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Expression of intermediate filaments (IF) in tissues and cultured cells

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ABSTRACT. Intermediate filaments are found in most nucleated cells as part of their cytoskeleton. Intermediate filaments are formed by different proteins in cells of major tissues types. Therefore, antibodies against intermediate filaments can be used in tissue typing, in the analysis of cell lineages during development and in the elucidation of the origin of unknown tumors.

KEY WORDS: *Intermediate filaments, mammalian cells, development, cytokeratins, cell culture*

Intermediate filaments are expressed in tissues in a cell type-specific manner

Actin-containing microfilaments, microtubules, intermediate filaments and the membrane skeleton together form the cellular cytoskeleton. While there are only some differences in the composition of microfilaments, microtubules and membrane skeletal proteins in diverse cells, the major cell types in mature mammalian tissues differ in their composition of intermediate filaments (Steinert and Roop, 1988). Intermediate filaments were first described as a separate cytoplasmic entity based on electron microscopic observations (Ishikawa *et al.*, 1968). In immunohistochemistry they were first characterized by using human (Kurki *et al.*, 1977) or animal autoantibodies. Interestingly, an autoantibody response against various types of intermediate filaments appears to be a common sign in many disease states (Kurki and Virtanen, 1985; Kurki *et al.*, 1986; for a review, see Kurki and Virtanen, 1984).

During recent years, intense research has focused on revealing the composition, structural organization, and functions of these cytoplasmic fibrils. In most cells they extend as fine fibrillar arrays between the nucleus and cell surface, and can be revealed easily in electron microscopy after extraction of cells with buffers containing nonionic detergent (Lehto *et al.* 1978). Based on this finding, it was proposed that they might function as nucleus anchoring elements in cells (Lehto *et al.*, 1978).

Most of our knowledge on the tissue content and distribution of intermediate filaments is based on immunohistochemical findings with specific polyclonal or monoclonal antibodies raised against their subunit proteins as well as on two dimensional gel electrophoretic analysis. Intermediate filaments of different cell types show a remarkable variation in both their composition and immunochemical properties. Most mesenchymal cells in mammalian tissues, including fibroblasts, histocytes, endothelial cells, and circulating blood cells, contain a 57 kilodalton (kD) polypeptide, vimentin, as their intermediate filament-forming protein (Virtanen *et al.*, 1981; Fig. 1a). Many muscle cells, on the other hand, contain a different 55 kD intermediate filament protein, termed desmin or earlier also as skeletin (see Thornell *et al.*, 1985). Astrocytes and most neuronal cells contain specific intermediate filament-forming proteins, designated as glial fibrillary acidic protein (GFAP) and neurofilament triplet proteins, respectively. Epithelial cells contain a more complicated, multigene-derived intermediate filament system, consisting of keratins or cytokeratins, present in both keratinizing epithelial cells and nonkeratinizing epithelial cells of internal organs. In cells of stratified squamous epithelia, intermediate filaments form typical dense arrays of fibrils, tonofilaments, that are easily identified in electron microscopy. In all epithelial cells, cytokeratin fibrils typically converge to desmosomes. Different normal epithelial cells appear to express distinct sets of keratin polypeptides, not necessarily shared by other epithelial cells. For example, the keratinizing epithelia differ quite typically in their keratin polypeptide content from the glandular epithelial cells. Such a distinction can also be utilized for the immunohistochemical identification of the different epithelial cells (Fig. 1b). Thusfar, 19 epithelial cytokeratin polypeptides have been identified in human tissues (Moll *et al.*, 1982). Recently, however, ca. 10 trichocytic keratins have additionally been characterized, first described in hair forming cells but found now also in other epithelial cells (Heid *et al.*, 1988).

Despite the immunochemical and biochemical heterogeneity of intermediate filament-forming proteins, the accumulated data points to their common evolutionary origin: all intermediate filament proteins and proteins of the nuclear lamina share a reminiscent rod-like core region in their primary sequence (Steinert and Roop, 1988). This may explain why a single monoclonal antibody can recognize all these polypeptides (Pruss *et al.*, 1981).

Monoclonal antibodies to intermediate filaments

The cell and tissue type-specific expression of intermediate filaments has led to the application of antibodies against intermediate filaments in tissue and tumor characterization and in the analysis of cell lineages during development. Although human tumors have been extensively analyzed for their intermediate filament content, there still are numerous unanswered questions to be solved. Yet, many general conclusions can now be made and many suggestions can be given concerning the classification and differential diagnosis of tumors on the basis of their expression of intermediate filaments. These conclusions and prospects are based on the well-established fact that the cell type-specific expression of intermediate filaments is for the most part faithfully maintained in tumor cells. Much of the recent knowledge about the differential expression of interme-

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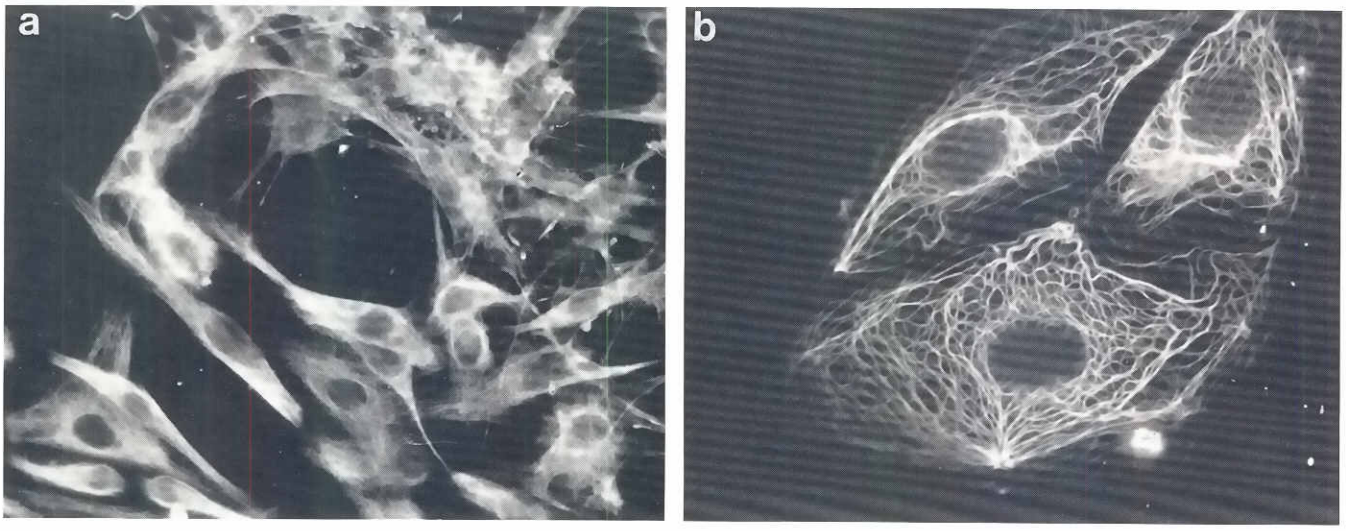

Fig. 1. *(a) Cultured human fibroblasts immunostained for vimentin by using a monoclonal antibody. Note the dense fibrillar reactivity in the cytoplasm. (b) Cytokeratin fibrils in rat kangaroo kidney epithelial cells. Note the thick cytoplasmic bundles of fibrils. 400 x.*

diate filaments is based on results obtained with monoclonal antibodies recognizing distinct subsets of intermediate filaments.

For instance, by using monoclonal antibodies it has been possible to specifically identify cytokeratins specific for simple, squamous and keratinizing squamous epithelium. Similarly, monoclonal antibodies may successfully differentiate between phosphorylated and non-phosphorylated forms of vimentin differentially expressed in normal and malignant cells (Virtanen *et al.*, 1988) as well as recognize a tissue-specific variant of desmin in Purkinje fibers of heart muscle, not found in cardiac muscle cells (Fig. 2).

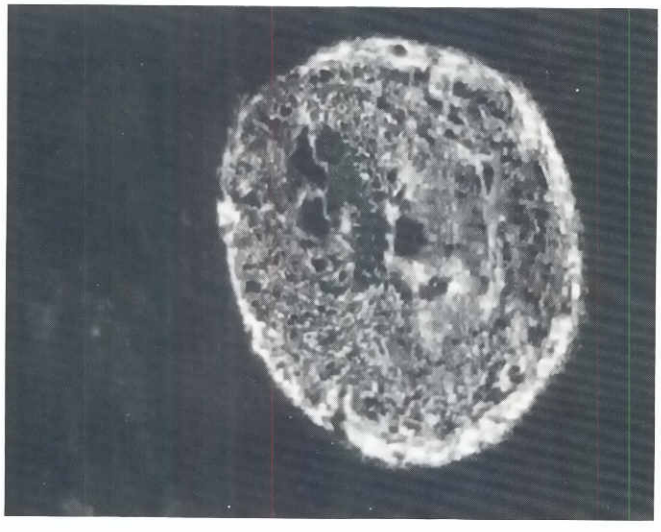

Fig. 2. *A monoclonal antibody PD 39AB6 reacts brightly with the Purkinje cells in cow cardiac muscle but does not react with the surrounding cardiac muscle cells. 300 x.*

Expression of IFs in developing tissues and in tumors

The major cell types in human tissues express intermediate filaments in a cell type-specific manner, mostly in line with our view on the histogenetic origin of cells.

However, many recent studies have revealed that during development the patterns of expression of intermediate filaments may be, in fact, more complex. Kidney tubular epithelial cells, although developing from the metanephric mesenchyme, appear to acquire expression of cytokeratins and lose the expression of vimentin during tubulogenesis (Holthöfer *et al.*, 1983). This can be induced also *in vitro* (Lehtonen *et al.*, 1985), suggesting that the onset of epithelial differentiation does not necessarily require tubule morphogenesis with its complex cell-cell interactions. In the kidney, the developing podocytes contain cytokeratin No. 19 during early development but later express only vimentin (Holthöfer *et al.*, 1984).

Earlier studies on the content of intermediate filaments in normal and tumor cells *in vivo* were based on the assumption that most cells may only express one type of intermediate filaments (e.g. Virtanen *et al.*, 1985). However, vimentin is often co-expressed with the cell type-specific intermediate filament protein. Such is the case with many cultured cells that more or less regularly contain vimentin (Virtanen *et al.*, 1981), although cell lines lacking vimentin are also common. During embryonal development some epithelial cells, as well as some neuronal cells, may display vimentin-immunoreactivity not found any more in the corresponding cells in adult tissues. Such a transient co-expression of two types of intermediate filaments may lead to the expression of an unexpected filament pattern in adult tissues: Sertoli cells in both adult rat and man contain only vimentin intermediate filaments (Virtanen *et al.*, 1986; Fig. 3a), although they are of epithelial origin. During embryonic and postnatal stages the Sertoli cells in rat contain cytokeratin Nos. 8 and 18, which disappear abruptly during hormonal maturation of the testis (Paranko *et al.*, 1986). Also human Sertoli cells contain cytokeratin during fetal stage (Fig. 3b). Such a dual expression of intermediate filaments during histogenesis may

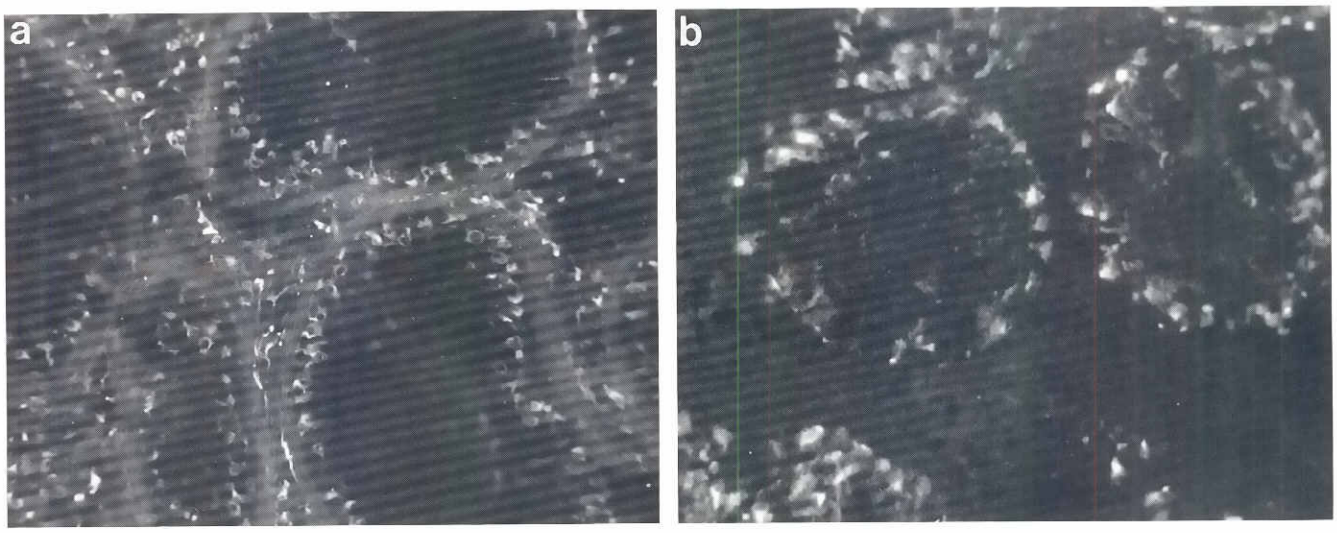

Fig. 3. *(a) Vimentin-immunoreactivity is seen in Sertoli cells of adult man. (b) In fetal human testis, monoclonal antibody reacting with cytokeratin No. 18 gives bright staining of the Sertoli cells. 200 x.*

explain why Sertoli cell-like tumors, for instance the tubular androblastomas, are cytokeratin-immunoreactive.

Co-expression of vimentin and cytokeratins is a frequent observation in carcinomas, for instance in thyroid, lung and renal carcinomas (Virtanen *et al.*, 1985; Lehto and Virtanen, 1986; Miettinen *et al.*, 1987). Ramaekers *et al.*, (1983) suggested that such a co-expression of vimentin and cytokeratin intermediate filaments may be typical of all metastatic carcinoma cells and could be due to aberrant gene expression caused by loss of normal cell-to-cell connections. Such a suggestion is supported by studies on some epithelial cells in embryonic tissues, for instance the fetal rat genital ducts (Paranko and Virtanen, 1986) and the stellate reticulum cells of fetal tooth germ (Heikinheimo *et al.*, submitted; Fig. 4). However, benign tumors like thyroid adenomas as well as ameloblastomas, neoplasms that display both abundant desmosomes and adherent junctions, also often express vimentin together with cytokeratins. Furthermore, our own results on different types of carcinomas suggest that the tumor cells in metastases only rarely express vimentin-immunoreactivity.

There are also other examples of co-expression of two or even three types of IFs in normal and malignant cells. Cytokeratins and neurofilament proteins are often co-expressed in many, but not in all neuroendocrine tumors (Virtanen *et al.*, 1985). The immunoreactive tumors include, for instance, lung carcinoid tumors (Lehto *et al.*, 1985), pancreatic islet cell tumors (Mietti-

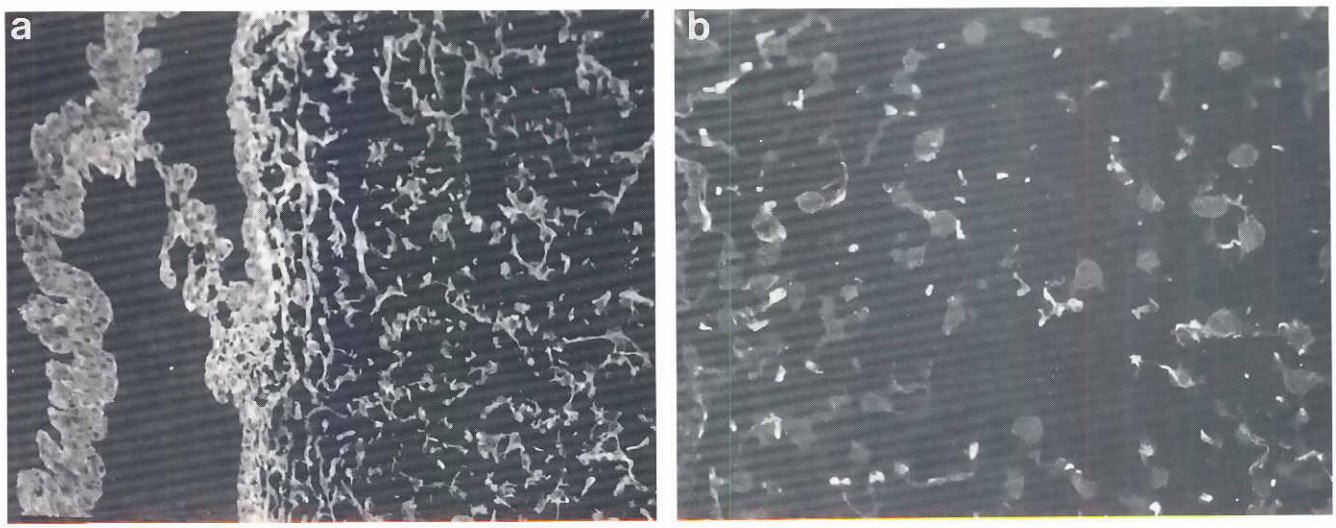

Fig. 4. *The stellate reticulum cells of human fetal tooth germ are cytokeratin-immunoreactive, (a) but also contain vimentin-immunoreactivity (b). 400 x.*

nen *et al.*, 1985), thyroid medullary carcinomas (Fig. 5a), and the Merkel cell carcinomas (Virtanen *et al.*, 1986; Fig. 5b). The latter tumors are interesting as it is now clear that Merkel cells in both embryonic and adult tissues only contain cytokeratin filaments (Figs. 5c, d).

Cardiac muscle cells appear to express complex patterns of intermediate filaments during development: embryonic heart muscle cells co-express desmin and cytokeratin (Fig. 6) while the developing Purkinje cells may co-express desmin and neurofilament proteins.

Distinct changes in the expression of cytokeratins also appear to take place during tissue maturation: during fetal development many squamous epithelial cells express simple epithelial cytokeratins (Regauer *et al.*, 1985) that are later lost but may again emerge during malignant change. Furthermore, for instance ameloblastomas frequently contain immunoreactivity for cytokeratin No. 18 (Fig. 7) not found in the odontogenic epithelium, giving rise to these tumors.

Expression of IFs in cultured cells

The above examples suggest that the expression of intermediate filaments in both developing tissues and tumors may be controlled by mechanisms leading to patterns of expression that cannot be fully explained on the basis of the concept of their tissue-specific expression. Such changes may suggest mechanisms of gene regulation in which the intermediate filament expression depends on the state of the cellular differentiation rather than on their derivation.

The pattern of expression of intermediate filaments has, however, been valuable in the analysis of the origin and developmental history of cultured cells. Thus, the content of desmin in a cultured cell line strongly suggests its derivation from mus-

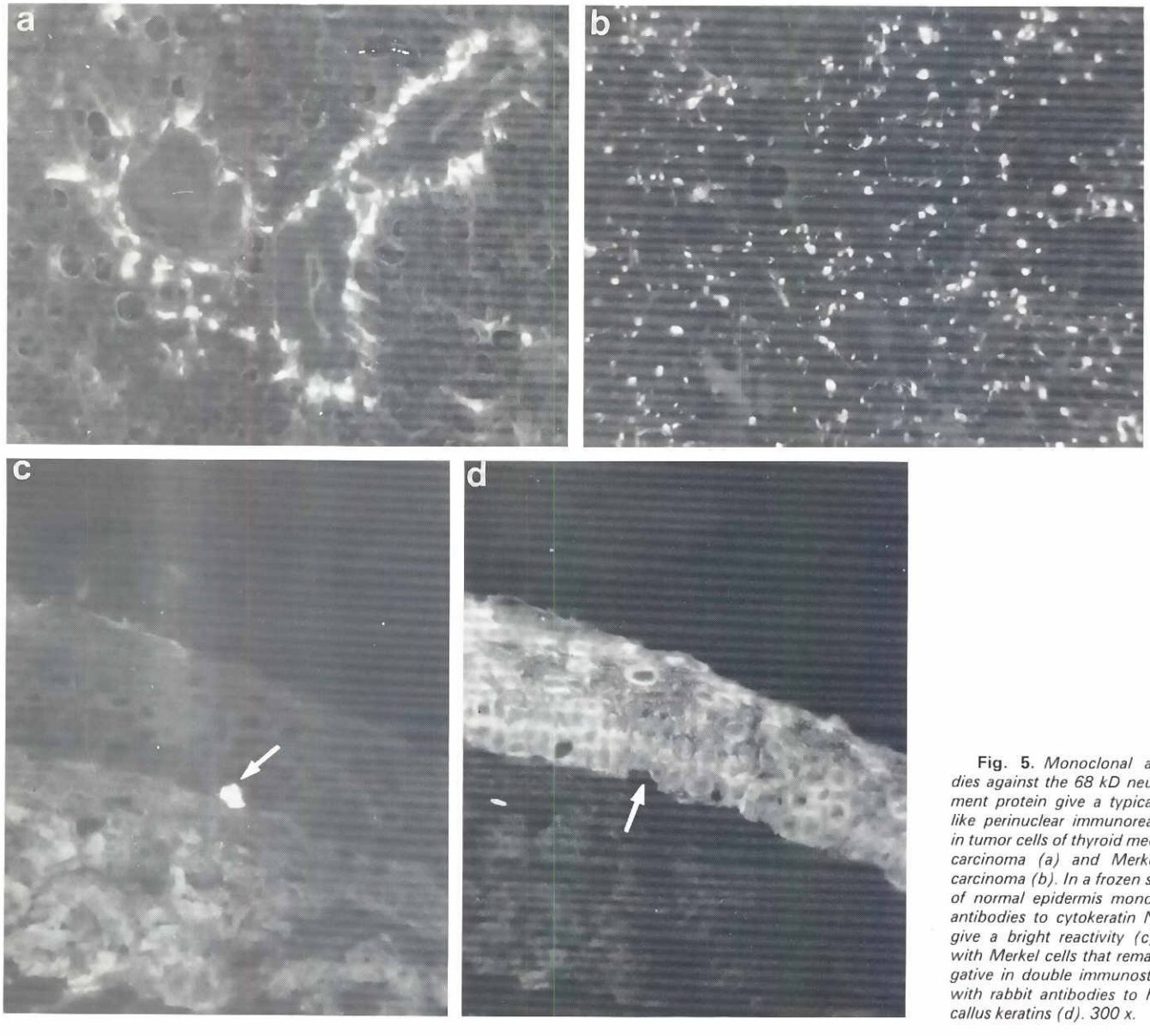

Fig. 5. *Monoclonal antibodies against the 68 kD neurofilament protein give a typical tuft-like perinuclear immunoreactivity in tumor cells of thyroid medullary carcinoma (a) and Merkel cell carcinoma (b). In a frozen section of normal epidermis monoclonal antibodies to cytokeratin No. 18 give a bright reactivity (c) only with Merkel cells that remain negative in double immunostaining with rabbit antibodies to human callus keratins (d). 300 x.*

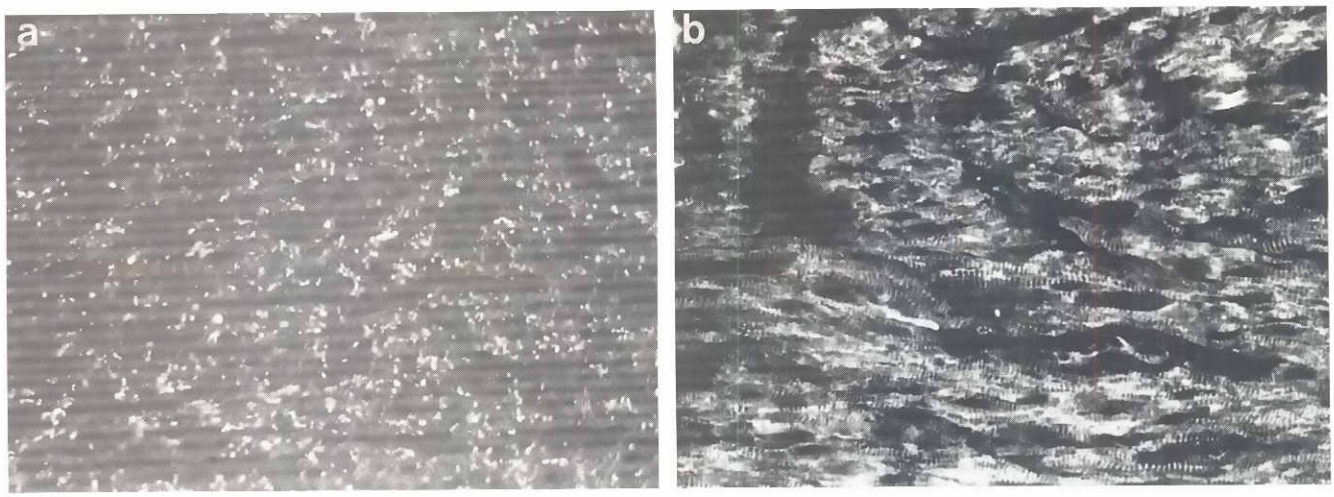

Fig. 6. *In frozen section of human fetal cardiac muscle a typical granular immunoreactivity for cytokeratin No. 18 can be detected (a), in addition to a different desmin-immunoreactivity (b). Specimens from skeletal muscle from the same fetus were not immunoreactive for cytokeratins. 300 x.*

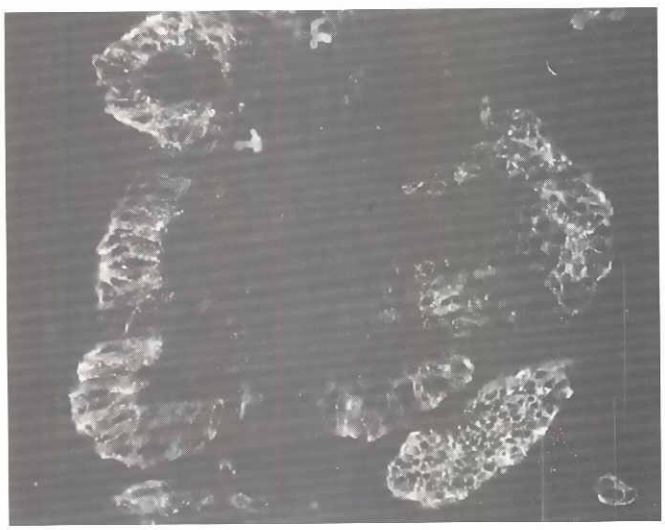

Fig. 7. *Cytokeratin No. 18-immunoreactivity in tumor cells of an ameloblastoma. Note the heterogenic staining of some of the cells. 200 x.*

cle tissue or rhabdomyosarcoma, respectively. However, there are examples that illustrate the complexity of such an analysis: Venetianer *et al.*, (1983) described, for instance, that cultured hepatoma cells may completely lose their intermediate filaments. In some cases, intermediate filament typing may allow re-evaluation of the derivation of even some widely used cell lines. Recent findings on PC12 pheochromocytoma cells, thought to be derived from a transplantable pheochromocytoma, showed that they co-express neurofilaments and cytokeratins (Franke *et al.*, 1986). As pheochromocytomas only contain neurofilaments (Miettinen *et al.*, 1985) this finding suggests that PC12 cells represent neuroendocrine, rather than neuronal cells.

The expression of intermediate filaments in mesenchymal cells may be more complex than anticipated: recently, we showed that in a complex culture medium fetal, but not adult, human fibroblasts can be induced to express cytokeratin Nos. 8 and 18 (von Koskull *et al.*, 1987; Fig. 8a). Similarly, co-expression of cytokeratins and vimentin appears to be common in human fibrosarcoma cells, such as A8387 (Fig. 8b), and HT 1080. Similar co-expression is seen in many mesenchymal tumors (Lehto and Virtanen, 1987; Figs. 8c, d). Furthermore, in cultured fibrosarcoma cells, cytokeratin immunoreactivity increased upon exposure of the cells to sodium butyrate and disappeared when the cells were exposed to a tumor promoting phorbol ester. K562 erythroleukemia cells, able to cause erythroid differentiation, were similarly shown to express cytokeratins 8, 18 and 19, together with vimentin (Jarvinen *et al.*, submitted). These results suggest that the regulation of expression of intermediate filaments in cultured cells is a complex phenomenon: different cell lines respond differently to various modulating exogenous agents. Futhermore, little is known as yet about the role of post-transitional modifications in the regulation of the expression and functions of intermediate filaments. In this respect, it is of interest that vimentin filaments in normal and malignant human mesenchymal cells appear to differ in their state of phosphorylation as well as susceptibility to the action of Ca^{++}-activated protease (Virtanen *et al.*, 1988). Such differences in the phosphorylation status of neurofilament and desmin polypeptides are found even in normal tissues (Lee *et al.*, 1987; Kjörell *et al.*, 1987).

In the characterization of retinoblastomas and small cell carcinomas of the lung, great effort has been devoted to cell culture studies to elucidate the histogenesis and state of differentiation of these tumors. First *in vivo* studies on retinoblastomas implied that these tumors would be able to differentiate into glial cells. Experiments with the Y79 retinoblastoma cell line suggested, on the other hand, that these cells are able to co-express glial and neuronal markers (Kyritsis *et al.*, 1984) although such features have not been found in surgically removed retinoblastomas (Kivelä *et al.*, 1987). Our recent results on Y79 retinoblastoma cells (Virtanen *et al.*, 1988) indicated that the previous suggestions on their dual capability to differentiate may be based on a cross-reaction of the polyclonal

GFAP antibodies used in those studies. The results with a panel of neurofilament antibodies revealed that during induced differentiation of the Y79 cells on laminin-coated growth substratum, the cells acquired neuronal characteristics as they expressed phosphorylated neurofilament proteins (Fig. 9a) but did not show GFAP-immunoreactivity under any culture conditions. However, when plated on fibronectin-coated growth substratum the cells rapidly began to express cytokeratin Nos. 8 and 18, but not 19, resembling in this respect pigmented retinal epithelial cells. Instead, another retinoblastoma cell line, Weri Rb1 appears to express neurofilament proteins more constitutively (Fig. 9b) and differs in this respect from the Y79 cells.

The expression of neurofilaments in small cell carcinomas of the lung has also been a topic of intense study (Lehto and Virtanen, 1986). Most earlier studies failed to reveal neurofilament-immunoreactivity in typical lung small cell carcinomas (for a review, see Lehto and Virtanen, 1986). The results with cultured

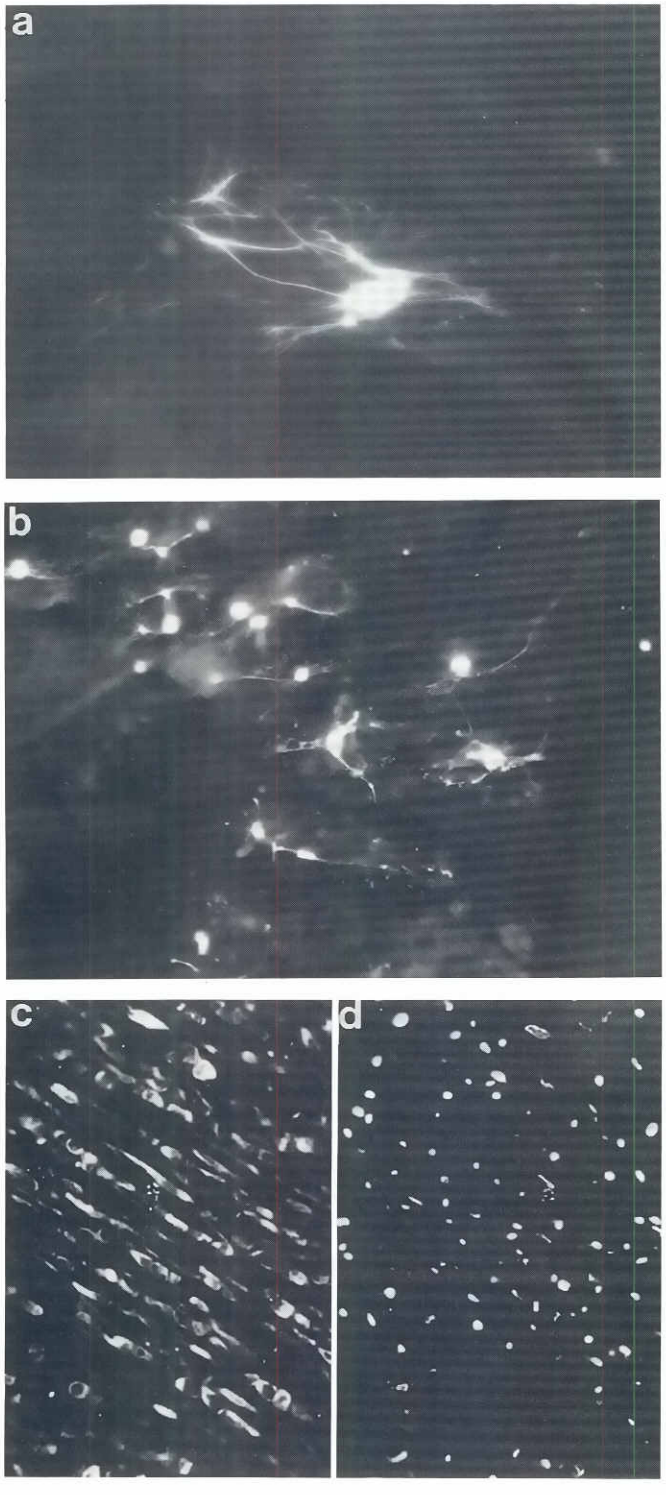

Fig. 8. *Fibrillar immunoreactivity for cytokeratin No. 18 is seen in human fetal fibroblasts cultured in Condimed® medium (a) and in human A8387 fibrosarcoma cells (b). In frozen section, human epithelioid sarcoma cells co-express vimentin (c) and cytokeratin (d). a, b x 400, c, d 250 x.*

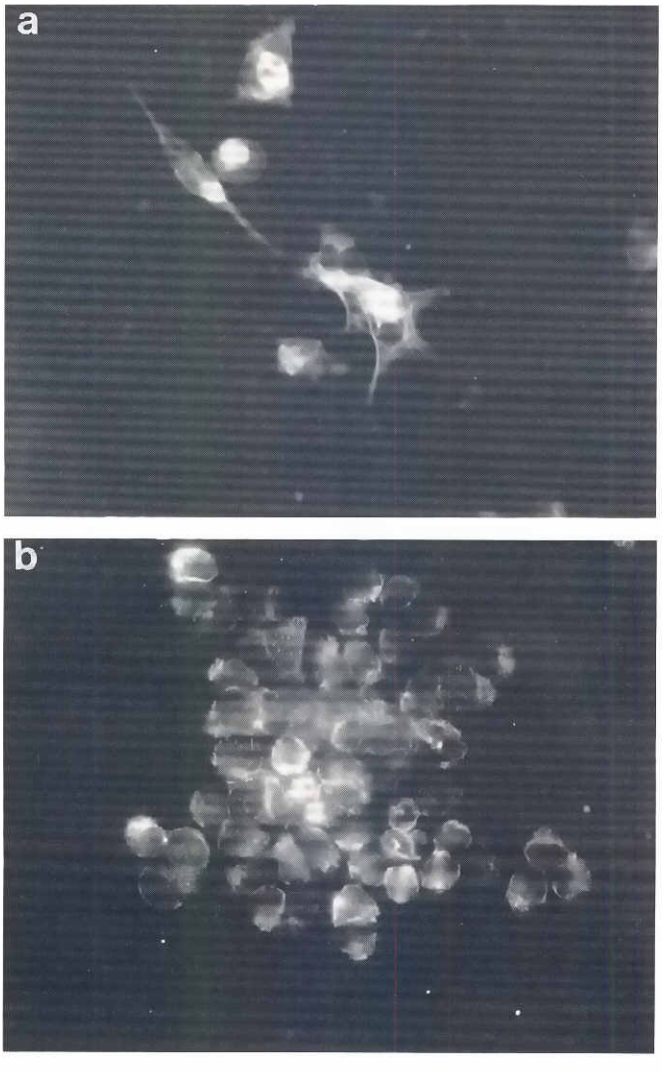

Fig. 9. *Y79 retinoblastoma cells start to express phosphorylated neurofilament proteins after exposure to dbcAMP (a) whereas also the suspension-cultured, undifferentiated Weri Rb1 retinoblastoma cells express neurofilament-immunoreactivity (b). 300 x.*

cells have, however, shown that distinct cell lines derived from lung small cell carcinomas constitutively express neurofilaments (Bergh *et al.*, 1984) and lack cytokeratins, suggesting that cells derived from these tumors are able to acquire and maintain neuronal differentiation.

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