was not statistically significant.
There are numerous techniques for CD44 identification and evaluation (7, 9, 12, 29). In our series, we tested the most frequently used method, namely the immunohistochemical identification of CD44v6, which is technically easier and cheaper compared to reverse transcription-polymerase chain reaction (RT-PCR) or in situ hybridization and is routinely applicable to paraffin-embedded, formalin-fixed tissue. However, on reviewing the literature, it was established that the use of various antibodies, different cut-off levels and intratumoral heterogeneity of expression adversely affect the reproducibility of IHC.

To test the reproducibility and to achieve higher data reliability, our specimens were evaluated independently at two institutions by two experienced investigators blinded both to the clinical outcome and other IHC results. Although the results from both institutions correlated closely, we did not confirm any statistically significant correlation of CD44v6 expression with either OS or DFI.

In accordance with others (7, 9, 16), we found a significant correlation between LVSI and CD44v6 positivity, which may be caused by an increased ability of the CD44v6-positive tumor cells to mimic lymphocytes and exploit their pathways.

We also found the strongest correlation between the tumor type (SCC) and CD44v6 expression, which is in agreement with most other large series (9, 11, 12). Consequently, some authors consider the expression of various CD44 isoforms as a reflection of the tumor heterogeneity of individual patients rather than an exact parameter of aggressive tumor behavior and they cite CD44v6 as a marker for cellular differentiation (9, 12, 30). Ayhan et al. (6) recommend considering only "nonbasal" (CD44v6-positive cells both in the basal and central portions of the neoplastic islands) positivity of CD44v6.

In conclusion, we found a statistically significant correlation between CD44v6 expression and LVSI, deep stromal invasion and SCC. We did not find any statistically significant correlation between CD44v6 expression and OS or DFI, either in the univariate or multivariate analysis. The most significant prognostic factors for OS and DFI in our patients series were regional lymph node status, followed by tumor diameter, LVSI positivity and GOG score.

In our study, we demonstrated that CD44v6 IHC evaluation is an easy and reproducible method, but CD44v6 expression is not a prognostic factor for OS and DFI in a population of 178 patients with FIGO IB cervical carcinoma.

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