Virchows Archiv A Pathol Anat (1989) 415:533–538

Virchows Archiv A
Pathological Anatomy and Histopathology
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Immunolocalization of the human basal epithelial marker monoclonal antibody 312C8-1 in normal tissue and mammary tumours of rodents

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Summary. Using immunoperoxidase staining of monoclonal antibody 312C8-1 against 51000 dalton human keratin polypeptide, immunolocalization was observed in frozen sections of normal tissue and mammary tumours of adult female mice and rats. In normal tissue, the epitope was recognized in myoepithelial cells of the mammary, sweat and salivary glands, and in basal and suprabasal cells of the epidermis. However, the antibody did not react with luminal epithelial cells of the above glands or with mesenchymal cells. In spontaneous mammary tumours of mice, marker-positive tumour cells were distributed only in the outer layer of adenocarcinoma Type A, while they were scattered in some foci of adenocarcinoma Type B, and encircled the epithelial foci of pregnancy dependent tumours (plaque). All layers of epidermoid structures in adenoacanthoma revealed positivity. In rat mammary tumours induced by local dusting with 7, 12-dimethylbenz(α)anthracene (DMBA) powder, the staining pattern of benign tumours was comparable to that of the normal mammary gland. But, in addition to basally situated cells, marker-positive tumour cells were found scattered in the foci of adenocarcinoma, and were not restricted to basal cells in squamous cell carcinoma. The marker was not found in sarcomatous tissue. This antibody can therefore also be applied to rodents, and the staining pattern can be used to identify the epithelial subclass specific marker in normal tissue and in mammary tumours.

Key words: Keratin – Mammary neoplasms – Mouse – Rat – Immunohistochemistry

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Introduction

The mammary gland epithelium is composed of luminal cells bordering the lumen and of myoepithelial cells situated between the luminal cells and the basement membrane. Although differentiating the two cell types is difficult by routine methods, their identification in the normal mammary gland and in mammary tumours would contribute to the comprehension of functional differentiation of the gland and the histogenesis of mammary tumours. Keratin filaments are a complex family of many different polypeptides, and a certain epithelial cells can be characterized by the specific pattern of their components (Moll et al. 1982). Luminal and myoepithelial cells express different keratin phenotypes (Franke et al. 1980). Moll et al. (1982) cataloged the major human keratins and identified them numerically as keratin 1–19. Several studies have indicated the presence of human keratin 5, 13 and 14 in myoepithelial cells (Nagel et al. 1986; Taylor-Papadimitriou and Lane 1987; Jarasch et al. 1988; Levy et al. 1988; Wetzels et al. 1989). Monoclonal antibody 312C8-1 against 51000 dalton keratin, reacting with human keratin 14, isolated from human mammary carcinoma tissue, has been shown to label the myoepithelial cells of the human mammary, sweat and salivary glands, as well as the basal cells of the human epidermis (Dairkee et al. 1985, 1987, 1988; Dardick et al. 1987). It is worth studying whether a certain keratin phenotype may or may not be immunohistochemically the same in rodents as in humans, since the function and structure of the mammary gland differs between the two species. Studies by Asch and Asch (1985) have identified at least five keratins in mouse mam-

mary gland. These were characterized according to murine keratin catalog defined by Schiller et al. (1982), as keratins 8, 11, 14, 20, and 22, which are probably homologous with human keratins 5, 8, 14, 18, and 19, respectively. Several antibodies have been reported that stain rodent myoepithelial cells (Asch et al. 1981; Taylor-Papadimitriou et al. 1983; Allen et al. 1984). However, no comparative study of a monoclonal antibody 312C8-1 have been undertaken in the rodent system. The tissue-specific patterns of keratin is usually preserved in tumour cells (Moll et al. 1982) and so application of the analysis of keratin expression might not only serve as an important indicator of cell phenotype but might also tell from which type of cell a tumour has originated. 312C8-1, a monoclonal antibody specific to human myoepithelial cells can be used to identify the myoepithelial cells in the normal mammary, sweat and salivary gland of rodents, as well as basal and suprabasal cells in the normal epidermis. In addition, this antibody allows the definition of the subclass of mammary tumours in rodents.

Materials and methods

BALB/c, C3H and GRS/A mice are maintained in our laboratory. Sprague-Dawley female rats were purchased from Japan CLEA Inc., Osaka. These animals were housed in an air-conditioned room with daily schedule of 14 h of light and 10 h of darkness, kept 4–6 animals per cage, and fed CMF pellets (Oriental Yeast Co., Tokyo) and water ad libitum.

Disk-shaped mammary lesions (plaque) were seen in pregnant GRS/A mice. BALB/cfC3H mice were established by foster-nursing BALB/c mice with C3H mother, and within a year, the multiparous females yielded a high incidence of spontaneous mammary tumours. These mammary tumours were classified according to Dunn's classification (Dunn 1959; Sass and Dunn 1979; Squartini 1979), as indicated in Table 1. In brief, adenocarcinoma Type A is characterized by a fine uniform acinar structure, and adenocarcinoma Type B includes a multiform group of tumours that are composed of glandular epithelial origin. Rat mammary tumours were induced by DMBA administration. Direct application of approximately 1 mg of DMBA powder (Eastman Chemical, Rochester) to the right inguinal mammary gland was carried out at the age of 30 days, and the locally growing mammary tumours were harvested 11 to 20 weeks after the DMBA dusting (Tsubura et al. 1988b). The induced tumours were classified according to the terminology used in several papers (Young et al. 1973; Komitowski et al. 1982; Tsubura et al. 1988b; Russo et al. 1989) as listed in Table 2.

Development of the monoclonal antibody 312C8-1 has already been described in detail (Dairkee et al. 1985). Mice were immunized with tumour cells in secondary cultures of human breast cancer specimen, and spleen cells from the immunized mice were fused with SP2/0 mouse myeloma cells. The immunoglobulin subclass as determined by Ouchterlony analysis was IgM.

Normal tissues of the mammary, sweat and salivary glands and of the skin, and the mammary tumour tissues, were obtained from mice and rats. The tissue slices were frozen with dry ice-acetone and stored at −80° C until use. Cryostat sections, 4 μm, were cut at −20° C, allowed to attach to albumin-coated slides, air-dried for more than 30 min at room temperature, fixed in acetone for 3 min, followed by 3 washes in ethyl ether to remove fat. The avidin-biotin-peroxidase system (ABC Vectastain Kit, Vector Lab., Burlingame) was performed on these frozen sections (Tsubura et al. 1988a). Ascites preparations of 312C8-1 were diluted 1:500 in Tris-saline buffer, pH 7.2, and reaction was carried out for 1 h at room temperature. The colour reaction developed in 4 min in a freshly prepared substrate solution of 3,3′-diaminobenzidine. The sections were weakly counterstained with Gill's haematoxylin (10 s), and mounted on glycerin-jelly. Negative controls were obtained by replacing the primary antibody with non-immune mouse serum.

Table 1. Staining patterns for 312C8-1 in spontaneous mammary tumours in mice

Type of mammary tumour	Staining pattern in tumour tissue
pregnancy-dependent tumour	positive in almost all basal cells
adenocarcinoma Type A	positive in almost all basal cells
adenocarcinoma Type B	positive in scattered foci
adenoacanthoma	positive in almost all epidermoid components

Table 2. Staining patterns for 312C8-1 in rat mammary tumours induced by local dusting with DMBA powder

Type of mammary tumour	Staining pattern in tumour tissue
adenoma	positive in myoepithelial cells
lactating adenoma	positive in myoepithelial cells
papilloma	positive in myoepithelial cells
fibroma	negative
fibroadenoma	positive in myoepithelial cells
adenocarcinoma	positive in basal, stem and myoepithelial cells
squamous cell carcinoma	positive in all layers of parenchymatous cells
phyllodes tumour	positive in myoepithelial cells
carcinosarcoma	positive in basal, stem and myoepithelial cells
stromal sarcoma	negative

Results

In normal rat mammary glands, constantly strong positivity with 312C8-1 was observed in epithelial cells situated between the luminal epithelial cells and the basement membrane. These positive cells showed a characteristic cytoplasmic staining of the myoepithelial cells in the ducts and acini. The luminal cells lining the glandular lumen were completely negative. A similar staining pattern was observed in the proliferating lobules of pregnant mammary glands, but secretory materials in the lumen were negative. In rat sweat glands, strong

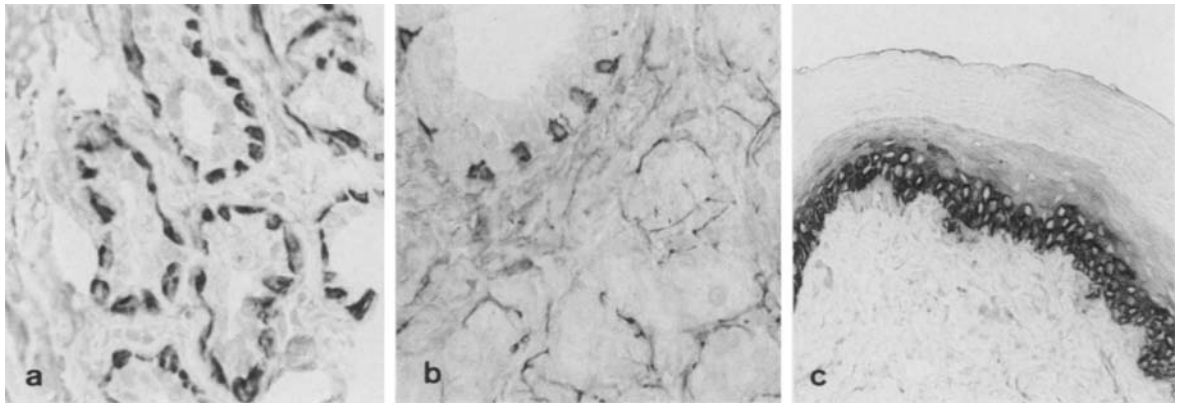


Fig. 1. a Normal rat sweat gland stained with 312C8-1. Myoepithelial cells in the acini are stained, while luminal cells are not. ×460. **b** Normal rat salivary gland stained with 312C8-1. Myoepithelial cells in the acini and triangular-shaped basal cells in the ducts are stained. ×460. **c** Normal rat skin stained with 312C8-1. The basal and suprabasal cell layers of the epidermis are strongly stained, but the other zones are not. ×230

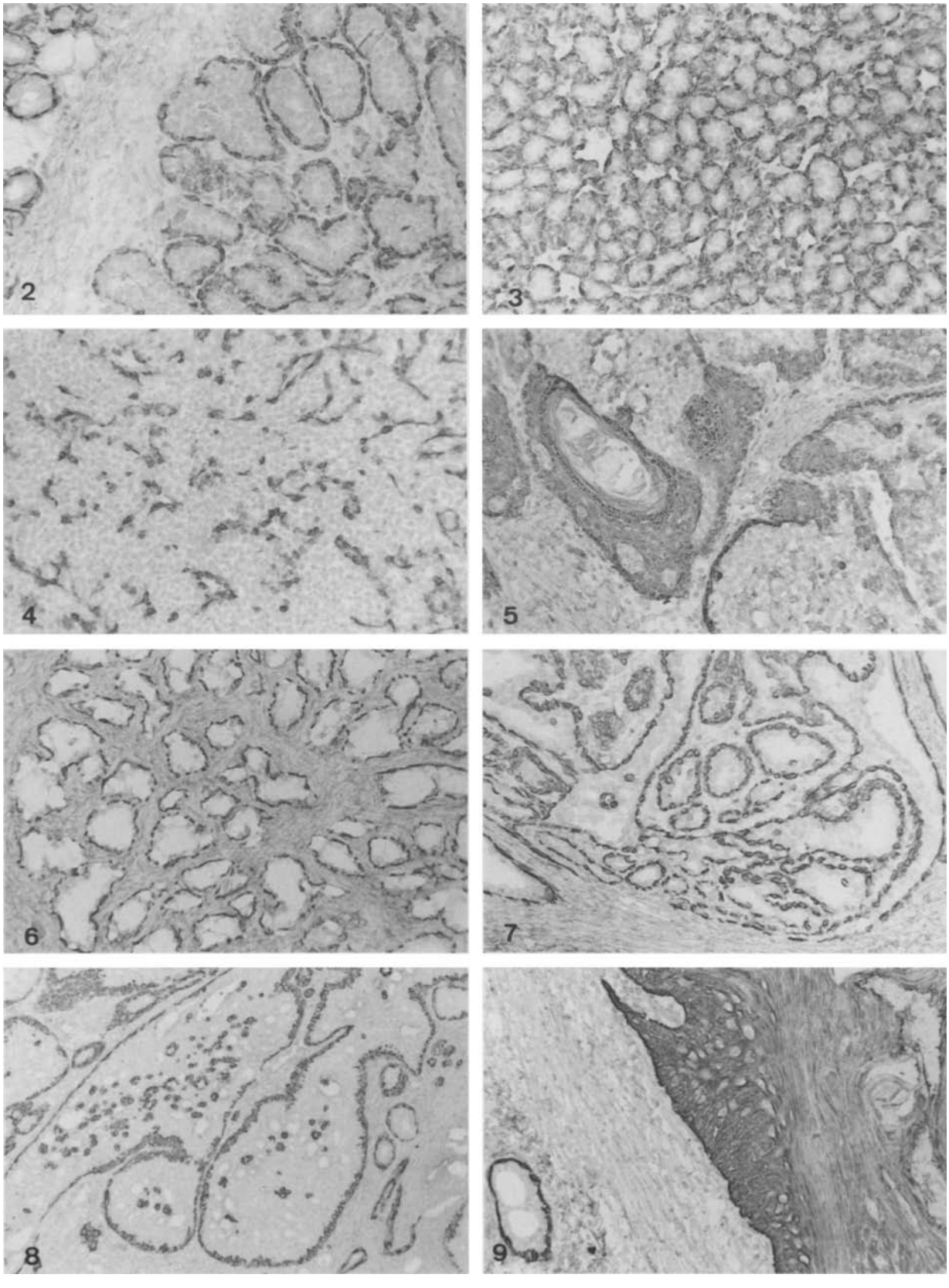
positivity with 312C8-1 was found in the myoepithelial cells of the secretory portions (Fig. 1a), but not in the luminal cells. It was also observed in outer layer cells of the ductal portions. In rat salivary glands, positivity to 312C8-1 was seen in the myoepithelial cells of the acini as well as the triangular-shaped outer layer cells of the ducts (Fig. 1b). Antibody 312C8-1 also reacted with the basal and suprabasal cells of the epidermis, but not with upper zonal cells (Fig. 1c). The staining patterns in mouse tissue were the same as those in rat tissue. In both species, positivity was restricted to epithelial cell types and never detected in stromal cells.

In the pregnancy-dependent mammary tumours (plaque) (Table 1), marker-positive tumour cells completely encircled the marker-negative tumour cells of the branching tubules (Fig. 2). In adenocarcinoma Type A, the acinar-like structure was regularly composed of marker-positive outer layer cells and marker-negative inner layer cells (Fig. 3). In adenocarcinoma Type B, marker-positive tumour cells were scattered in cancer foci (Fig. 4). In adenoacanthomas, almost all tumour cells in epidermoid structures were marker-positive (Fig. 5) in addition to the marker-positive basally situated tumour cells.

In benign tumours such as adenoma, lactating adenoma (Fig. 6), papilloma (Fig. 7) and fibroadenoma, myoepithelial cells stained with 312C8-1 were observed in DMBA treated animals (Table 2). In adenocarcinomas and carcinosarcomas, a mosaic staining pattern was seen in the proliferating epithelial cell nests, in addition to the marker-positive basally situated tumour cells (Fig. 8). In squamous cell carcinomas, the marker was observed, not only in basally situated tumour cells, but also in almost all cells in the cancer foci, except for the keratinizing zones (Fig. 9). Neoplastic mesenchymal cells in fibromas, phyllodes tumours, stromal sarcomas and carcinosarcomas were invariably marker-negative.

Discussion

As the mammary gland is derived from the epidermis, it is thought to belong to the same tissue type as the sweat and salivary glands and the epidermis. Human basal epithelial marker, demonstrated with a monoclonal antibody, 312C8-1, against the keratin polypeptide of human breast cancer, could be detected in cells between luminal cells and basement membrane in normal mammary, sweat and salivary glands where the myoepithelial cells are present. The antigen positive cells were also positive with actin which demonstrated the myoepithelial cells distinctly (Tsubura and Morii 1988). In our present study, this antibody demonstrated similar staining patterns in the mouse and rat. The monoclonal antibody 312C8-1 show cross-reactivity between rodents and humans. This epitope is conserved along the phylogenic line. In normal tissue of rodents, it recognized a certain keratin in myoepithelial cells of the glands and the basal and suprabasal cells of the epidermis specifically, while it was absolutely unreactive with the luminal epithelial cells of these glands. The similarity between myoepithelial cells of these glands and basal cells of the epidermis is due to the epidermal origin of the former. In rodents as well as in humans, the myoepithelial cells have a characteristic phenotype shared with basal cells of the epidermis.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The significance of antigen-positive cells in tumour tissue must be assessed with extreme caution. In benign mammary tumours in the rat, the distribution of tumour cells positive for 312C8-1 was similar to that of those stained with anti-actin antibody (Tsubura and Morii 1988), which clearly labelled the myoepithelial cells. However, 312C8-1 completely encircled the branching tubules in plaque, the pregnancy-dependent mouse mammary tumour, where no myoepithelial cells were seen (Tsubura and Morii 1981). Therefore, 312C8-1 may be reactive with the basal cell layer, which consists of undifferentiated precursor cells. The basal epithelial marker would be exhibited not only in differentiated myoepithelial cells, but also in undifferentiated precursor cells. This antibody should thus be defined simply as being basal epithelium specific. The majority of human cancers lack this basal epithelial marker (Dairkee et al. 1985, 1987, 1988; Wetzels et al. 1989), but many tumour cells in rodents were labelled. An interesting feature was seen in mouse adenocarcinoma Type A, where the outer layer tumour cells of a uniform acinar-like structure were all positive. This staining picture mimics normal acinar stainability. It is reasonable to interpret the marker-positive tumour cells as basal cells rather than mature myoepithelial cells, as this type of tumour metastasizes readily (Squartini 1979). Furthermore, transplanted adenocarcinoma Type A revealed two cell types on routine histology (Slemmer 1974). Groups of the marker-positive tumour cells, which could not be ascertained as neoplastic basal or residual myoepithelial foci were always encircled by marker-positive tumour cells and the proliferating cancer foci revealed a mosaic staining pattern. The positive tumour cells might be the "intermediate cell" described by Ozzello (1971), which lacks the characteristics of both the luminal and myoepithelial cells and possesses a more primitive character. Different keratin expressions can be seen in squamous cell carcinogenesis when compared with its benign counterparts (Tatemoto et al. 1987). In the present study, 312C8-1 was not restricted to basal or suprabasal cells in the malignant epidermoid element in rodents. None of the examined stromal tumour cells expressed the human basal epithelial marker. We concluded that 312C8-1 is useful in distinguishing cell phenotype in mice and rats, and has interesting implications for defining the cell types from which rodent mammary tumours originate.

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Received April 25, 1989 / Accepted June 14, 1989