

Detection of Intermediate Filament in Testicular Seminoma Through Immunohistochemical Expressions of Keratins

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Abstract: Intermediate filament was detected in human testicular seminoma using immunohistochemical expression of keratin. Commercially available Cytokeratin of varying molecular weight were applied to 32 seminomas along with 22 embryonal carcinomas (pure and mixed form). Detection of intermediate filaments was correlated with age, tumor size, and stage. Seminomas showed Intermediate filaments in number of cases. Cytokeratin of varying molecular weight showed positivity. Of 32 seminomas, 37.5 % show presence of intermediate filaments through positive expression with AE1/AEIII, 37.5 % with 34bE12, 43.7% with Cytokeratin 7. There were no differences in patient age, stage, tumor size, between CK-positive and CK-negative seminomas. Of 22 Embryonal carcinomas 86% shows positivity with AE1/AEIII, whereas 34bE12 and Cytokeratin 7 was positive in 86% and 100% of cases. Epithelial membrane antigen was positive in 9% cases of Embryonal carcinomas and 8.6% cases of seminomas.

Keyword: Intermediate filament, Seminoma, Keratin

I. Introduction

Intermediate filaments (IFs), comprising of Cytokeratins, desmin, vimentin. Glial and neurofilaments (1) a class of cytoskeletal proteins, have been considered a powerful tool for objective identification of tumor histogenesis and have gained wide application in differentiation of tumor elements (2-9, 15, 23, 37, 38,). Each IF class has generally been supposed a unique histogenetic marker of cell origin (3), therefore, IFs are especially helpful in demonstrating heterogeneity of cell population in tumors. Testicular germ cell tumors are such tumors having the propensity of heterogeneity. Classification and histogenesis of testicular germ cell tumors have been of great interest in the pathologic field (40, 41), not only for treatment of these tumors, but also for understanding of tumorigenesis. It is believed that identifying individual components of the tumor is a very important step in considering the behavior of these tumors. Tissue demonstration of IFs in testicular germ cell tumors might provide a good approach to revealing different tumoral components and in making classification of these tumors.

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 (10). 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 (11-21). 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 (2, 18, 20). Whether these seminomas with cytokeratin positive intermediate filaments behave more aggressively remains unknown.

II. Objective

The objective of the present study was to detect Intermediate Filaments through 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. In addition, a group of embryonal carcinomas in pure and mixed germ cell tumors also was studied.

III. Materials And Method

We retrieved 32 pure seminomas, 8 pure embryonal carcinomas, 9 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 2010 and 2014 in Medical college, Kolkata. Immunohistochemical stains CAM 5.2, AE1/AE3, high-molecular-weight keratin (HMWK), 'Keratin Cocktail', 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 sections were deparaffinized in xylene, and rehydrated in a graded ethanol series. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. The slides were subsequently incubated at room temperature with reagents. The antibodies, and clones Molecular weight and Moll No. of Cytokeratin are given in Table 1.

Table 1

Details of Immunohistochemical stains applied			
Primary Antiserum	Clone	Molecular weight (of Keratin)	Moll number (of Keratin)
AE1/AE3	AE1 and AE3	56,5,50,48,40,65,67,64,59,58,56,,52	10,14,16,19,2,3,4,5,6,8
'Keratin Cocktail'	Polyclonal	60,58,56,52,51,48	5,6,8,4,14,16
High Molecular Weight Keratin	34bE12	68,58,56,5,50	1,5,10,14
Cytokeratin 7	OV-TL 12/30	54	7
Cytokeratin 20	Ks 20.8	46	20
Epithelial Membrane Antigen	E 29		
Placental Alkaline Phosphatase	8A9		
CD 30	Ki-1		
CAM 5.2	CAM 5.2	52,46	8,18

AE1/AE3, CK7, CK20, CAM 5.2, and 'Keratin Cocktail' 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. 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 of the entire section of tumor. Clinical histories were reviewed for age at diagnosis, stage at diagnosis (including radiographic studies consisting of abdominal computed tomography). Slides and pathology reports were reviewed for diagnosis and tumor stage. Tumors were staged using the stage grouping of the World Health Organization TNM system (22). 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 and 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 immunostaining was evaluated by two pathologists by determining the percentage of positively staining cells as follows (0) no staining; (+) 1-10% of staining cells; (++) 11-50% of staining cells; (+++) more than 50% of staining 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.

IV. Results

Pure seminoma (n = 32) occurred in men 26 to 74 years of age (mean, 39.4 years; median, 36.6 years). 26 patients (81.25%) had stage I tumors, 6 (18.75%) stage II, and 6 (18.5%) stage III. 9 patients (28.1%) were treated by radical orchiectomy, 8 (25%) by radical orchiectomy followed by regional lymph node radiation, 3 (9.4%) by radical orchiectomy and systemic chemotherapy, and 2 (6.25%) by radical orchiectomy, regional lymph node radiation, and systemic chemotherapy. Tumors ranged in size from 0.3 to 13 cm (mean, 4.4 cm; median, 3.6 cm).

Pure embryonal carcinoma (n = 8) occurred in men 20 to 41 years of age (mean, 34.1 years; median, 36.2 years). 2 patients (25%) had stage I tumors, and 3 (75%) had stage II. Two (25%) patients were treated by radical orchiectomy alone, and 1 (12.5%) underwent radical orchiectomy followed by systemic chemotherapy. Tumors ranged in size from 0.4 to 3.7 cm (mean, 2.5 cm; median, 2.5 cm). Mixed germ cell tumors (n = 14) occurred in men 24 to 46 years of age (mean, 30.1 years; median, 28 years). 6 patients (42.8%) had stage I tumors, 3 (21.4%) had stage II, and 5 (35.7%) had stage III tumors. 4 patients (21.4%) underwent radical

orchiectomy alone, 6 (42.8%) underwent radical orchiectomy followed by systemic chemotherapy, 1 (7%) underwent radical orchiectomy followed by retroperitoneallymph node dissection, and 3 (21.4%) underwent radical orchiectomy and received systemic chemotherapy followed by resection of residual masses. Tumors ranged in size from 2 to 11 cm (mean, 5.5 cm; median, 5 cm). The majority of seminomas did not express cytokeratin intermediate filaments; the number of negatively staining cases ranged from 18 (56.2%) to 32 (100%), depending on the primary antikeratin antibody(**Table 2**).

Table 2

Immunohistochemical staining of pure Seminoma(n=32)									
Positively staining Cells(%)	AEI/AEII	`Keratin Cocktail`	High Molecular Weight Keratin	Cytokeratin 7	Cytokeratin 20	Epithelial Membrane Antigen	Placental Alkaline Phosphatase	CD 30	CAM 5.2
0	20(62.5)	20(62.5)	32 (100)	18(56.2)	32(100)	30 (93.7)	0	30(93.7)	22(68.75)
<1	5(15.6)	5(15.6)	0	4(12.5)	0	2(8.6)	1(4.3)	2(8.6)	5(15.6)
1-10	4(12.5)	4(12.5)	0	5(15.6)	0	0	3(9.3)	0	3(9.3)
11-50	2(8.6)	2(8.6)	0	2(8.6)	0	0	8(25)	0	1(4.3)
>50	1(4.3)	1(4.3)	0	1(4.3)	0	0	20(62.5)	0	1(4.3)

A minority of cases stained positively; 5 cases (15.6%) contained 5% or more cytokeratin-positive cells. Immunohistochemical staining most often was focal with a discontinuous membranous and/or cytoplasmic globular staining pattern (**Image 1**).CK20 and HMWK did not stain any of the seminomas. EMA was identified in only 2 tumors, and the positivity was very focal. CD30 was identified in 2 (6%) of the cases, and staining was very focal. Monoclonal PLAP was identified in 32 tumors (100%); the majority of seminomas expressed more than 50% positively staining cells. 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. The majority of embryonal carcinomas expressed cytokeratins (except CK20 and HMWK); the percentage of positively staining cases ranged from 19 (86.3%) to 22(100%)(**Table 3**).

Table 3

Immunohistochemical staining of Embryonal Carcinoma(n=22)									
Positively staining Cells(%)	AEI/AEII	`Keratin Cocktail`	High Molecular Weight Keratin	Cytokeratin 7	Cytokeratin 20	Epithelial Membrane Antigen	Placental Alkaline Phosphatase	CD 30	CAM 5.2
0	3(13.6)	3(13.6)	22(100)	0	22(100)	20(90.9)	0	1	3(13.6)
<1	0	0	0	3(13.6)	0	1(4.5)	0	1(4.5)	1
1-10	6(27.2)	5(22.7)	0	6(27.2)	0	1(4.5)	0	6(27.2)	2
11-50	2(9)	4(18.8)	0	3(13.6)	0	0	5(22.7)	6(27.2)	6(27.2)
>50	11(50)	10(45.4)	0	10(45.4)	0	0	17(77.2)	8(36.3)	10(45.4)

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,(**Image 2**)AEI/AEIII, `Keratin Cocktail` and CD30 was significantly different between seminoma and embryonal carcinoma (P < .001; Wilcoxon rank sum test).

V. Discussion

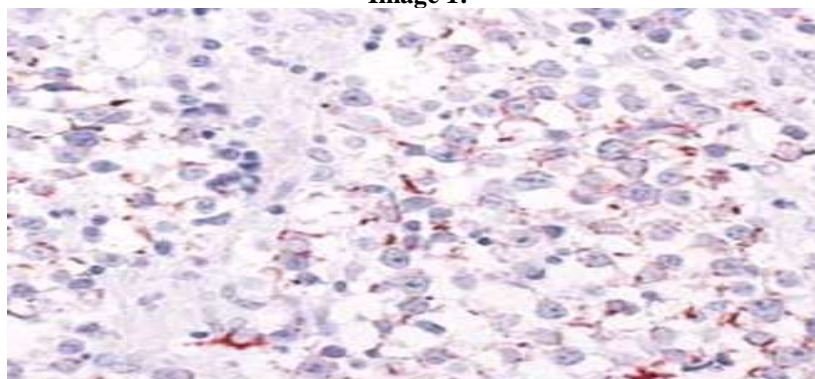
Immunohistochemically IFs have not been found in germ cells, such as spermatocytes and spermatids (**3**),whereas when cell differentiation begins, IFs gradually appeared. IFs have been proposed to possess functions in the maintenance of cell integrity, shape and organelle positioning and in the regulation of the cellular and intracellular movements, but some authors believed that IF expression might be the result of a series of interactions between the microenvironment and synthetic mechanisms rather than as a function of histogenesis(**35,39**).

Some studies have shown that seminoma has the potential to differentiate into nonseminomatous germ cell tumors, and cytokeratin positive Intermediate filaments are present in some seminomas(**11-21**).Fogel et al,(**20**)using immunohistochemical techniques, identified cytokeratin positive Intermediate filaments in 19 of 26seminomas. In their study, cytokeratins 8 and 18 were identified in all cases. Denk et al (**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(24,25). Battifora et al(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(11). However, the consistent expression of cytokeratins 8 and 18 in seminoma recapitulates early epithelial differentiation and these keratins are the first 2 keratins in mouse embryogenesis(24,25). We identified cytokeratin filaments in seminoma of the human testis. Approximately 9.4% of the seminomas contained more than 10% cells that stained positively for 1 of the following antikeratin antibodies: CK7, CAM 5.2, AEI/AEIII, or 'Keratin Cocktail'. There were no associations between cytokeratin staining in seminoma and stage, tumor size, patient age, stage at presentation. CK20 and HMWK were consistently negative in seminoma. Embryonal carcinoma was positive for cytokeratin positive Intermediate 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 was indicative of embryonal carcinoma, and this staining pattern was not seen in seminoma. In regard to our study, CAM 5.2, AEI/AEIII, and 'Keratin Cocktail' 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(18,20). Similar to seminoma, embryonal carcinoma contains cytokeratins 8 and 18, and more complex cytokeratins are seen less commonly (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.

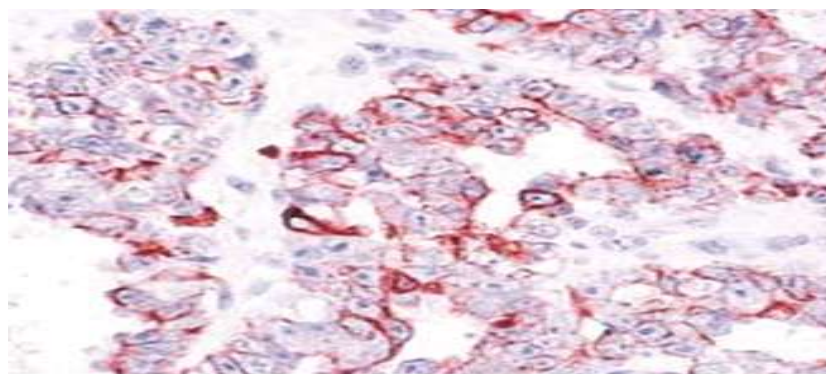
Our study showed that use of CD30 in combination with cytokeratin may be especially useful. CD30 staining was identified very focally in 2 seminomas, whereas in embryonal carcinoma, staining usually is diffuse. Such finding is helpful in needle biopsy specimens. In needle biopsy specimens, on many occasions it is difficult to distinguish seminoma from embryonal carcinoma or poorly differentiated Adenocarcinoma. In this setting, positive cytokeratin staining should not be considered diagnostic of embryonal carcinoma or Adenocarcinoma. In difficult cases, the use of PLAP, cytokeratin, CD30, and EMA may be useful for separating seminoma, embryonal carcinoma, and Adenocarcinoma (27-34). The present study showed that seminoma can contain cytokeratin positive Intermediate filaments, and these can be identified with anti-CK7 antibodies or "cocktails" that contain anticytokeratin 8, anti-cytokeratin 18, or both. There were no differences in patient age, stage at presentation or tumor size between patients whose seminomas contained cytokeratin positive Intermediate filaments and whose seminomas did not. Cytokeratin, CD30, and PLAP stains may be useful for separating seminoma, embryonal carcinoma and Adenocarcinoma in difficult cases. Immunostaining for IFs helps to discover cell changes in the tumors, which are not apparent by light microscopical observation of H&E stained sections, and two patterns of mixed tumors are distinct, which may assist us in understanding the complicated combination of the germ cell tumors and in making an accurate diagnosis.

Image 1:



Cytokeratin AEI/AEIII showing positive staining(x400)

Image 2:



Pure Embryonal carcinoma showing Cytokeratin CAM 5.2 showing intense diffuse positivity (x400)

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