Differentiation of Human Retinoblastoma in Vitro *Into Cell Types With Characteristics Observed in Embryonal or Mature Retina*

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The capacity of a primitive human retinoblastoma cell line (Y-79) to differentiate into several cell types of normal human retina was investigated. Cells were studied in suspension and monolayer cultures, in serum-free or serum-supplemented medium, and in the presence or absence of differentiating agents such as N^6O^{12}-dibutyryl adenosine 3',5'-cyclic monophosphate (dbc-AMP) and sodium butyrate (Nabut). Electron microscopy, immunohistochemistry for detection of myelin basic protein (MBP), and formaldehyde-induced fluorescence (FIF) for catecholamines were performed. Treated cells exhibited morphologic characteristics supportive of differentiation toward photoreceptors, conventional neurons and glial cells, increased FIF reactivity, and MBP expression. Growth in serum-free medium without differentiating agents led to a similar but less enhanced morphologic differentiation. These results confirm the concept that human retinoblastoma originates from a primitive neuroectodermal multipotential cell. (Am J Pathol 1986, 123: 542–552)

WE HAVE recently postulated that retinoblastoma originates from a primitive multipotential retinal cell.[1] This was based on experiments *in vitro* which showed simultaneous expression of a neuronal (neuron-specific enolase [NSE]) and a glial (glial fibrillary acidic protein [GFAP]) marker by the undifferentiated cells of the human retinoblastoma cell line Y-79 and preferential expression of the appropriate marker after morphologic differentiation toward neuronal- or glial-like cells. The morphologic changes which were observed in the Y-79 cells by light microscopy occurred after addition of N^6O^{12}-dibutyryl adenosine 3', 5'-cyclic monophosphate (dbc-AMP)[2] and sodium butyrate (Nabut)[3] in long-term monolayer cultures. Other authors have also reported dual properties in retinoblastoma cells *in vitro* (ie, simultaneous expression of dopamine-β-hydroxylase and GFAP).[4]

In the present study we evaluated the ultrastructural appearance of the Y-79 cells before and after treatment with dbc-AMP and Nabut and further studied their ability to 1) express myelin basic protein (MBP), which is normally present in Müller cells,[5] oligodendrocytes, and Schwann cells,[6] and 2) produce catecholamines or catecholamine precursors, as shown previously for the amacrine cells.[7,8]

Materials and Methods

Cell Cultures—Differentiation Experiments

Monolayer cell cultures from the Y-79 cell human retinoblastoma cell line were obtained as previously described.[2] The plastic tissue culture flasks were treated with poly-D-lysine (0.2 mg/ml) (Sigma Chemical Co., St. Louis, Mo) in sodium borate, pH 8.2, for 6 minutes, washed in serum-free medium, and coated with human fibronectin (5 μg/ml) (Sigma) in serum-free medium for 20 minutes at 37 C. Y-79 cells were grown on these prepared substrata in the presence of serum-supplemented or serum-free medium. Eagle's minimum essential medium with Earl's salts, supplemented with 10% fetal calf serum, 2 mM glutamine, and 100 U/ml penicillin (GIBCO, Grand Island, NY), constituted the serum-supplemented medium, whereas the serum-free medium was composed of the same medium with 5

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μg/ml insulin, 10 μg/ml transferrin, 8.8 ng/ml putrescine, 4 ng/ml sodium selinite, and 6.3 ng/ml progesterone.[9]

The differentiating agents dbc-AMP (4 mM) and Nabut (2 mM) (Sigma) were added at 3-day intervals beginning on Day 8 (3 doses total) as previously described.[2,3]

Electron Microscopy

For electron microscopy, cells from each condition were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4) for 1–24 hours, postfixed in OsO_4, and embedded in Maraglas 655 (Ladd Research Industries, Burlington, Vt). In some experiments the cells were exposed to 1% tannic acid for 30 minutes prior to fixation with glutaraldehyde for enhancement of the membranes. Sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 electron microscope.

Immunocytochemistry

For detection of MBP, the avidin–biotin peroxidase complex (ABC) method of Hsu et al,[10] as well as immunofluorescence,[1] were employed. Cytospin smears from all the conditions (ie, in serum-free and serum-supplemented medium, with or without differentiating agents) were fixed in cold acetone (−20 C) for 5 minutes and washed in phosphate-buffered saline (PBS) for 10 minutes. When the ABC method was employed, the endogenous peroxidase activity was blocked by incubation in 30% H_2O_2 in methanol for 30 minutes. The smears were then washed in PBS, and the nonspecific antigenic sites were blocked by incubation in 10% egg albumin solution in PBS for 20 minutes. Excess albumin was blotted from the sections, and the primary antiserum, a monoclonal antibody against monkey MBP (Hybritech Inc., San Diego, Calif), was applied (concentration 1:100) overnight at 4 C. On the next day the primary antibody was washed off with PBS, and a biotinylated anti-mouse IgG (concentration 1:50) was applied for 30 minutes at room temperature. After washing in PBS, the smears were subsequently covered with ABC for 30 minutes at room temperature.

For immunofluorescence, the smears were fixed similarly (cold acetone), washed in PBS (20 minutes), and covered directly with the anti-MBP monoclonal antibody at the same concentration and conditions as for the ABC method. On the next day, the primary antibody was washed off in PBS, and a fluorescein-conjugated anti-mouse IgG (concentration 1:20) was applied for 30 minutes at room temperature. The smears were then washed well in PBS (20 minutes) and coverslipped with 50% glycerol in PBS. Fluorescence was evaluated with a Zeiss standard microscope equipped with an epifluorescence illuminator and FITC narrow-band filter. Both secondary antiserum and ABC were purchased from Vector Lab., Inc., Burlingame, California. The reaction was developed with 0.05% diaminobenzidine tetrahydrochloride (Sigma) in PBS with 0.01% H_2O_2. At the end, the smears were washed, counterstained with hematoxylin, and mounted.

In addition to the Y-79 cells, several other human tissues and neoplasms were investigated for the presence of MBP by the ABC method and immunofluorescence, serving as positive and negative controls. Lung, skin, retina, spinal cord, Ewing's sarcoma, rhabdomyosarcoma, primitive sarcoma of bone, a glioma cell line, and four neuroblastoma cell lines constituted the control tissue.

Formaldehyde-Induced Fluorescence (FIF)

For FIF, cytospin smears from each condition were dried immediately over phosphorus pentoxide under vacuum, placed over paraformaldehyde powder (70% humidity) in a dessicator, and heated in an oven at 80 C for 1 hour.[11] The cells were then examined under a Zeiss standard microscope equipped with a catecholamine filter pack.

Results

Morphology of Y-79 Cells by EM

The ultrastructural features of the Y-79 cells ranged from very primitive (cells in suspension) to ones supporting differentiation into various cell types occurring normally in the adult or embryonal human retina, ie, neuronal, photoreceptor, and glial cells. The percentages of these cell types in the various studied conditions, estimated grossly from the examined grids, are shown in Table 1, and their morphologic characteristics are analyzed in detail for each condition.

Cells in Suspension

The overall ultrastructural appearance of the cells growing in suspension was similar to that previously described.[12] The cytoplasm contained sparse organelles, ie, mitochondria, Golgi apparatus, multivesicular bodies, polyribosomes, very short strands of rough endoplasmic reticulum (RER) microtubules, and occasional basal bodies. Cilia were not encountered. The nuclei exhibited prominent nucleoli, infoldings of the nuclear envelope, and occasional triple membrane structures. The tumor cells were closely apposed but exhibited delicate cytoplasmic membranes with primitive

Table 1—Degree of Morphologic Differentiation of the Y-79 Retinoblastoma Cells Under Various Tissue Culture Conditions

Growth condition	Agent	Cell type			
		Undifferentiated	Photoreceptor	Conventional neurons	Glial cells
Suspension culture	None	100%	0	0	0
Attachment culture					
Serum-supplemented medium	None	100%*	0	0	0
	dbc-AMP	>75%	<10%	1–5%	<1%[a]
	Nabut	>70%	10–20%	1%	<1%[a]
Serum-free medium	None	>90%*	0	<10%†	<1%[b]
	dbc-AMP	70%	<1%	20–30%	<1%[b]

* Cells showed minor signs of differentiation such as low nuclear-cytoplasmic ratio, occasional intermediate cytoplasmic filaments, and enhanced rosetting.
† Neuronal differentiation was subtle, characterized mainly by long processes, although NSG were not identified.
a, astrocyte-like; b, oligodendrocyte-like.

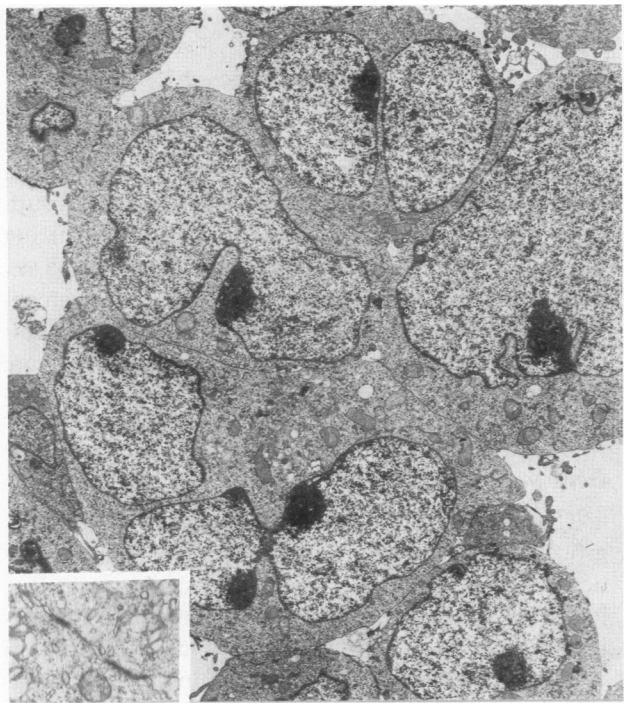
intercellular attachments of the macula adherens type (Figure 1).

Cells Growing in Monolayers and Serum-Supplemented Medium

Mere attachment of the cells to the substrate resulted in changes suggestive of minor differentiation. Specifically, very few cells showed abundant cytoplasm and a higher number of mitochondria and intracytoplasmic microtubules. Focal aggregates of intermediate intracytoplasmic filaments were also noted (Figure 2). Rosette formation was more obvious than in cells growing in suspension. The intercellular junctions did not increase in number.

Cells Growing in Monolayers and Serum-Free Medium

Growth of the Y-79 cells in serum-free medium resulted in 1) the development of long cytoplasmic processes which were devoid of organelles and contained occasional aggregates of intermediate filaments (Figure 3a) and 2) appearance of lamellated membra-



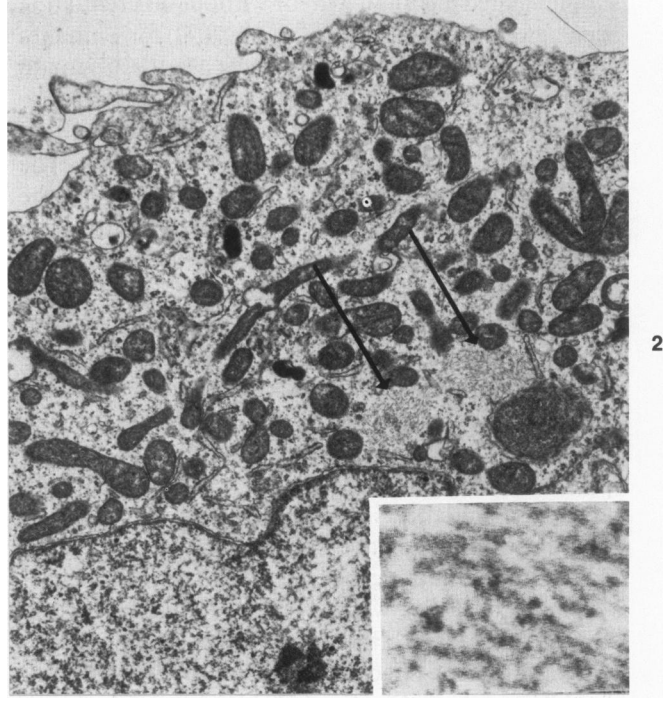


Figure 1—Y-79 retinoblastoma cells growing in suspension. The cells show a high nuclear to cytoplasmic ratio and lack specialized cytoplasmic structures. The cytoplasmic membranes are smooth and delicate. (×3300) Occasional primitive attachments of the macula adherens type are present. (**Inset**, ×200,000) **Figure 2**—Cytoplasmic detail of a retinoblastoma cell from a monolayer culture on poly-D-lysine- and fibronectin-coated substratum. Mere attachment of cells resulted in cytoplasmic increase and appearance of a fair number of cytoplasmic organelles (mitochondria and RER). Focal aggregates of intracytoplasmic filaments (*arrows*) were also observed. These filaments were of the intermediate size (8–10 nm) (**Inset**). (×13,000; **Inset**, ×120,000)

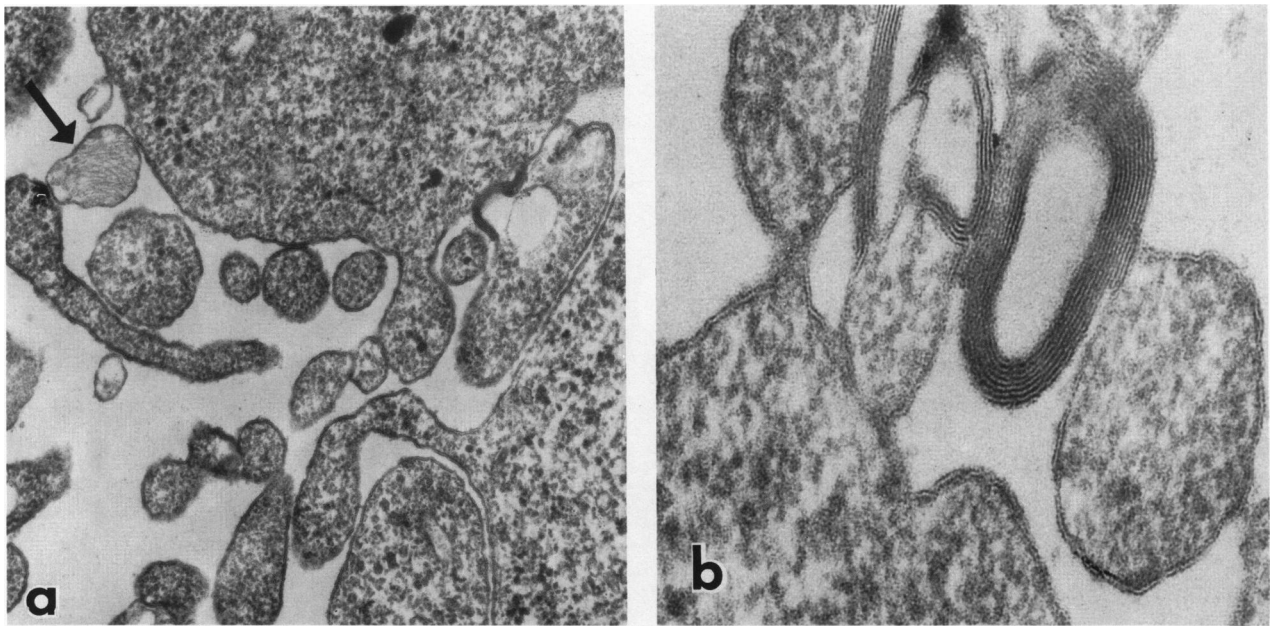


Figure 3—Retinoblastoma cells growing in serum-free medium. **a**—Cytoplasmic processes on cross and longitudinal sections. Most of them were devoid of organelles and contained only polyribosomes. They occasionally contained filaments measuring up to 7 nm in diameter (*arrow*). (×44,000) **b**—Some of the retinoblastoma cells in serum-free medium showed membranous proliferations with a lamellated appearance. These membranous structures were present both on the surface of the cells as seen in this figure as well as inside the cytoplasm. They were reminiscent of myelin but lacked the intraperiodic lines of myelin. They probably represent an excessive membranous flow consistent with early atypical forms of myelin. (×13,000)

nous structures, both in the cytoplasm and on the surface of the cells (Figure 3b). The former cells were considered as evidence of early neuronal differentiation and constituted approximately 10% of the total cellular population. The latter cells constituted less than 1% of the cellular population and were interpreted as possible early forms of myelin-producing (Müller-type) glial cells. However, fusion of the outer leaflets of these membrane units was not seen. Therefore, although reminiscent of myelin, the membranous structures lacked the characteristic intraperiodic lines of myelin; and the possibility of their being artifactual cannot be excluded entirely. Changes in the number of cytoplasmic organelles or the intercellular attachments were not observed in the cells grown in serum-free medium. However, the number of rosettes was slightly increased when compared to that in the cells growing in suspension or even in monolayers with serum-supplemented media.

Cells Treated With dbc-AMP and Nabut in Serum-Supplemented and Serum-Free Media

Y-79 cells treated with dbc-AMP and Nabut showed ultrastructural features of differentiation toward photoreceptors, conventional neurons, and glial cells.

EM Evidence for Photoreceptor Cell Differentiation

Rosette Formation. Rosettes were formed to a much greater extent by Y-79 cells treated with Nabut or dbc-AMP than by cells growing without the above agents. Rosette formation was especially prominent in cells grown in serum-supplemented versus serum-free medium and treated with dbc-AMP and Nabut (10–20%). The rosette-forming cells showed microvillous processes on their surface facing the lumen and a fair number of mitochondria (Figure 4). In addition, well-developed intercellular attachments of the zonula adherens type were observed between the rosette-forming cells, particularly at their apical borders (Figure 4, inset).

Focal Microvillous Cytoplasmic Projections. These projections were seen in both dbc-AMP- and Nabut-treated cells grown in serum-supplemented as opposed to serum-free medium (Figure 5a and b). They were present at the cytoplasmic surfaces of apposed cells with or without an intervening space and were accompanied by well-developed junctional complexes bilaterally. Occasionally, centrioles and cilial rootlets were present at the base of these microvillous projections (Figure 5a). Well-developed cilia were not observed. Most of the microvillous cytoplasmic projections contained actin filaments measuring up to 7 nm in diameter, whereas others were filled with smooth membrane-bound vesicles of various sizes (Figure 5b). Numerous membrane-bound vesicles of smaller size were also present in the cytoplasm of these cells (Figure 5a).

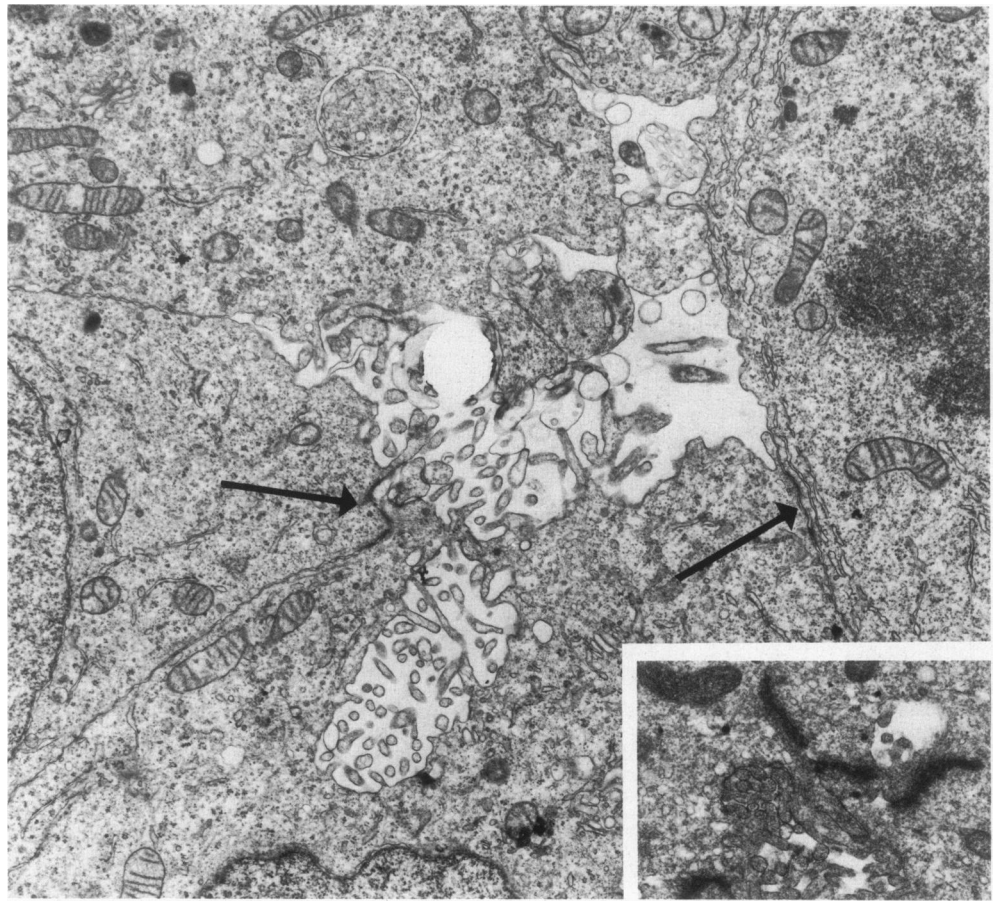


Figure 4—A rosette formed by four cells. The free apical surfaces of the cells facing the lumen show multiple microvilli, whereas the apical surfaces of contiguous cells show several dense junctional complexes (*arrows* and **Inset**) of the zonula adherens type. (×13,100; **Inset**, ×10,600)

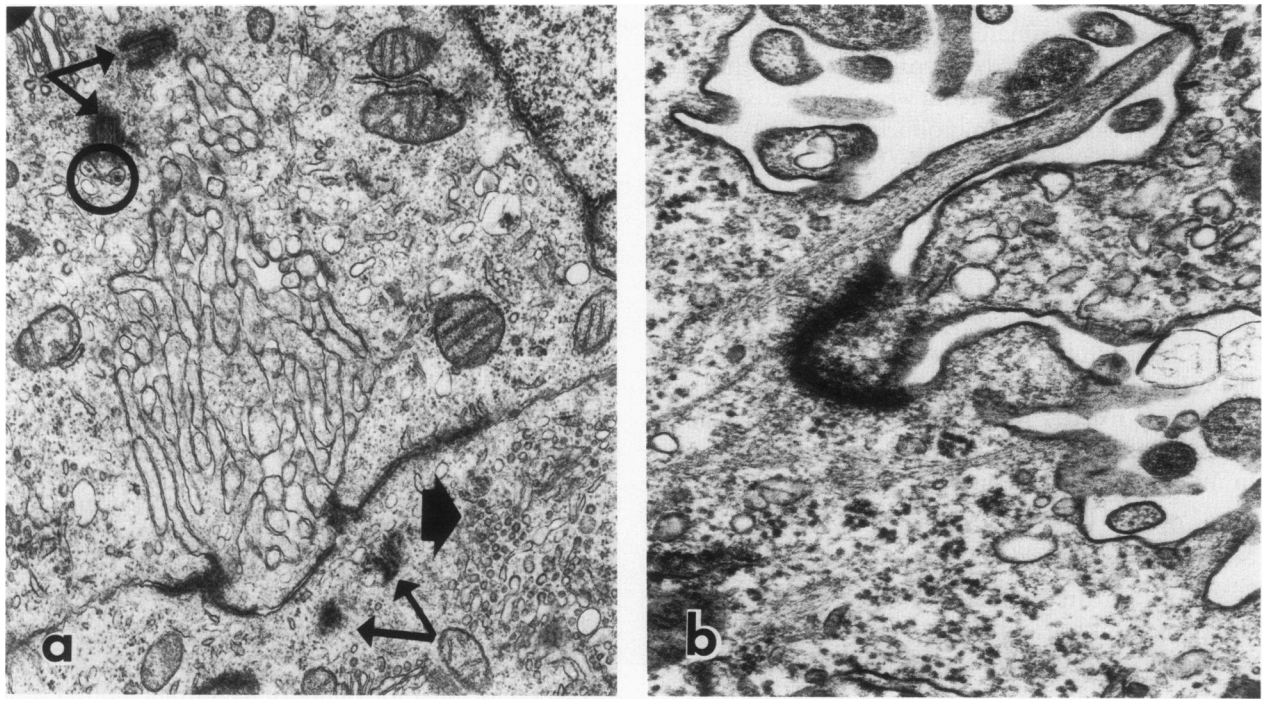


Figure 5—Lateral cytoplasmic specializations in treated retinoblastoma cells. **a**—Cell treated with Nabut in serum-supplemented medium. Microvillous cytoplasmic formations are noted at a site of close apposition to another cell; well-formed junctional complexes of the zonula adherens type are also observed. At the base of these villous processes on both sides, one can see centrioles (*angled arrows*) and the early formation of a ciliary rootlet (*circle*). The adjacent cytoplasmic area contains aggregates of small membrane-bound vesicles (*short arrow*). (×17,000) **b**—Cell treated with dbc-AMP in serum-supplemented medium. The lateral cytoplasmic projections protrude in a free intercellular space, are longer, vary in width, and contain thin actin-like filaments which measure up to 7 nm in diameter or smooth membrane-bound vesicles of various sizes. A macula adherens type of junction is noted as well. (×40,000)

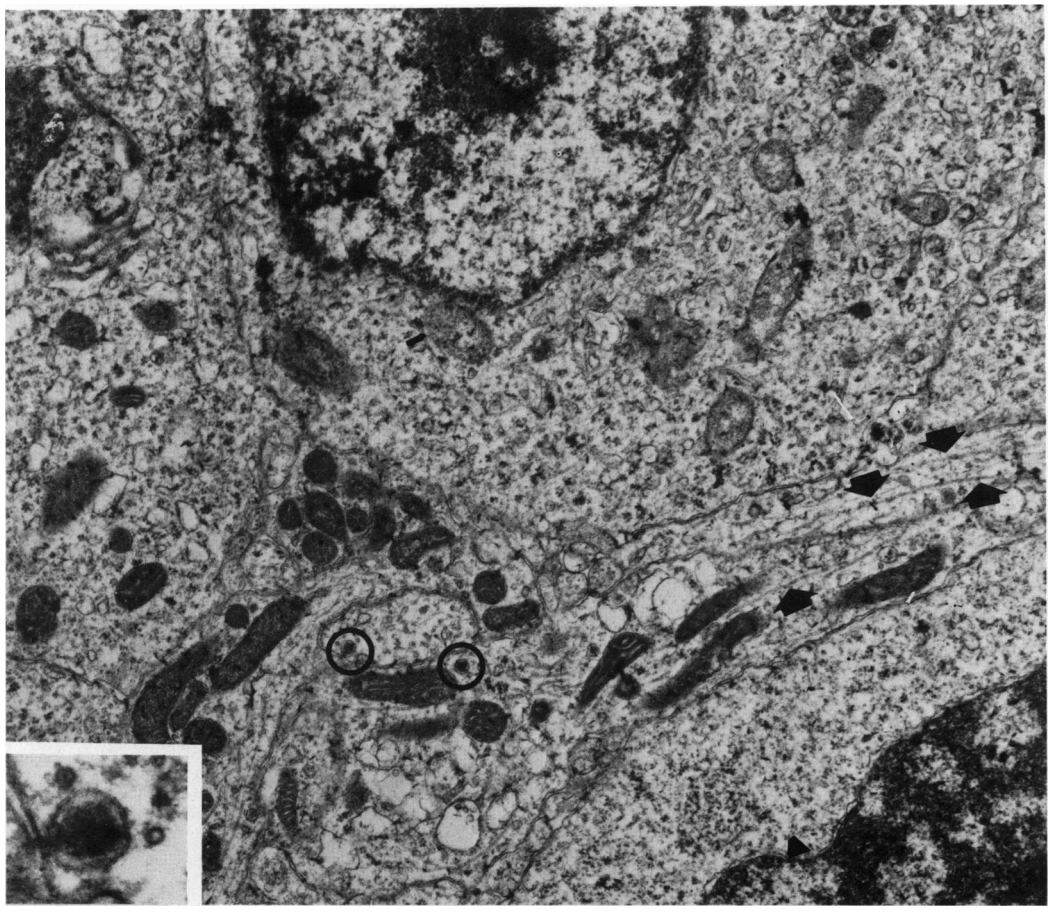


Figure 6—Retinoblastoma cells treated with dbc-AMP in serum-free medium. Cytoplasmic processes with microtubules (*short arrows*) and dense-core (neurosecretory) granules (*circles*) are seen in gross and longitudinal sections. (×16,000) The **inset** shows one of the granules at higher magnification. (×80,000)

EM Evidence for Conventional Neuronal Differentiation

Cells with parallel arrays of RER, as well as cells with cytoplasmic processes containing microtubules and occasional neurosecretory granules (NSGs) (Figure 6), were considered as more conventional neurons, because photo receptors of normal retina do not contain NSGs. The NSGs were seen predominantly in Y-79 cells treated with dbc-AMP (5–30%) and only rarely (1%) in Nabut-treated cells. Growth in serum-free medium further increased the number of NSGs in the dbc-AMP-treated cells (from 5% to 30%). On the other hand, cells grown in serum-free medium, but not treated with dbc-AMP, showed long processes but no NSGs.

EM Evidence for Glial Differentiation

Cells With Glial-like Filaments and Myelinlike Figures. Occasionally, cells with a darker cytoplasm ensheathed other tumor cells in the Y-79 cell line. These cells contained focal aggregates of oriented intermediate cytoplasmic filaments, consistent with glial filaments (Figure 7). The number of cells with glial-like filaments was very small (<1%). Cells with glial-like filaments were not seen in serum-free conditions; however, sparse cells with myelin-like figures were seen in Y-79 cells grown in serum-free conditions and with dbc-AMP, similar to the untreated cells, but to a lesser extent.

Sequential Apicolateral Development of Intercellular Attachments. Junctions of the zonula adherens type were seen not only between the rosette-forming cells, as reported above, but also in other cells which arranged themselves in a row. These junctions had an apicolateral distribution (Figure 8) and were reminiscent of the ones seen between Müller cells or between Müller and photoreceptor cells of human retina at the sites of formation of the outer limiting membrane.

Immunocytochemistry

Staining for MBP was detected specifically in the white matter and nerves of the spinal cord, which was

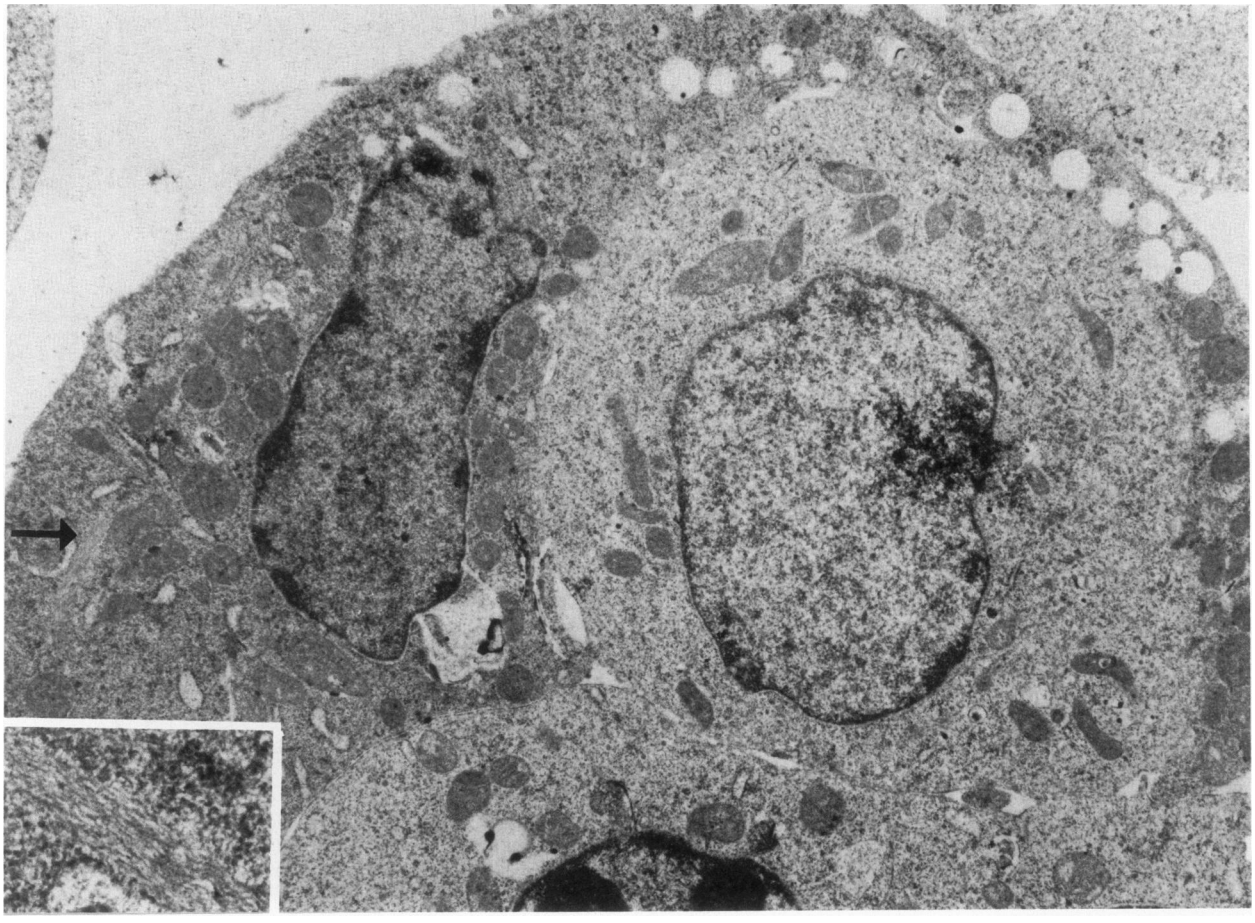


Figure 7—Retinoblastoma cells after treatment with Nabut in serum-supplemented medium. The elongated cell with the darker cytoplasm ensheaths two other cells and contains a cluster of longitudinally oriented filaments (*short arrow*). The same filaments are shown in detail in the inset. They measure approximately 8–10 nm in diameter. (×13,000; **Inset**, ×50,000)

used as positive control (gray matter was consistently negative). Normal tissues and tumors were consistently negative for MBP, except for Müller cells of human retina. The Y-79 cells grown in suspension or in monolayer cultures in serum-supplemented media did not contain detectable MBP by the ABC method (Figure 9a). However, after treatment with Nabut and dbc-AMP or deprivation of serum, occasional cells (1%) showed cytoplasmic staining for MBP (Figure 9b). Similar results were obtained by immunofluorescence and immunoperoxidase.

FIF by Y-79 Cells Before and After Differentiation

Although some cells of the Y-79 cell line were negative or equivocally positive for FIF when in suspension or in monolayers without treatment with differentiating agents (Figure 10a), after treatment they were definitely positive for FIF. Both dbc-AMP and Nabut promoted the development of FIF-positive cells, but the reaction was more pronounced after treatment with dbc-AMP (Figure 10b).

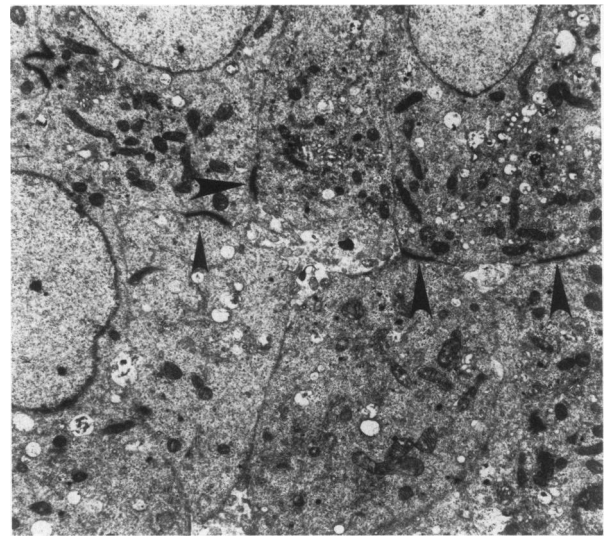


Figure 8—Sequential apicolateral intercellular attachments of the zonula adherens type (*arrowheads*) were occasionally present among linearly arranged retinoblastoma cells. These junctional complexes resemble the interrupted outer limiting membrane of the human retina (between Müller and photoreceptor cells). (×6300)

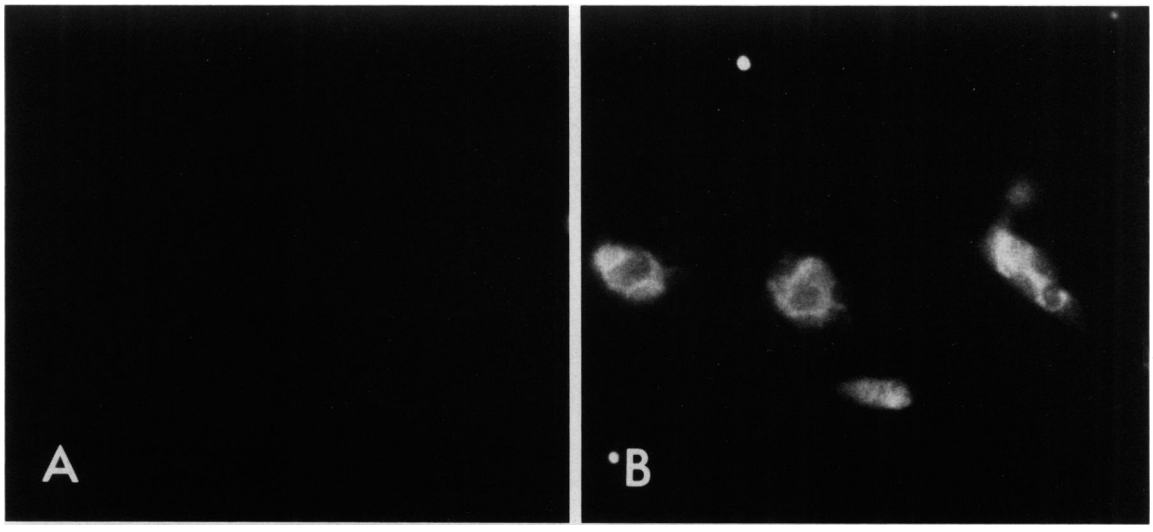


Figure 9a—Y-79 cells in suspension, all negative for MBP. (×500) **b**—Y-79 cells in monolayer cultures treated with Nabut. Some cells show cytoplasmic staining (*curved arrows*). (×250)

Discussion

In the present study we have demonstrated morphologic differentiation of the human retinoblastoma cell line Y-79 into cell types with characteristics similar to neuronal, photoreceptor, and glial cells or normal human retina. The data were obtained by modulation of the tissue culture conditions (growth in serum-free versus serum-supplemented medium and addition of differentiating agents, ie, dbc-AMP and Nabut). An additional favorable factor for the appearance of morphologic differentiation was the recently accomplished attachment of Y-79 cells on polylysine-coated substrata,[2] because mere attachment resulted in some degree of differentiation in the present study and changes in gene expression in a former study.[13] Dbc-AMP and Nabut are known differentiating agents for neuroectodermal tumors and neurons of embryonal chick retina.[14-17]

Cells simulating photoreceptors in the present study were characterized by the formation of rosettes with apical junctions of the zonula adherens type and intraluminal villi, as well as a fair number of mitochondria, as previously described.[18,19] Rosettes have been considered to represent recapitulation of primitive neural tube formation and have been reported to be composed of cells with photoreceptor cell differentiation.[18,19] Photoreceptor cell differentiation of the Y-79 cells by Nabut has been confirmed by increased synthesis and expression of interphotoreceptor retinoid-binding protein (IRBP),[20] a protein which is normally synthesized by rods.[21] In addition to rosette-forming cells, some Y-79 cells treated with dbc-AMP and Nabut showed focal microvillous cytoplasmic projections with centrioles at sites of close apposition. We interpret these microvillous projections to be an attempt by the cells to form outer segments of photoreceptors, because in primitive organisms (invertebrates) the outer segments of the photoreceptors are formed by microvillous projections of a ciliary shaft,[22] in contrast to vertebrates, in which repeated organized evaginations of the plasma membrane of a connecting cilium are required.[23] The absence of cilia is not unusual in retinoblastomas *in vitro*.[12,24,25] Moreover, smooth membrane-coated vesicles similar to the ones seen in some of these microvillous projections after treatment with dbc-AMP have been described in early stages of photoreceptor morphogenesis in chick retina,[26] and the actin filaments seen in some of the filopodial protrusions are normally present in the inner segment of the photoreceptors extending into the pedicles.[27]

Cells with features of conventional neurons seen in the differentiating Y-79 cell line were characterized by the presence of parallel arrays of RER and cytoplasmic processes containing occasional NSGs. The NSGs, in addition to being present in peripheral adrenergic neurons of neural crest origin, are also present in ganglionic cells of the inner plexiform layer of the retina.[8,28] The NSGs in our study were sparse and appeared after treatment with dbc-AMP in serum-free medium, whereas the putative photoreceptors appeared after treatment with both agents. Cells in serum-free medium alone did not exhibit NSGs but did express the neuronal marker NSE[1] and showed long processes containing actin filaments. Serum-free medium and dbc-AMP have been independently reported to induce neurites and increased numbers of NSGs in other neuroectodermal tumors, such as neuroblastoma[14] and a primitive neu-

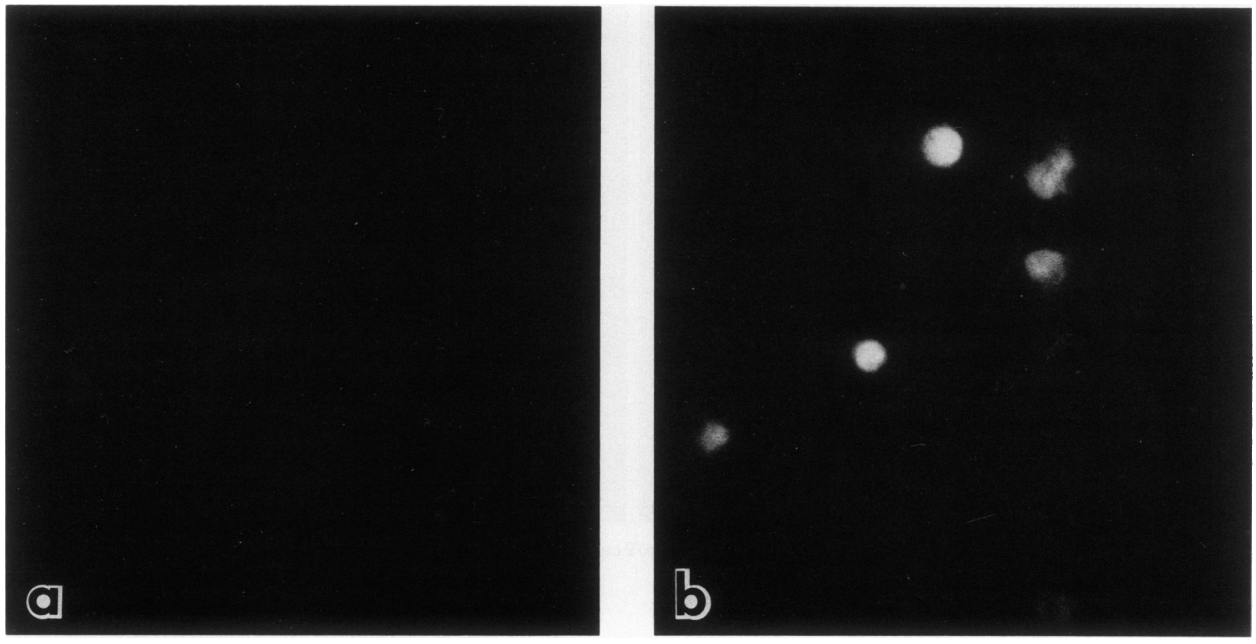


Figure 10a—Negative FIF of untreated Y-79 cells growing in suspension. (×350) **b**—The same cells fluoresced intensely by the FIF method when treated with dbc-AMP. (×350)

roectodermal tumor of spinal nerve root.[15] NSGs are occasionally seen in retinoblastomas.[24,25] However, the original cells of the Y-79 cell line did not contain NSG,[12] and therefore the appearance of these granules after treatment connotes a further stage of neuronal differentiation of the Y-79 cells. Moreover, neurotransmitter enzymes (ie, acetyl-cholinesterase and choline-acetyltransferase) have been detected previously in the Y-79 cells and two other retinoblastoma cell lines by biochemical methods.[29] The finding of FIF-positive Y-79 cells prior to addition of any agent and their increase after treatment with dbc-AMP and Nabut in the present study are in agreement with previous studies[30] and argue for the presence of catecholamines or catecholamine precursors in these cells.[11] Cholinergic and adrenergic neurotransmitter enzymes are usually produced by the amacrine cells of normal retina,[7,8] and therefore these results suggest the potential of the Y-79 retinoblastoma cells to differentiate toward amacrine-like cells. Further supportive evidence for the latter speculation was provided by the detection of substance P, which is usually seen in amacrine cells and nerve fibers of the inner plexiform layer,[31] in the neoplastic retinoblastoma cells of an *in vivo* tumor.[32] The recent findings of β-adrenergic receptors in the Y-79 cells[33] are also in agreement with the above, since such receptors should be localized in cells which interact with amacrine cells, ie, other amacrine or ganglion cells of human retina.

The glial differentiation of the Y-79 retinoblastoma cell line was characterized ultrastructurally by the presence of cells ensheathing other cells and expressing aggregates of oriented intermediate filaments or lamellated lipid-containing membranous structures reminiscent of an atypical form of myelin. The aggregated filaments are consistent with those seen in astrocytes and explain the previously reported presence of GFAP in these cells.[1] The presence of myelinlike structures suggested myelinogenic potential, which was further confirmed by the presence of MBP. The latter has been associated with Schwann cells and oligodendrocytes,[7] even prior to myelin sheath formation,[34] and has been detected in Müller cells of human retina.[6] Given that Müller cells have been considered as intermediate forms of astrocytes and oligodendrocytes, based on their morphologic[35,36] and immunocytochemical[6,37,38] characteristics, the detection of GFAP and MBP in the retinoblastoma cells suggests differentiation into Müller-like cells. On the other hand, given that a primitive glial cell capable of differentiation into astrocytes in serum-supplemented medium and oligodendrocytes in serum-free medium has been described,[39] the dual astrocytic and oligodendrocytic properties of some Y-79 cells may reflect the existence of such a glial cell progenitor. An additional piece of evidence for differentiation of retinoblastoma cells into glial cells is the remarkable increase in intercellular attachments of zonula adherens type after treatment with dbc-AMP and Nabut. Although intercellular attachments have been previously reported in retinoblastomas *in vitro* and *in vivo*,[12,18,19,24,25] they are usually limited to the areas of

rosette formation, being very primitive (punctae adherentiae) in the remaining sites. Such sequential appearance of apicolateral junctions of the zonula adherens type, as seen in the Y-79 cells, is reminiscent of the multiple junctions seen between Müller cells[40] as well as of the ones in the interrupted outer limiting membrane, seen originally between the pigmented and primitive cells of outer retinal surface in the embryonic retina[26,41] and later on between the process of Müller cells and the bodies of the photoreceptors.[40]

Previous studies have only shown differentiation of retinoblastoma to photoreceptor cells *in vitro*.[18,19,25] In this study we have presented morphologic evidence of differentiation to cells of not only neuronal but also glial lineage. Although smaller numbers of cells showed morphologic differentiation by electron microscopy, sufficient to characterize them as neuronal or glial, when compared with those detected by immunocytochemistry,[1] the identification and description of their specific ultrastructural features further proved the dual properties of retinoblastoma cells and confirmed the immunocytochemical findings. In that respect, retinoblastoma parallels pathways of differentiation of other neuroectodermal tumors, ie, neuroblastoma[42] and medulloblastoma.[43] Also, a primitive neuroectodermal tumor of the spinal nerve root has been shown to differentiate into neuronal and glial cells *in vitro*.[15] Differentiation of neuroectodermal tumors along these lines supports the concept that they originate from a primitive neuroectodermal cell. In respect to retinoblastoma, additional evidence has been provided by glycolytic enzyme studies, which show that retinoblastoma cells and embryonic retinal cells share similar enzymes.[44] Such a primitive cell has been recently localized in the ventricular zone of mouse hypothalamus.[45]

In summary, we have demonstrated morphologic and biochemical differentiation of human Y-79 retinoblastoma cells into various cell types of the retina, ie, putative photoreceptors, amacrine or ganglion and Müller cells, as well as the potential of these cells to interact in a fashion which recapitulates normal structures, such as the outer limiting membrane. In addition, we found expression of MBP, which confirms the glial phenotypic differentiation of retinoblastoma and supports the unifying concept of origin from a primitive multipotential cell. This *in vitro* system appears to provide a model for studies of cell differentiation. It may also provide information on the effectiveness of therapeutic agents in human retinoblastoma.

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