Cytokeratin Expression in Seminoma of the Human Testis

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Key Words: Seminoma; Cytokeratin; Outcome; Embryonal carcinoma; CD30

Abstract

We studied cytokeratin (CK) expression immuno-histochemically in 64 seminomas using a panel of commercially available antikeratin antibodies and tested for association of CK expression with patient age, tumor size, stage, and outcome. Seventeen embryonal carcinomas were compared with seminoma. CK7, CAM 5.2, AE1/AE3, and wide-spectrum screening keratin (WSK) were positive in 41%, 30%, 36%, and 36% of the seminomas, respectively. CK20 and high-molecular-weight keratin (HMWK) were negative in all cases. CD30, placental alkaline phosphatase (PLAP), and epithelial membrane antigen (EMA) were positive in 6%, 100%, and 2% of cases, respectively. There were no differences in patient age, stage, tumor size, or outcome between CK-positive and CK-negative seminomas. CK7, CAM 5.2, AE1/AE3, and WSK were positive in 100%, 88%, 94%, and 88% of embryonal carcinomas, respectively. CK20 and HMWK were negative in all cases. CD30, placental, alkaline phosphatase (PLAP), and epithelial membrane antigen (EMA) were positive in 6%, 100%, and 2% of cases, respectively. There were no differences in patient age, stage, tumor size, or outcome between CK-positive and CK-negative seminomas. CK7, CAM 5.2, AE1/AE3, and WSK were positive in 100%, 88%, 94%, and 88% of embryonal carcinomas, respectively. CK20 and HMWK were negative in all cases. CD30, placental, alkaline phosphatase (PLAP), and epithelial membrane antigen (EMA) were positive in 6%, 100%, and 2% of cases, respectively. CKs are present in seminoma, and their presence is not associated with a difference in patient age, stage, or outcome. In cases such as small needle biopsy specimens, CK and CD30 stains may be useful in separating seminoma from embryonal carcinoma.

The intermediate filaments are an integral part of the cytoskeleton, and the family of intermediate filaments consists of desmin, vimentin, cytokeratin, glial, and neurofilaments. The cytokeratins are the largest and most diverse group of intermediate filaments in human cells. Their presence is indicative of epithelial differentiation, and they are present in normal and neoplastic epithelia. The cytokeratins are expressed in a characteristic pattern in different epithelia, a pattern that often is maintained during neoplastic transformation. Cytokeratin filaments typically are not found in germinal, mesenchymal, neuroectodermal, or hematopoietic tissues.

Seminoma arises from undifferentiated germ cells. There are conflicting reports about intermediate filament expression in seminoma, particularly cytokeratin expression. Early studies suggested that seminoma lacks cytokeratin, a finding that supported the hypothesis that seminoma cells were incapable of somatic differentiation. Subsequently, chromosomal and molecular studies have shown that seminoma is capable of differentiation into embryonic (embryonal carcinoma, teratoma) and extraembryonic (yolk sac tumor, choriocarcinoma) tissues and that some seminomas show epithelial differentiation with the formation of desmosomes and expression of cytokeratin filaments. The finding of epithelial differentiation in seminoma has suggested to some that there are seminomas with epithelial features that are “transitional tumors” between typical seminoma and embryonal carcinoma. Whether these seminomas with cytokeratin filaments behave more aggressively remains unknown.

The objective of the present study was to examine cytokeratin expression in a large series of seminomas using a wide panel of commercially available antikeratin antibody stains. Immunohistochemical cytokeratin expression was tested for an association with patient age, tumor size, tumor...
stage, and outcome. In addition, a group of embryonal carcinomas in pure and mixed germ cell tumors also was studied.

Materials and Methods

We retrieved 64 pure seminomas, 5 pure embryonal carcinomas, 7 mixed germ cell tumors composed of embryonal carcinoma and seminoma, and 5 mixed germ cell tumors composed of embryonal carcinoma and various combinations of teratoma and yolk sac tumor obtained between 1990 and 1998 from the Mayo Clinic Tissue Registry, Rochester, MN. The immunohistochemical stains CAM 5.2, AEI/AEIII, high-molecular-weight keratin (HMWK), wide-spectrum screening keratin (WSK), cytokeratin 7 (CK7), cytokeratin 20 (CK20), placental alkaline phosphatase (PLAP), epithelial membrane antigen (EMA), and CD30 were performed on a representative block of formalin-fixed paraffin-embedded tissue using the avidin-biotin complex technique. The antibodies, dilutions, suppliers, and clones are given in Table 1. The cytokeratin stains detected Moll cytokeratin numbers 1 through 8, 10, 14, 16, and 18 through 20 (Table 2). AEI/AEIII, CK7, CK20, CAM 5.2, and WSK stains were pretreated with protease; PLAP, CD30, and EMA stains were steam pretreated in citrate buffer (pH 6) for 30 minutes; and the HMWK stain was steam pretreated in EDTA buffer (pH 8) for 30 minutes. All slides were placed on a Ventana ES or Nexes autostainer (Ventana, Tucson, AZ). Slides were stained with labeled streptavidin-biotin detection chemistry after primary antibody incubation. The chromogen was 3-amino-9-ethylcarbazole. Immunostaining was evaluated by determining the percentage of positively staining cells in 5% increments over the entire section of tumor. Clinical histories were reviewed for age at diagnosis, stage at diagnosis (including radiographic studies consisting of abdominal computed tomography, and, in some cases, bipedal lymphangiogram), treatment, length of follow-up, and outcome. Slides and pathology reports were reviewed for diagnosis and tumor stage. Tumors were staged using the stage grouping of the American Joint Committee on Cancer TNM system.22,23 Stage I tumors were confined to the testicle, rete testis, epididymis, spermatic cord, or scrotum; stage II tumors involved ipsilateral, contralateral, or bilateral abdominal or groin lymph nodes without distant metastases; and stage III tumors consisted of all tumors with distant metastases. Staging was based on pathologic and clinical (radiographic) findings.

Associations between the extent of cytokeratin expression with patient age, tumor size, and stage were evaluated using the Wilcoxon rank sum test, chi-square test, and Spearman rank correlation. Cytokeratin expression was defined and evaluated in 2 groups: tumors exhibiting any positivity (<1% to 100%), and tumors containing 5% or more positive cells. All significance tests were 2-sided, and a type I error level of 0.05 was used. Comparisons with respect to cytokeratin, CD30, PLAP, and EMA expression between seminoma and embryonal carcinoma were made using the Wilcoxon rank sum test.

Results

Pure seminoma (n = 64) occurred in men 22 to 71 years of age (mean, 37.3 years; median, 35.5 years). Forty-
four patients (69%) had stage I tumors, 12 (19%) stage II, and 8 (12%) stage III. Nine patients (14%) were treated by radical orchiectomy, 43 (67%) by radical orchiectomy followed by regional lymph node radiation, 6 (9%) by radical orchiectomy and systemic chemotherapy, and 4 (6%) by radical orchiectomy, regional lymph node radiation, and systemic chemotherapy. Tumors ranged in size from 0.2 to 12 cm (mean, 4.1 cm; median, 3.5 cm). Patients were followed up for a mean of 41.0 months (range, 1-103 months). One patient (2%) died of metastatic seminoma. 1 patient (2%) is alive with metastatic seminoma and undergoing chemotherapy, and 59 patients (92%) are alive and without tumor. For 3 patients (5%), follow-up information was not available.

Pure embryonal carcinoma (n = 5) occurred in men 21 to 39 years of age (mean, 33.0 years; median, 35 years). Two patients (40%) had stage I tumors, and 3 (60%) had stage II. Two (40%) patients were treated by radical orchiectomy alone, and 3 (60%) underwent radical orchiectomy followed by systemic chemotherapy. Tumors ranged in size from 0.5 to 3.5 cm (mean, 2.1 cm; median, 2.1 cm). Patients were followed up for a mean of 37.2 months (range, 8-62.2 months). All patients were alive without tumor at last follow-up.

Mixed germ cell tumors (n = 12) occurred in men 20 to 42 years of age (mean, 29.5 years; median, 28 years). Seven patients (58%) had stage I tumors, 2 (17%) had stage II, and 3 (25%) had stage III tumors. Four patients (33%) underwent radical orchiectomy alone, 5 (42%) underwent radical orchiectomy followed by systemic chemotherapy, 1 (8%) underwent radical orchiectomy followed by retroperitoneal lymph node dissection, and 2 (17%) underwent radical orchiectomy and received systemic chemotherapy followed by resection of residual masses. Tumors ranged in size from 2 to 13 cm (mean, 5.3 cm; median, 5 cm). Patients were followed up for a mean of 72.1 months (range, 1-153 months). On last follow-up, 1 patient (8%) was receiving chemotherapy for recurrent mixed germ cell tumor, and the remaining 11 (92%) were alive without tumor.

The majority of seminomas did not express cytokeratin intermediate filaments; the number of negatively staining cases ranged from 38 (59%) to 64 (100%), depending on the primary antikeratin antibody. A minority of cases stained positively; 18 cases (28%) contained 5% or more cytokeratin-positive cells. Immunohistochemical staining most often was focal with a discontinuous membranous and/or cytoplasmic globular staining pattern. CK20 and HMWK did not stain any of the seminomas. EMA was identified in only 1 tumor, and the positivity was very focal. CD30 was identified in 4 (6%) of the cases, and staining was very focal. Monoclonal PLAP was identified in 64 tumors (100%); the majority of seminomas expressed more than 50% positively staining cells. For the 2 definitions of cytokeratin expression (<1% and 5% and greater cytokeratin positivity), tumors that were not organ-confined did not differ in their cytokeratin expression from confined tumors. There also were no differences in cytokeratin-negative and cytokeratin-positive tumors in regard to patient age, tumor size, and outcome.

The majority of embryonal carcinomas expressed cytokeratins (except CK20 and HMWK); the percentage of positively staining cases ranged from 15 (88%) to 17 (100%). Immunohistochemical staining intensity typically was intense with a membranous staining pattern. CD30 was positive in all cases; most tumors showed more than 10% positively staining cells. EMA rarely was positive but was focal in distribution in positively staining cases. Staining for CK7, CAM 5.2, AEI/AEI, WSK, and CD30 was significantly different between seminoma and embryonal carcinoma (P < .001; Wilcoxon rank sum test).

**Table 3**

<table>
<thead>
<tr>
<th>Positive Staining Cells (%)</th>
<th>CK7</th>
<th>CK20</th>
<th>CAM 5.2</th>
<th>AEI/AEI</th>
<th>WSK</th>
<th>34:E12</th>
<th>EMA</th>
<th>CD30</th>
<th>PLAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38 (69)</td>
<td>64 (100)</td>
<td>45 (70)</td>
<td>41 (64)</td>
<td>41 (64)</td>
<td>64 (100)</td>
<td>63 (98)</td>
<td>60 (94)</td>
<td>0 (0)</td>
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<tr>
<td>&lt;1</td>
<td>10 (16)</td>
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<td>11 (17)</td>
<td>11 (17)</td>
<td>11 (17)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>4 (6)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>1-10</td>
<td>13 (20)</td>
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<td>6 (9)</td>
<td>8 (12)</td>
<td>8 (12)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>11-50</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>3 (5)</td>
<td>3 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>16 (25)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>40 (62)</td>
</tr>
</tbody>
</table>

CK, cytokeratin; EMA, epithelial membrane antigen; PLAP, placental alkaline phosphatase; WSK, wide-spectrum screening keratin.

* Data are given as number (percentage) of cases.

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Table 4
Immunohistochemical Staining in Embryonal Carcinoma (n = 17)*

<table>
<thead>
<tr>
<th>Positively Staining Cells (%)</th>
<th>CK7</th>
<th>CK20</th>
<th>CAM 5.2</th>
<th>AEI/AEIII</th>
<th>WSK</th>
<th>34βE12</th>
<th>EMA</th>
<th>CD30</th>
<th>PLAP</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0)</td>
<td>17 (100)</td>
<td>2 (12)</td>
<td>1 (6)</td>
<td>2 (12)</td>
<td>17 (100)</td>
<td>15 (88)</td>
<td>0 (0)</td>
<td>3 (18)</td>
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<td>&lt;1</td>
<td>2 (12)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>1-10</td>
<td>5 (29)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>5 (29)</td>
<td>4 (24)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>4 (24)</td>
</tr>
<tr>
<td>11-50</td>
<td>2 (12)</td>
<td>0 (0)</td>
<td>5 (29)</td>
<td>2 (12)</td>
<td>3 (18)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (35)</td>
<td>4 (24)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>8 (47)</td>
<td>0 (0)</td>
<td>8 (47)</td>
<td>9 (53)</td>
<td>8 (47)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (59)</td>
<td>5 (29)</td>
</tr>
</tbody>
</table>

CK, cytokeratin; EMA, epithelial membrane antigen; PLAP, placental alkaline phosphatase; WSK, wide-spectrum screening keratin

* Includes 6 pure embryonal carcinomas and 12 embryonal carcinomas in mixed germ cell tumors. Data are given as number (percentage) of cases.

Image 1
A, Pure seminoma showing uniform cells with distinct cell membranes (×350). B, Cytokeratin AEI/AEIII showing positive staining (×350).

Image 2
A, Pure embryonal carcinoma showing glandular differentiation and marked cytologic atypia (×400). B, Cytokeratin CAM 5.2 showing intense diffuse positivity (×400).
HMWK were consistently negative in seminoma. Embryonal carcinoma was positive for cytokeratin filaments, and, like seminoma, embryonal carcinoma lacked staining for CK20 and HMWK. Unlike seminoma, CD30 was positive in all cases of embryonal carcinoma. Diffuse cytokeratin and CD30 staining were indicative of embryonal carcinoma, and this staining pattern was not seen in seminoma.

Battifora et al\textsuperscript{4} studied 18 seminomas with specific antikeratin antibodies to keratin classes of 40, 50, and 56.5 kD (Moll keratin classification numbers 19, 14, and 10, respectively) and found no cytokeratin positivity. This finding supported the hypothesis that seminoma was unable to exhibit embryonic or extraembryonic differentiation.\textsuperscript{11} Subsequent studies have shown that seminoma has the potential to differentiate into nonseminomatous germ cell tumors, and cytokeratin filaments are present in some seminomas.\textsuperscript{11-21} Fogel et al,\textsuperscript{20} using immunohistochemical techniques, identified cytokeratin filaments in 19 of 26 seminomas. In their study, cytokeratins 8 and 18 were identified in all cases. Denk et al\textsuperscript{18} identified cytokeratins 8 and 18 in seminoma using gel electrophoresis of cytoskeleton proteins. Cytokeratins 8 and 18 are the cytokeratins of simple epithelia, and these are the first 2 keratins expressed in mouse embryogenesis.\textsuperscript{24,25} The consistent expression of cytokeratins 8 and 18 in seminoma recapitulates early epithelial differentiation. In regard to our study, CAM 5.2, AE1/AEIII, and WSK contain cytokeratin 8, 18, or both. Seminoma also is capable of expressing more complex cytokeratins (the cytokeratins of stratified epithelia), such as cytokeratins 4, 17, 19, and, as demonstrated in our study, CK7,\textsuperscript{18,20}

Similar to seminoma, embryonal carcinoma contains cytokeratins 8 and 18, and more complex cytokeratins are seen less commonly.\textsuperscript{26} This finding supports the close relationship of seminoma and embryonal carcinoma. Our study shows that cytokeratin filaments in seminoma are not associated with more aggressive behavior, as determined by stage at presentation or outcome.

In orchietomy specimens, the distinction between seminoma and embryonal carcinoma usually is not difficult. However, in limited specimens, such as needle biopsy specimens, it may be difficult to distinguish seminoma from embryonal carcinoma or somatic carcinoma. In this setting, positive cytokeratin staining should not be considered diagnostic of embryonal carcinoma or metastatic carcinoma without supporting morphologic features. In difficult cases, the use of PLAP, cytokeratin, CD30, and EMA may be useful for separating seminoma, embryonal carcinoma, and somatic carcinoma.\textsuperscript{27-34} The use of CD30 in combination with cytokeratin may be especially useful. CD30 staining was identified very focally in 5 seminomas, and in embryonal carcinoma, staining usually was diffuse.

The present study showed that seminoma can contain cytokeratin filaments, and these can be identified with anti–CK7 antibodies or “cocktails” that contain anti–cytokeratin 8, anti–cytokeratin 18, or both. There were no differences in patient age, stage at presentation, outcome, or tumor size between patients whose seminomas contained cytokeratin filaments and whose seminomas did not. Cytokeratin, CD30, and PLAP stains may be useful for separating seminoma, embryonal carcinoma, and somatic carcinoma in difficult cases.

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References


