HISTOPATHOLOGY AND GROWTH PATTERNS OF NOVEL MICRONEOPLASMS IN EYES WITH RETINOBLASTOMA

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In this retrospective study, all available stained tissue sections of 91 enucleated eyes known to contain retinoblastoma, obtained from the files of two medical centers, were examined under the light microscope. In 17 of these eyes, one or several microneoplasms with distinctive histopathological features, differing from those of both retinoblastoma and retinocytoma, were discovered within remnants of histologically normal, mature retina, whether attached or detached. These microneoplasms seemed to originate within either the outer or the inner nuclear layers and sometimes occurred in crops. Their presumed manner of growth, as inferred from their differing histopathological appearances in the various specimens, is analysed in detail. The clinical significance of these tumors remains to be elucidated. (Saudi J. Ophthal 1993;7:11-16)

Key words crops of microspherules - microneoplasms, retina - retinoblastoma - retinocytoma - subset, retinoblastoma - tenting

RETINOCYTOMA has been categorized by Margo and coworkers as a benign variant of retinoblastoma (Rb).[(1)] This paper provides histopathological evidence that suggests the existence of yet another, currently unrecognized subset of retinoblastoma consisting of microspherules of neoplastic cells with certain characteristic features.

Materials and Methods

All available tissue sections from a total of 91 globes enucleated for retinoblastoma (74 from patients of King Khaled Eye Specialist Hospital (KKESH) and 17 from the Montefiore Medical Center) were re-examined under the light microscope. On average, four hematoxylin and eosin (H&E) stained sections were available from each globe. A systematic search of all available segments of mature retinal tissue, not involved by the retinoblastoma (Rb), was made. Some of the cases were found to be unsuitable for a search for microneoplasms, since no remnants of uninvolved retina could be detected.

In the 74 cases from KKESH, corresponding to 81% of all cases, clinical records corresponding to the sectioned eyes were available and were reviewed.

Results

Histopathological Findings

In 17 of the examined globes (15 eyes from KKESH and 2 eyes from Montefiore Medical Center), derived from 16 patients, microneoplasms with histopathological features distinctly different from both Rb and retinocytoma were detected. Figures 1 – 8 illustrate many of these features. Descriptive terms including "microspherule", "oblate microspherule", and "micropolypoid

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structure" are used by us to characterize morphological differences (presumably induced by particular local events or forces) among these microneoplasms. Oblate microneoplasms were seen only rarely and are not displayed in any of our photomicrographs. The lesions coexisted with, but were clearly separate from Rb elsewhere within the same globe. They occurred exclusively within mature retinal tissue which was otherwise histologically unremarkable, even though it might occasionally be displaced or detached. No difficulty was encountered in differentiating between Rb, Rb implants or seedlings, and the microneoplasms here discussed. In all cases where serial sections were available, no evidence of any direct continuity with Rb could be demonstrated.

The term "tenting" aptly describes a diagnostically important histopathological feature of passive spatial accommodation provided by adjacent segments of retinal limiting membranes and/or layers, as a consequence of preemptive spherical expansion of microspherules in situ. Such tenting is evident in several of the photomicrographs of our sections (Figs. 1, 2, and 4 – 8) and provides a significant clue leading to the accurate determination of the retinal layer of origin of the tumor, as well as the direction of its growth. A hypothetical flow-chart regarding the possible transformations of the microspherules, and the morphogenesis of the various structural forms of the microneoplasms seen by us, is displayed in Figure 9.

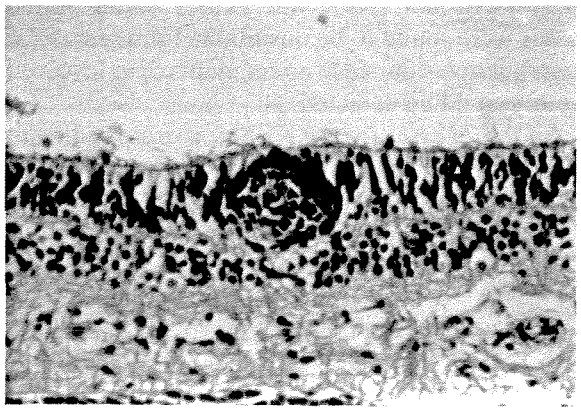


Figure 1. Single, nonencapsulated, circumscribed microspherule arising within the ONL. Features: cellular atypism, centrifugal growth in situ with compression displacement of adjacent parenchymal cells, tenting of both the OLM and the outer plexiform layer (OPL), mild compression displacement and/or atrophy of the INL. (H&E; original magnification (O.M.) × 200).

Figure 2. Nonencapsulated, circumscribed microspherule arising within the ONL. The OPL is markedly compressed and tented (arrow). Extensive cystoid degeneration in the INL. OLM intact but very slightly tented. (H&E; O.M. × 100).

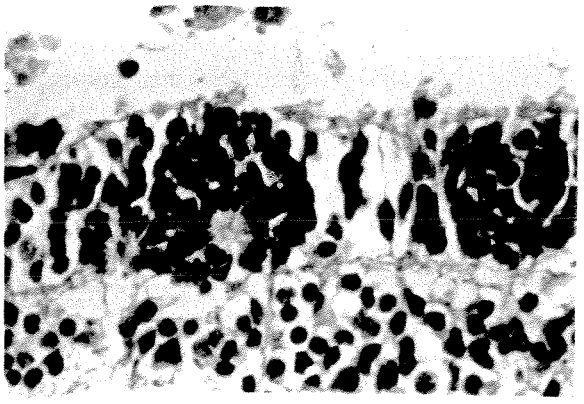


Figure 3. Two adjacent microspherules of similar size within the ONL, forming a crop. Minimal compression and displacement of adjacent parenchymal cells, but very little or absent tenting of OPL or OLM. Homer Wright rosettes within the left microspherule. (H&E; O.M. × 400)

From their histopathological appearance, microneoplasms were determined to originate as microspherules within either the inner nuclear layer (INL) or the outer nuclear layer (ONL) of mature and otherwise intact remnants of retina. Characteristic features, apart from those listed as helping in differentiation from either retinocytoma or retinoblastoma (Table 1), included non-encapsulation, a generally monomorphic cytology, and occasional compression atrophy of adjacent parenchyma. Homer Wright rosettes were infrequently observed (Figs. 3 and 8A), Flexner-Wintersteiner rosettes were occasionally seen (Figs. 7, 8B, 8C), but fleurettes were not found in any of these tumors.

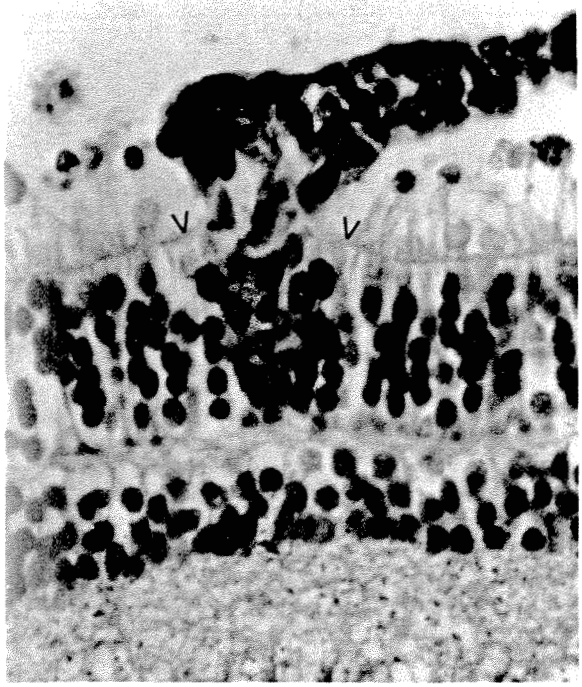


Figure 4. Micropolypoid structure, presumably resulting from an antecedent microspherule arising within the ONL which has perforated the OLM and spread into the subretinal space. Note the subtle but definite tenting of the OLM, and eversion of its edges at the perforation site (arrowheads), indicating the direction of growth. The neoplastic cells are of atypical shape and have larger nuclei than the adjacent normal parenchyma, with prominent nucleoli. (H&E; O.M. × 200)

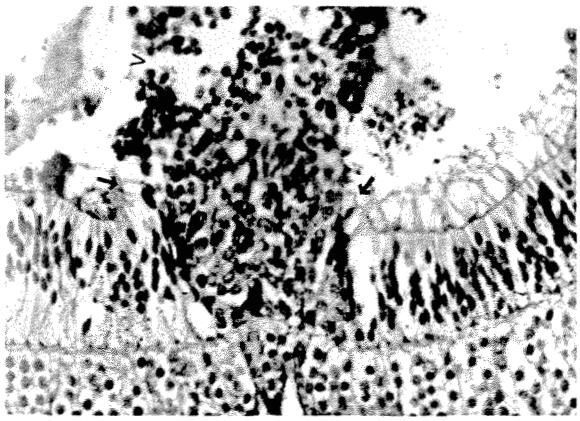


Figure 5. Similar situation as in Figure 4, but in this case the perforation of the OLM has left clearer traces: the arrows point to the both everted and retroflexed OLM. The arrowhead indicates a miniscule infarct. Note the "negative tenting" in the OPL. (H&E; O.M. × 200)

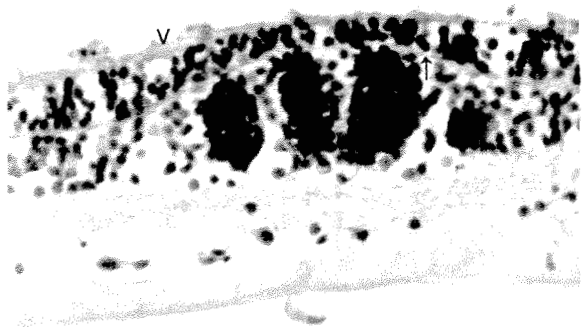


Figure 6. Crop of 4 microspherules of identical cytological atypism, within the INL. Note the tenting of the OPL (arrow) and early compression atrophy of the ONL, commencing at the arrowhead. Serial sections revealed the apparently smaller microspherule on the right to in fact be of equal size to its companions. (H&E; O.M. × 300)

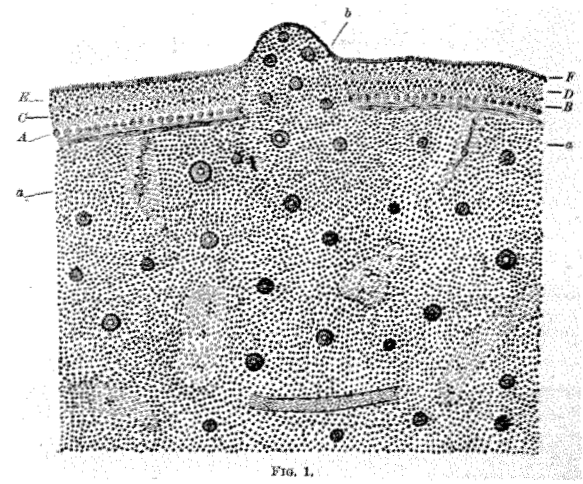
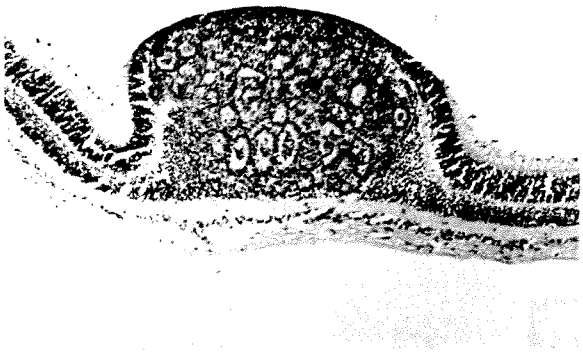


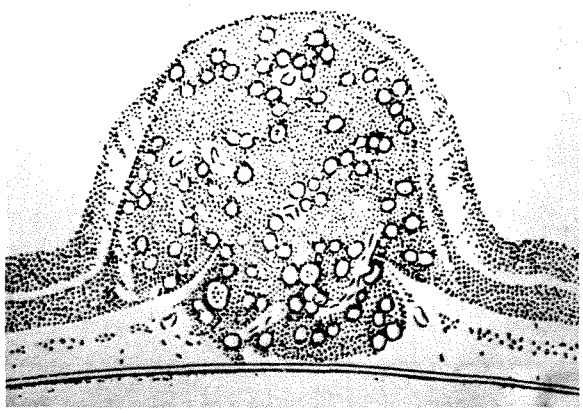
Figure 7. Reproduction of drawing by Flexner (1891) – see text. Flexner identified the site marked "b" as the point of origin of the tumor in the ONL, adjacent to the OLM and the photoreceptor layer, and presumed further growth into the vitreous cavity after breaching of all other retinal layers. We use the term tenting for what Flexner called "pushed upwards". Multiple Flexner-Wintersteiner rosettes are drawn. (Magnification not stated)

Clinicopathological Correlation

The clinical records for those eyes that were found to contain microneoplasms concurrently with the Rb were found not to differ in any respect from those that did not.



A



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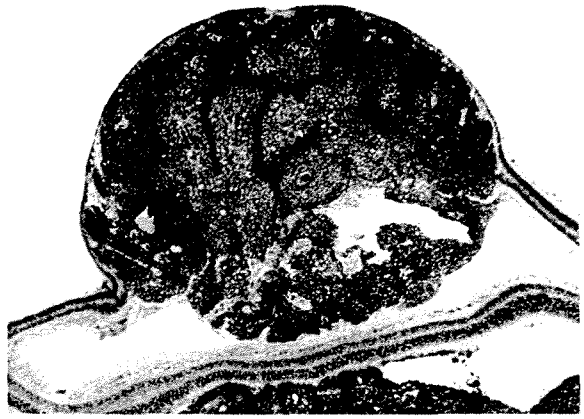

C

Figure 8. Three examples of microneoplasms with many comparable features, namely: nonencapsulation, circumscription, epicenter and origin in the INL, centrifugal expansion in situ, compression atrophy of contiguous ONL.
Figure 8A. This specimen from our collection additionally shows compression atrophy of the photoreceptor layer; Homer Wright rosettes are present. (H&E; O.M. × 100)
Figure 8B: Wintersteiner's drawing of his case shows perforation of the tumor into the nerve fiber and ganglion cell layers; Flexner-Wintersteiner rosettes are indicated by the empty circles. (Magnification not stated)
Figure 8C: One of our cases similarly shows complete replacement of the nerve fiber and ganglion cell layer by the expansile neoplasm, and compression atrophy of the photoreceptor layer, as well as numerous Flexner-Wintersteiner rosettes. (H&E; O.M. × 100)

TABLE 1. *This comparison summarizes the several exclusionary clinical, histoarchitectural, and histopathological features differentiating the three subset entities of retinoblastoma.*

	Proposed subsets of retinoblastoma		
Features	Retinocytoma	Microneoplasm	Retinoblastoma
Clinically benign	+	–	–
Plaque formation	+	–	–
Microspherules	–	+	–
Fixed epicenter	–	+	–
Centrifugal expansion in situ	–	+	–
Circumscription	–	+	–
Tenting	–	+	–
Micropolypoid structure	–	+	–
Crop formation	–	+	–
Cytological malignancy	–	±	+
Implants/seedlings	–	–	+
Perivascular cuffing	–	–	+
Vascular invasion	–	–	+
Metastases	–	–	+
Necrosis	–	–	+

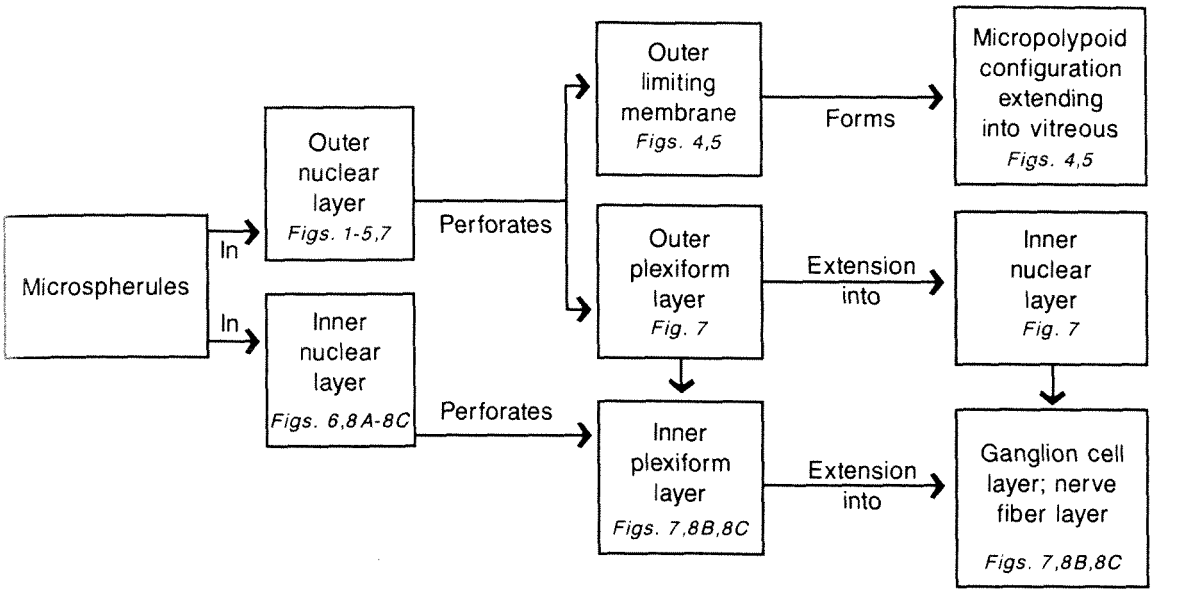

Figure 9. Hypothetical flow chart of the growth patterns of microspherules, with references to Figures 1 to 8.

Discussion

Our findings indicate that while the described microneoplasms do coexist with retinoblastoma (Rb) within the same globe, they appear to comprise a distinctly identifiable, separate histopathological subset of Rb.

In our study we found no histopathologic evidence that the origin of the microneoplasms was the consequence of Rb-emboli within the retinal microcirculation. Embolization as a pathogenetic mechanism for these microspherules does not explain either their origin within the ONL (which is devoid of retinal vasculature), nor their occurrence in crops (such as seen in Figures 3 and 6).

The morphologic feature of crops of identical contiguous microspherules has never been reported for either Rb or retinocytoma. Like the microspherules themselves, we found it to occur more frequently in the ONL than in the INL. The striking, mimetic identity of cytology and histoarchitecture of all microspherules within a particular crop implies a synchronous rate of growth, and points to the possible existence of regulatory mechanisms that control the entire set. We propose that each microspherule within a crop may be the clonal progeny of a singular set of identical but segregated ancestral cells, one for each microspherule. At some subsequent time, these separate ancestral cells may be simultaneously activated by a hypothetical, unidentified, soluble metabolite to replicate synchronously.

Historial Overview

Since Wardrop first established Rb as an entity in 1809[2], no mention has been made in the literature regarding the morphological relationships between the microneoplasms described by us, and the clinically obvious disease. In spite of this, we believe that the entity, though unrecognized, has been recorded by two observers in the last century:

Flexner[3], in 1891, stressed the feature of tenting of the outer limiting membrane of the retina (OLM) and appreciated its significance as giving an indication of origin and direction of growth of a tumor (which, in his case, he hesitantly labelled "neuroepithelioma of the retina"). This may be seen from the following two quotes:

1. "For in our case there cannot be the least doubt as to the place of origin of the tumor,

that it was in the external layer, and that far from invading other layers of the retina it has not spread out in this one to any extent."

2. "... the tumor in the external nuclear layer ... at the point of origin is noticed to be pushed upwards."

It is apparent from both his text and his drawing (Fig. 7) that Flexner's use of the phrase "pushed upwards" means that the OLM and the photoreceptor layer are focally displaced towards the choroid, which corresponds to what we call tenting. Both Flexner and we interpret the site of tenting to point to the epicenter of tumor origin.

Wintersteiner[4], also in the nineteenth century (1897), described "... a miliary nodule that has developed in the inner nuclear layer." His drawing is reproduced in Figure 8B. These three microneoplasms (Figs. 8 A-C), separated by a century and the Atlantic Ocean, replicate distinctive histopathological features and are virtually interchangeable.

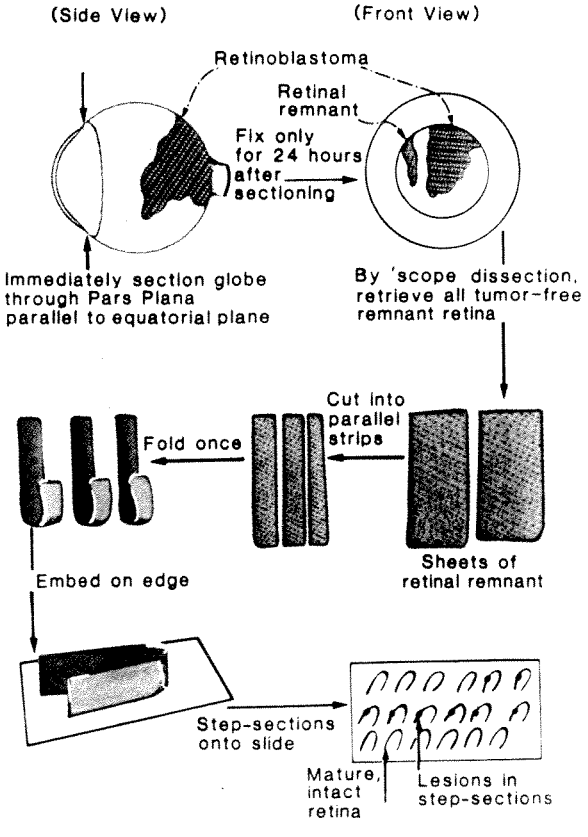

Figure 10. Proposed method of histopathological processing of eyes with retinoblastoma and normal retinal remnants, to optimize search for microneoplasms without compromizing traditional diagnostic objectives.

Future Outlook

Based on our experience, an improved method for gross and microscopic processing of Rb-containing globes (Fig. 10) would be highly desirable for future clarification of the true incidence of the described microneoplasms, as well as further elucidation of their features. A prospective study, concurrent with use of this improved method of processing, would be most helpful for determining the clinico-pathological significance of the presence or absence of these microneoplasms in any one case of Rb, as well as possibly differentiating and elucidating genetic aspects of Rb, with a view toward improving treatment and prognosis for the patient.

Conclusions

The histoarchitectural and histopathological evidence presented in this paper suggests that, in addition to retinocytoma, microneoplasms might constitute a second, as yet unrecognized subset within the entity of retinoblastoma. Appropriately designed and sufficiently large prospective studies and correlated clinico-pathological analyses are now in order.

Acknowledgement

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