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Evidence of Interepithelial Seminoma Spread into the Rete Testis by Immunostaining of Paraffin Sections with Antibodies Against Cytokeratin and Vimentin

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Summary. Seminomas are tumors of high proliferative activity and show a marked tendency towards local invasion with the capacity for interepithelial spread within the seminiferous tubules as well as into the rete ductules. Immunohistologic investigations were carried out on paraffin sections of 47 typical seminomas. Immunostaining with antibodies against cytokeratin and vimentin allows the convenient detection of even small rete residuals in cases of subtotal rete destruction as well as the identification of discrete interepithelial seminoma spread within the rete ductules, thus facilitating seminoma staging.

Key words: Seminoma – Rete testis – Intermediate filaments – Cytokeratin – Vimentin

Introduction

Seminomas are tumors of high proliferative activity [4, 15, 16, 19] and show a marked tendency towards local invasion [27]. Examination of conventionally stained paraffin sections may occasionally fail to identify small rete residuals, thus making it difficult to define the tumor stage when the rete testis has been destroyed by infiltrating tumor masses. Furthermore, seminomas are capable of interepithelial infiltration into the rete testis by lifting the normal rete epithelium from the basement membrane [12, 20]. When infiltration is very discrete, the identification of tumor cells can be difficult, which risks understaging.

Since the expression of intermediate filaments differs in seminoma cells, Sertoli cells and the rete epithelium [3, 4, 9, 10], immunostaining of rete sites should facilitate morphological staging. 47 seminomas were examined immunohistologically to investigate this assumption.

Material and Methods

Hematoxylin- and eosin-stained (H&E) sections of 47 typical seminomas were reexamined. The tumor staging referring to the International Union Against Cancer classification system [6] is shown in Table 1. The sites of the rete testis were investigated immunohistologically using monoclonal antibodies against cytokeratin (KL1; 23; recognizing cytokeratin subgroups #2–6, 9–12 referring to 11; Lu5; 15; designated as pankeratin antibody) and vimentin (V9; 14; molecular weight of the antigen = 57,000 daltons). The staining procedure was carried out on formaldehyde-fixed and paraffin-embedded material, using the alkaline phosphatase anti-alkaline phosphatase (APAAP) staining procedure [2] modified by Stein et al. [21].

Results

Conventional Light Microscopy

27 of the cases showed infiltration induced rete destruction to a varying degree. Some cases showed only a very circumscribed rete infiltration by the seminoma, whereas, in six cases, the rete was nearly totally destroyed; thus difficulties arose in the identification of rete residuals. In numerous other cases, parts of the rete were completely intact and well separated from the seminoma by connective tissue, but other parts showed a degree of destruction which also gave rise to problems in identifying of rete structures. Spindle-shaped cells intermingled with tumor cells showed no distinct tubular feature and resembled the endothelium of blood vessels (Fig. 1a). In these cases, a pT3 stage was difficult to define.

In addition to obvious rete destruction, seven cases showed unusually large cells between the basement membrane of rete ductules and the normal rete epithelium. These large polyhedral or round cells had a distinct cell border. The cytoplasm was clear or granular, and a large, centrally located nucleus with up to four nucleoli was present (Fig. 2a–c). Morphologically, these cells could not

Table 1. Histological features of the seminomas

		IUC 3/1979[a]			IUC 4/1987[b]		
		pT1	pT2	pT3	pT1	pT2	pT3
Total number	47	13	2	32	22	23	2
Solely destructive invasion of the rete	22	–	–	22[c]	4	18	–
Solely interepithelial spread into the rete	2	–	–	2	2	–	–
Combination of destructive invasion and intraepithelial spread	7	–	–	7	2	5	–
Solely intraluminal growth within rete ductules	1	–	–	1	1	–	–
No rete invasion	15	13[d]	2	–	13	–	2

[a] International Union Against Cancer classification system, 3rd edn, 1979
[b] International Union Against Cancer classification system, 4th edn, 1987
[c] 5 cases with N3, 1 additional with N3M1; all others with NOMO
[d] 1 case with N2, 1 with N3; all others with NOMO; the N-stage cannot be transferred to the new system without additional information about the size of the lymph node metastases

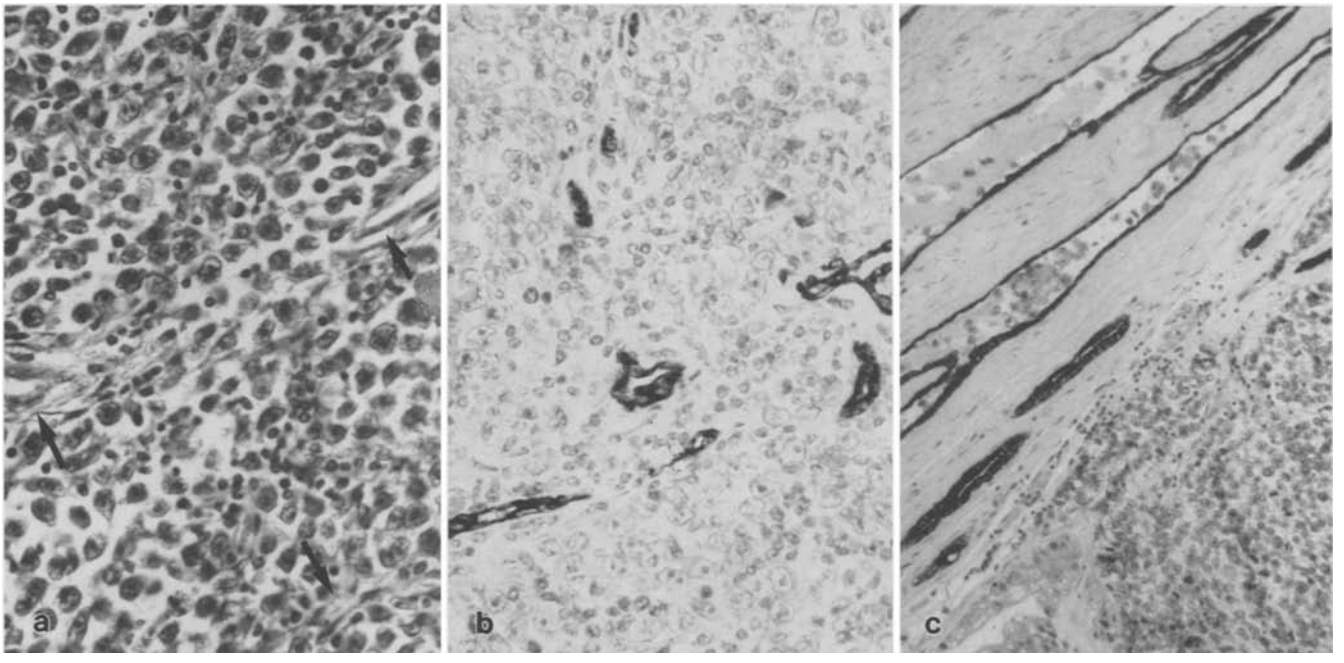

Fig. 1. **a** Subtotal destruction of the rete ductules by infiltrating seminoma masses. Only small tubular residuals can be detected (arrows), which can easily be confused with vascular endothelium (H&E), x200). **b** In contrast, small rete residuals are easily detected within subtotal destruction by the infiltrating seminoma when stained with the cytokeratin antibody (KL1, x200). **c** Intraductular seminoma cell complexes in the rete and solid tumor masses in the vicinity of the rete (lower right corner), both negative for cytokeratin (KL1, x200)

be confused with Sertoli cells or spermatogonia and were suspected of being seminoma cells. In these cases, the cell complexes were within the vicinity of destructively growing tumor masses (Fig. 2c), whereas, in two additional ones, this type of rete involvement was not accompanied by rete destruction, and the ductules were separated from other tumor sites by sheets of connective tissue (Fig. 2a, b). Since the rete ductules did not seem to have been invaded by these cells from outside in any of these cases, the phenomenon could not be interpreted as a result of continuing destruction; an intact or at least not completely destroyed ductular basement membrane separated the interepithelial cell complexes from the surrounding tumor masses (see Fig. 2c), suggesting primary interepithelial growth.

One case showed only intraductular expansion of the seminoma into the rete testis without involvement of the epithelium or of the stroma (Fig. 1c).

The results are summarized in Table 1.

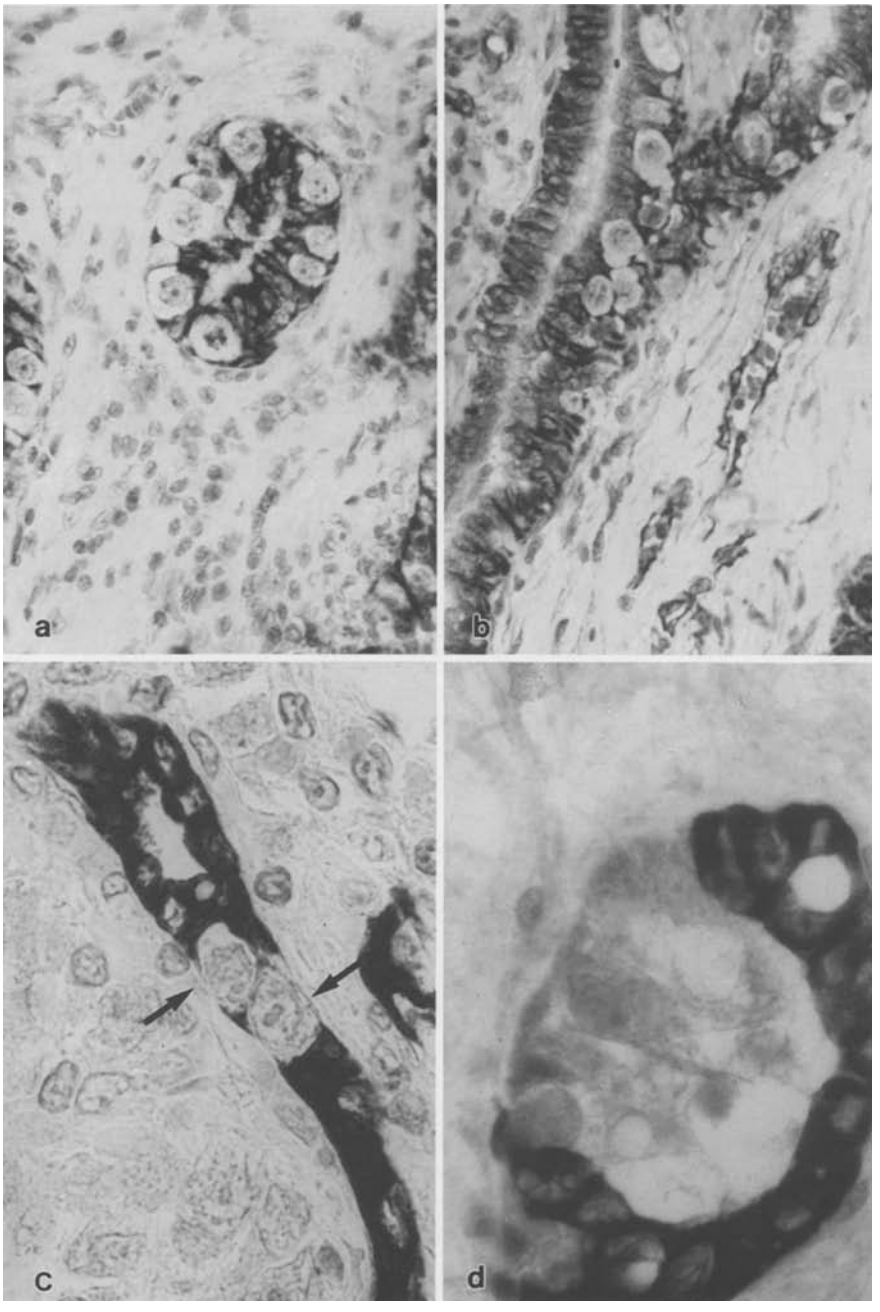

Fig. 2. a Complexes of large round cells with a fairly central nucleus and up to four nucleoli within the rete epithelium. They resemble the seminoma cells of other sites of the same testicle. The cell complexes do not express cytokeratin; the rete epithelium reacts strongly (KL1, x200). **b** The same feature as in A, reaction with the vimentin antibody V9. The rete epithelium expresses vimentin, but the interepithelial cell complexes do not. Note the vimentin expression of the vascular endothelium as compared to their lack of cytokeratin expression shown in A (V9, x200). **c** Some large cells with large nuclei, hyperchromatic nucleoli and granular cytoplasm. Located within the rete epithelium, they are morphologically identical to the surrounding cells of the destructively growing seminoma. They seem to be separated from the tumor masses in the vicinity by a basement membrane (arrows). The rete epithelium expresses cytokeratin, while the seminoma does not (KL1, x630). **d** Continuous change from the rete epithelium to Sertoli cells within the same tubule. Note the marked decrease and lack of cytokeratin expression in Sertoli cells (KL1, x200)

Immunohistology

The rete epithelium coexpressed cytokeratin and vimentin (Figs. 1b, c, 2a–d) with a slight preponderance of cytokeratin. In contrast, the seminoma cells were negative for both intermediate filaments (Figs. 1b, c, 2a–c). All Sertoli cells expressed vimentin but were almost all cytokeratin-negative (Fig. 2d; Table 2). However, we consistently found some seminiferous tubules which contained numerous Sertoli cells with distinct expression of cytokeratin. This was confirmed by our results on tumor free testicles removed from patients with carcinoma of the prostate as well as on fetal gonads (in preparation). The tubules were scattered throughout the testicle, not concentrated in the vicinity of the rete. Several specimens showed some tubules which were lined by a cylindrical epithelium resembling that of the rete and coexpressed both intermediate filament, which also showed no topographic relation to the rete.

In those cases where the large cells were detected within the rete epithelium, as mentioned above, immunostaining did not reveal expression of either keratin or vimentin (Fig. 2a–c) which suggested association with the tumor. In the one case with solely intraluminal tumor spread into the rete, the intraductular tumor formations shared the lack of cytokeratin and vimentin expression with the infiltrating seminoma sites within the testicle (Fig. 1c).

Even the smallest residuals of the rete testis in infiltrating seminoma sites were easily detected, even at low mag-

Table 2. Immunohistologic features of seminomas and normal cell components of the male gonad using the monoclonal antibodies KL1 and V9

Antigen	Seminoma	Sertoli cells	Rete testis	Efferent ductules	Endothelium
Cytokeratin	–	–/++	+++	+++	–
Vimentin	–	+++	+++	–	+++

– = negative; ++ = moderately positive; +++ = strongly positive reaction. Sign before the slash = majority, sign after the slash = minority of cells. The results refer to paraffin-embedded material

nifications, by cytokeratin or vimentin staining (Fig. 1b) and could be distinguished from vascular endothelium by the expression of cytokeratin (see also Fig. 2a, b).

Discussion

According to our results, there are three ways for seminomas to spread into the rete testis: intraluminal growth, interepithelial infiltration and destructive invasion. They may be combined, i.e. destructive invasion may occur with or without interepithelial infiltration, and interepithelial infiltration may occur with or without destructive invasion. The greater number of advanced pT- and N-stages within the group of destructively growing seminomas, as shown in Table 1, may indicate that interepithelial infiltration is an early or mild stage of tumor spread. But the number of cases with solely interepithelial spread into the rete is too small for this interpretation. However, no relation was found between tumor size, ranging from 1 to 970 ccm, and tumor stage or kind of infiltration (see also [4]).

The ability of seminoma cells to migrate into evaginations of the seminiferous tubules without initial disturbance of the basement membrane has been investigated by electron microscopy [5, 18]. Intraductular seminoma spread into the rete with lifting of the normal epithelium from the basement membrane has been described [12]. This was also documented by Skakkebaek et al. [20] for atypical germ cells within seminiferous tubules and within the rete ductules (see [20], Figs. 1, 3). It can easily be identified in cases of massive invasion, as demonstrated by Mostofi and Price ([12], Fig. 13) and was observed in several cases in this study. According to our results, immunostaining facilitated the identification of discrete interepithelial spread of seminomas into the rete. Moreover, Sertoli cells, which are occasionally observed within one tubule in addition to the normal rete epithelium (Fig. 2d), were easily distinguished from seminoma cells by their expression of vimentin. While the identification of seminoma cells or atypical cells of spermatogenesis with ferritin-antibodies produced some exceptions [7, 8], the differentiation of seminoma cells, Sertoli cells and the rete epithelium was not restricted when the cytokeratin- and vimentin-antibodies KL1 and V9 were used, as in this study.

On the other hand, subtotal destruction of the rete by an infiltrating seminoma will give rise to difficulties in the identification of rete residuals, and these may be confused with the endothelium of small blood vessels, as shown in Fig. 1a, hence impairing staging. The demonstrated differences in cytokeratin and vimentin expression allow a convenient distinction between these cell populations.

Thus, immunohistology makes some contribution to pathological tumor staging.

The usual lack of cytokeratin in seminoma cells is confirmed by previous reports [1, 3, 9, 10, 17]. While vimentin expression to varying degrees has been detected by the authors cited, we never found either cytokeratin or vimentin expression in seminomas, vascular endothelium serving as control for the vimentin reaction and the rete testis as control for that of cytokeratin (see Figs. 1a, b, 2a–c). According to our results, occasional marked differences in vimentin expression of seminomas were related to the use of frozen or paraffin-embedded sections (in preparation). However, for practical reasons, the use of paraffin-embedded material from sites of possible tumor infiltration is preferable for tumor staging, because the morphology is not always preserved to the same degree in cryostat sections. For that reason, immunofluorescence methods, which have been used for systematic studies [3, 9, 10], are not applicable for routine diagnosis of tumor infiltration. Finally, cytokeratin and vimentin antibodies not reacting with seminoma cells are very useful for their identification within the cytokeratin-positive and vimentin-positive rete testis.

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