Virchows Archiv A Pathol Anat (1992) 421:105–113

Virchows Archiv A
Pathological Anatomy and Histopathology

The pathobiology of salivary gland

II. Morphological evaluation of acinic cell carcinomas in the parotid gland of male transgenic (MMTV/v-Ha-*ras*) mice as a model for human tumours

Irving Dardick [1], Aileen P. Burford-Mason [1], David S. Garlick [2], and Walter P. Carney [3]

[1] Department of Pathology, University of Toronto, Banting Institute, 100 College Street, Toronto, Ontario M5G 1L5, Canada
[2] TSI Mason Research Institute, 57 Union Street, Worcester, MA 01608, USA
[3] Oncogene Science Incorporated, 80 Rogers Street, Cambridge, MA 02142, USA

Received December 20, 1991 / Received after revision February 14, 1992 / Accepted February 20, 1992

Summary. In the transgenic TG.SH (mouse mammary tumour virus/v-Ha-*ras*) mouse, designed to develop mammary tumours, occasional spontaneous salivary gland tumours have been reported, predominantly in males. The incidence and histomorphology of salivary gland tumours in 73 TG.SH mice were surveyed and in total, 21.9% developed both overt and microscopic parotid tumours. The majority developed between 73 and 150 days of age. In 31.5% of the TG.SH mice, occasional unilateral, but more frequently bilateral exophthalmos due to hyperplasia of the intraorbital (Harderian) lacrimal gland was observed. In 70% of these animals, parotid tumours developed later. Since Harderian gland hyperplasia, occurring as early as 5 weeks of age, preceded the development of palpable salivary gland lesions, this stigma is useful for the early selection of animals likely to progress to tumour formation. Before tumour-bearing transgenic mice are considered to be suitable models of human neoplastic disease, morphological characterization is necessary to ensure that the tumours are histologically representative of the human lesions for which they are potential models. In this study, all parotid tumours consisted of acinar-like glandular structures with central lumina discernible by electron microscopy. Ultrastructurally, secretory granules evident in the apical cytoplasm of the tumour cells resembled the zymogen granules of the normal parotid acinar cell, and some cells had a prominent complement of rough endoplasmic reticulum. These features, along with focal amylase expression detected immunohistochemically in some parotid tumours, identified these neoplasms as acinic cell carcinomas that mimic the human salivary gland acinic cell carcinoma faithfully.

Key words: Parotid gland – *ras* – Transgenic mouse – Electron microscopy – Immunocytochemistry

Correspondence to: I. Dardick

Introduction

Transgenic mice, express foreign DNA in their somatic cells, making them a unique resource in which to examine the complex molecular effects of a gene in a living animal (Cuthbertson and Klintworth 1988). Substantial efforts have been devoted to the development of transgenic animals as models of human neoplasia, since aetiological factors and pathogenetic processes relating to many neoplastic diseases are so poorly understood. The role of oncogenes in tumorigenesis and tumour progression is now well established (Hoffler 1991), and it is not surprising that the introduction of activated oncogenes into the genome of mice has resulted in tumour-bearing animals (Andres et al. 1987; Hanahan 1986; Neilsen et al. 1991; Pattengale et al. 1989).

The high incidence of human breast cancer and the lack of significant progress in therapeutic salvage, have promoted the production of strains of transgenic mice using oncogenes coupled to various mammary gland-specific promoter/enhancer DNA sequences. Mice expressing activated oncogenes such as c-*myc*, v-Ha-*ras* and c-*neu* coupled to either the mouse mammary tumour virus (MMTV) (Leder et al. 1986; Muller et al. 1988; Pattengale et al. 1989; Sinn et al. 1987; Stewart et al. 1984) or the murine whey acidic protein (*wap*) (Andres et al. 1987; Neilsen et al. 1991) promoter sequences result in mammary adenocarcinomas.

A low incidence of salivary gland tumours, primarily in males, has been reported in transgenic mice created for breast cancer research. These have been mainly adenocarcinomas and have occurred in mice bearing either the MMTV/v-Ha-*ras* transgene alone (Pattengale et al. 1989) or combined with the MMTV/c-*myc* construct (Pattengale et al. 1989; Sinn et al. 1987) and the *wap-ras* (human c-Ha-*ras* gene) transgene (Andres et al. 1987; Neilsen et al. 1991). The MMTV/*erb*-B2(*neu*) transgene, although expressed in salivary and breast tissues, only produces tumours in the latter, but results in bilateral

salivary gland hyperplasia (Muller et al. 1988). Occasional adenocarcinomas develop in the salivary glands of mice bearing the *int*-1 oncogene (MMTV LTR/c-*int*-1) (Tsukamoto et al. 1988). In such transgenic mice, salivary gland tumours have been well characterized in terms of oncogene expression (Andres et al. 1987; Neilsen et al. 1991; Pattengale et al. 1989; Sinn et al. 1987) but have little or no morphological characterization with respect to the human salivary gland tumours for which they are potential models. Poorly differentiated adenocarcinomas and undifferentiated carcinomas similar to those occurring in mice with the *wap-ras* transgene (Neilsen et al. 1991) are included in the WHO international classification of human salivary gland tumours Seifert 1991; Seifert et al. 1990) but are not representative of the more typical malignant tumours of these glands. In contrast, spontaneously occurring adenocarcinomas developing in TG.SH (MMTV/v-Ha-*ras* transgene) and TG.M/SH (both MMTV/v-Ha-*ras* and MMTV/c-*myc* transgenes) strains of transgenic mice have been reported as having an acinar-type organization and histology (Pattengale et al. 1989; Sinn et al. 1987). The object of the present study was to survey the incidence and histomorphology of salivary gland tumours occurring in male TG.SH mice and to use immunohistochemistry and electron microscopy to further characterize these neoplasms as a possible model of human salivary gland tumours.

Materials and methods

The investigation used 73 male transgenic mice of the TG.SH strain (MMTV/v-Ha-*ras*) originally developed and reported by Sinn et al. (1987). The mice were obtained from Charles River (Wilmington, Mass, USA), and were certified as viral antibody free. Known homozygous MMTV/v-Ha-*ras* males were bred to FVB females not carrying the gene. All male offspring were checked and those used in the study were heterozygous for the transgene. At least at weekly intervals, the mice were examined for evidence of exophthalmos and salivary and/or mammary gland tumours. When tumours were palpable or grossly visible, the mice were sacrificed and autopsied. Transgenic mice without exophthalmos or gross evidence of tumours were observed until 35 weeks of age and then sacrificed for histological assessment. Similarly, mice with exophthalmos but no palpable tumours at 35 weeks of age were sacrificed at this time. All mice were examined for histological evidence of tumours in the salivary glands and other organs, and for tissue pathology such as corneal ulceration and intraocular lesions.

All material for histological examination was fixed in a modified Carnoy's solution (90% methanol and 10% glacial acetic acid) and embedded in paraffin. Sections were stained using haematoxylin and eosin. Immunohistochemical studies used a polyclonal anti-amylase antibody (1:50; Dako, Santa Barbara, Calif., USA), an indirect peroxidase-antiperoxidase technique, and the chromogen 3,3′-diaminobenzidine. The proliferative capacity of tumour cells was studied using a monoclonal antibody against proliferative cell nuclear antigen (PCNA, 1:50; Coulter, Burlington, Ontario, Canada). Tissues for electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols and embedded in epon/araldite resin. Thin sections stained with uranyl acetate and lead citrate were examined and photographed using a Philips EM400 electron microscope.

Results

The pathology detected in 73 male MMTV/v-Ha-*ras* transgenic mice is summarized in Table 1. The earliest and perhaps most striking feature was the development of occasional unilateral, but most often bilateral, exoph-

Table 1. Tissue pathology and number of tumours or hyperplastic glands in male MMTV/v-Ha-*ras* (TG.SH strain) mice ($n=73$) and percentage of mice developing each type of lesion

Tissue	Number	Total	%
Salivary gland neoplasms			
Number with 3 tumours	5	15	
Number with 2 tumours	4	8	
Number with 1 tumour	7	7	
Totals	16	30	21.9
Mammary gland neoplasms			
Number with 3 tumours	0	0	
Number with 2 tumours	2	4	
Number with 1 tumour	5	5	
Totals	7	9	9.6
Harderian gland hyperplasia	23	–	31.5
Exorbital lacrimal gland hyperplasia	13	–	17.8

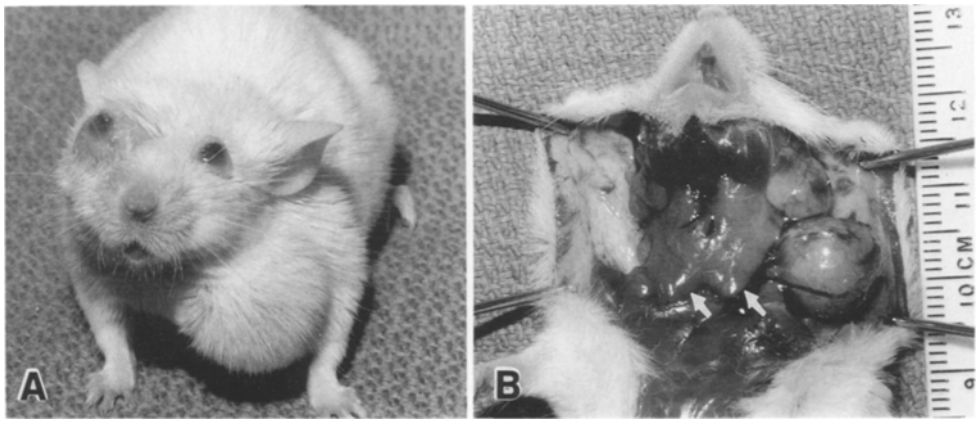

Fig. 1. A TG.SH (MMTV/v-Ha-*ras*) male transgenic mouse with bilateral exophthalmos (more marked on the right side) and a tumour mass in the left neck. **B** Exposure of the salivary glands of this mouse reveals two tumour nodules associated with the left parotid gland and no gross abnormalities of either submandibular/sublingual gland complex (*arrows*)

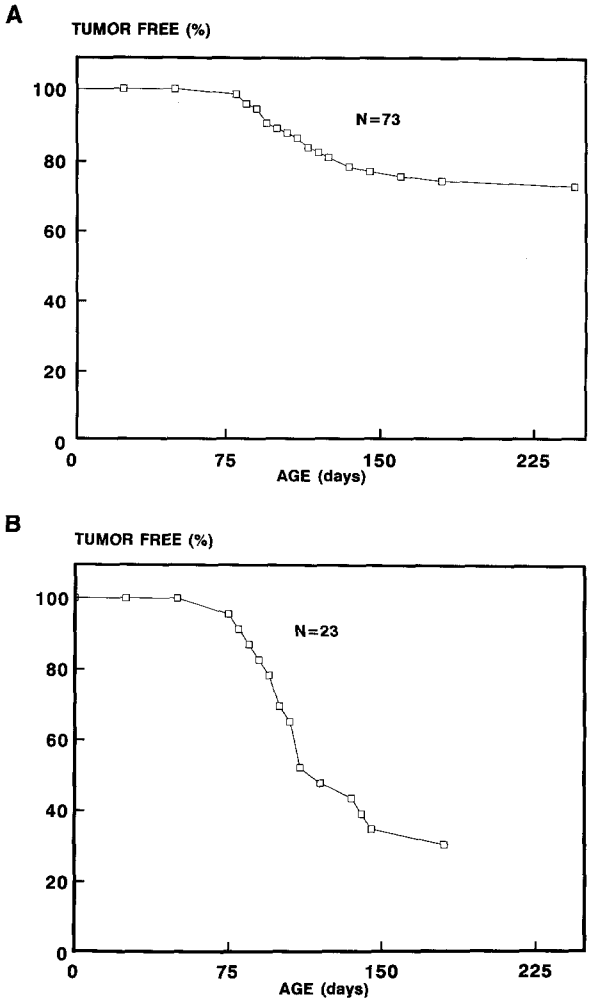

Fig. 2. **A** Time course and proportion of male TG.SH mice, carrying the MMTV/v-Ha-*ras* transgene, developing parotid gland tumours. The majority of tumours occurred between 73 and 150 days of age. **B** Time course for the development of parotid gland tumours of those male TG.SH mice carrying the MMTV/v-Ha-*ras* transgene, with Harderian (intraorbital) gland hyperplasia

thalmos due to marked hyperplasia of the intraorbital lacrimal (Harderian) gland (Fig. 1). This developed as early as 5 weeks and was apparent in all mice that developed this sign by 10 weeks of age. Twenty-three of the 73 (31.5%) male MMTV/v-Ha-*ras* transgenic mice (Table 1) developed exophthalmos, which in all cases preceded the development of palpable salivary gland and mammary tumours. Of the 23 males with Harderian gland hyperplasia, 16 (approximately 70%) developed salivary gland tumours (Table 2) with the time course displayed in Fig. 2B.

All salivary gland tumours occurred in the parotid gland, the earliest at 73 days of age, the majority (87.5%, 14 of 16 mice) occurring by 150 days, and 2 others at 181 and 258 days, respectively. Fragments of a parotid tumour obtained from one of the MMTV-v-Ha-*ras* male mice were finely minced and inoculated subcutaneously in a male nu/nu mouse. Both the light and electron microscopy of the resultant tumour was identical to in situ parotid gland tumours. The kinetics of tumour development are provided in Fig. 2A. Palpation of the neck at regular intervals resulted in the detection of small, tumour nodules in the neck region. In 7–10 days these grew to a diameter of 0.5–1.0 cm. Autopsy of male mice with definitive neck masses revealed more than one tumour grossly or microscopically in some animals but did not reveal tumours of internal organs other than the parotid gland or evidence of metastases. Periparotid lymph nodes showed no evidence of metastatic tumour. The lack of metastases may result from the timing of sacrifice of salivary gland tumour-bearing mice, because of the need to survey early stages of tumour development. The extensive degree of exophthalmos with its potential for corneal ulceration was another reason for intervention. Histological review of excised globes, however, showed no ophthalmological abnormality. Of the 7 males developing breast tumours (Table 1), 3 had no gross or microscopic evidence of parotid or other salivary gland tumours and 2 (both without salivary gland tumours) also had no Harderian gland hyperplasia. Of the 73 male MMTV/v-Ha-*ras* mice, 7 (9.6%) developed breast tumours (Tables 1, 2) which were cystadenomas. In 17.8% of the 73 male TG.SH mice and 56.5% of those with Harderian gland hyperplasia (Tables 1, 2), the exorbital lacrimal gland showed proliferation of ductal elements and an overall modest hyperplasia.

Table 2. Glandular pathology developing in the 23 male MMTV/v-Ha-*ras* mice with Harderian hyperplasia

Pathology	Number	%
Salivary or mammary tumours	23	100.0
Parotid acinic cell carcinoma	16	69.6
Mammary cystadenoma	7	30.4
Exorbital lacrimal gland hyperplasia	13	56.5

Dramatic differences could be seen between the intraorbital (Harderian) lacrimal glands of the TG.SH transgenic mice with exophthalmos and those with normal eyes. Whereas the normal Harderian gland measured 1–2 mm in greatest diameter, a hyperplastic gland was 8–12 mm long and virtually filled the entire orbital fossa. Microscopically, glandular alterations were also dramatic. The normal Harderian gland contained compactly arranged and fairly uniformly sized acini formed by a single layer of cuboidal to low columnar cells (Fig. 3A). Hyperplastic glands had larger acinar structures, some with microcystically dilated lumina. Judging from the nuclear distribution, the epithelium in many areas was bilayered, and still lined by low columnar cells with the apical regions filled with secretory granules (Fig. 3B). No dysplasia was observed and there were no definitive tumour nodules either grossly or microscopically.

In non-transgenic and transgenic mice, the normal parotid gland is multilobed and lies with the exorbital lacrimal gland on its supero-posterior aspect and the submandibular/sublingual complex on its inferior and anterior portions. It is important to recognize these anatomical features and relationships in assessing the gland

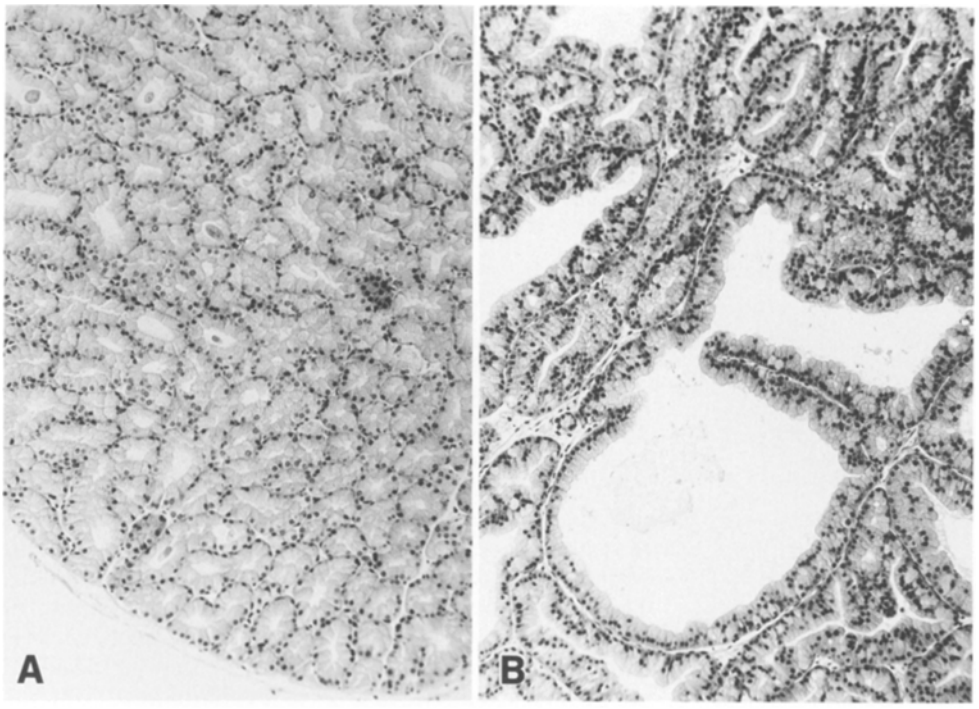

Fig. 3A, B. Harderian glands from male TG.SH mice bearing the MMTV/v-Ha-*ras* transgene. **A** Normal Harderian gland with relatively uniformly sized and compactly arranged acinar units composed of a single layer of cuboidal cells. **B** Hyperplastic Harderian gland with irregularly sized and shaped glandular structures lined by a more crowded and often pseudostratified epithelium with papillary projections. No epithelial dysplasia is evident. H & E, ×130

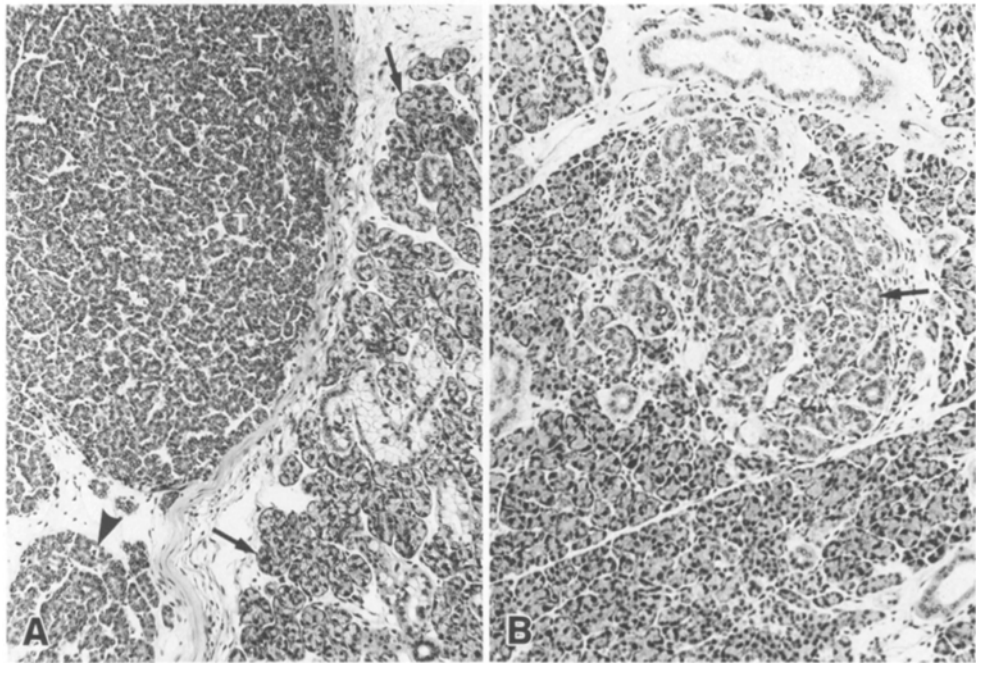

Fig. 4. A Tumour nodule (*T*) that is encapsulated but abuts on the fibrous capsule separating residual parotid salivary gland (*arrowheads*) from the adjacent submandibular gland (*arrows*). **B** Another male TG.SH transgenic mouse with a gross tumour nodule in which sections of the parotid gland revealed a microscopic focus of unencapsulated tumour (*arrow*) infiltrating adjacent parenchyma. H & E, ×130

of origin of tumours, since there is such a variety of lacrimal and salivary glands in the head and neck region.

In the 16 male mice with a total of 30 salivary gland tumours, all developed within or in immediate relation to the parotid gland (Tables 1, 2). Furthermore, all had the same general histological features (Fig. 4). Most tumours consisted of a single, circumscribed nodule with a thin fibrous capsule (Fig. 4A). Some, particularly when less than 1.0 cm in diameter, were still partially surrounded by atrophying lobules of parotid gland. In 11 of the 16 mice with parotid tumours, one or two small microscopic neoplastic foci were identified within the parotid glands (Fig. 4B), usually in a lobule separate from the grossly visible tumour or on the contralateral side. Such intraglandular tumours were generally unencapsulated and tended to infiltrate into the adjacent parotid tissue. Multiple tumours occurred in 9 of the transgenic mice (Table 2). Whether gross or microscopic, the parotid tumor consisted of small acinar-like glandular structures with or without apparent central lumena (Fig. 5). Some tumour cells were aligned in a more tubular fashion and frequently anastomosed with neighbour-

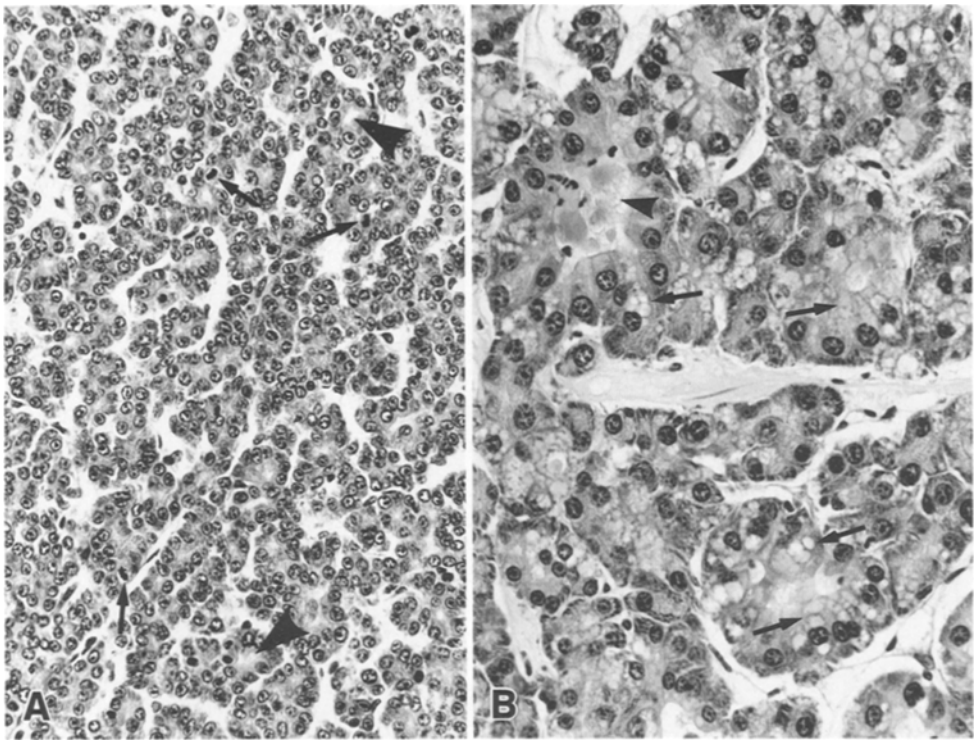

Fig. 5. A All parotid tumours were composed of clusters and anastomosing cords of cells forming glandular structures with small lumens (*arrowheads*). Mitotic figures (*arrows*) were frequent. **B** In some parotid gland tumours, secretory granules were readily identified within the apical cytoplasm of tumours cells (*arrows*) and secretory product distended glandular lumens (*arrowheads*). H & E. **A** ×340; **B** ×430

ing glands. Mitotic figures were frequent and occasionally abnormal in structure (Fig. 5A). Depending on the degree of differentiation, secretory granules were evident in the apical portion of the cells and excreted secretory product filled the lumen (Fig. 5B).

Ultrastructural features confirmed the glandular differentiation (Fig. 6). Polygonal to pyramidal shaped tumour cells were arranged to form small lumina. Tight junctions joined apical regions, but surface microvilli were sparse. Although variable in degree, two features were consistently present. One was the presence of relatively large secretory granules and the other moderate to extensive rough endoplasmic reticulum at times with an orderly and somewhat parallel arrangement (Fig. 6A). Some secretory granules were homogeneous in appearance, while others had a distinct central dot and a continuous margin or crescentic zone of more darkly staining material (Fig. 6A). The combination of these cellular features should be compared with acinar cells, containing typical zymogen granules and abundant arrays of rough endoplasmic reticulum, from a transgenic mouse without salivary or Harderian gland pathology (Fig. 6B).

Anti-amylase antibodies strongly and diffusely decorated acinar cells of the normal parotid gland, but duct cells were negative (Fig. 7A), along with all cells in the submandibular and sublingual glands. Most parotid tumours were entirely negative when stained with this antibody (Fig. 7A), but a few revealed foci with single or small groups of tumour cells with positively stained cytoplasm (Fig. 7A, inset). The intralobar microscopic foci of tumour were also negative for amylase (Fig. 7B).

When normal salivary gland and the portions of parotid gland adjacent to the tumour nodules were immunostained using anti-PCNA antibodies, occasional cells (almost exclusively acinar) had labelled nuclei. In contrast, the parotid tumours generally displayed a considerable proportion of the cells with stained nuclei (Fig. 8). As is evident from this figure, the percentage of cycling cells varied from area to area, but overall at least 25–30% of the tumour cells had strongly positive nuclei indicating peak expression of PCNA. Still other nuclei (Fig. 8) had weak to moderate staining representing tumour cells in phases of the cell cycle with lesser amounts of PCNA (Celis and Celis 1985).

For comparative purposes with the parotid tumours of TG.SH mice, electron microscopic appearances of a typical human acinic cell carcinoma of parotid gland are illustrated in Fig. 9. Acinar-like structures, with central lumina and zymogen-type secretory granules in the apical regions, are obvious and similar to those seen in all of the transgenic mice. As in the tumours of transgenic mice, some secretory granules are homogeneous, but a small number have what appear to be compartmentalization of secretory products within the granules (Fig. 9, inset). Rough endoplasmic reticulum is variably present but, at least in some cells, rows of endoplasmic reticulum are obvious in basal aspects of the cells (Fig. 9).

Discussion

Salivary gland tumours are probably the most morphologically complex of human neoplasms. The numerous distinctive types of these tumours have an extraordinarily wide histological spectrum. The latter appears to result from the differentiation of more than one kind of

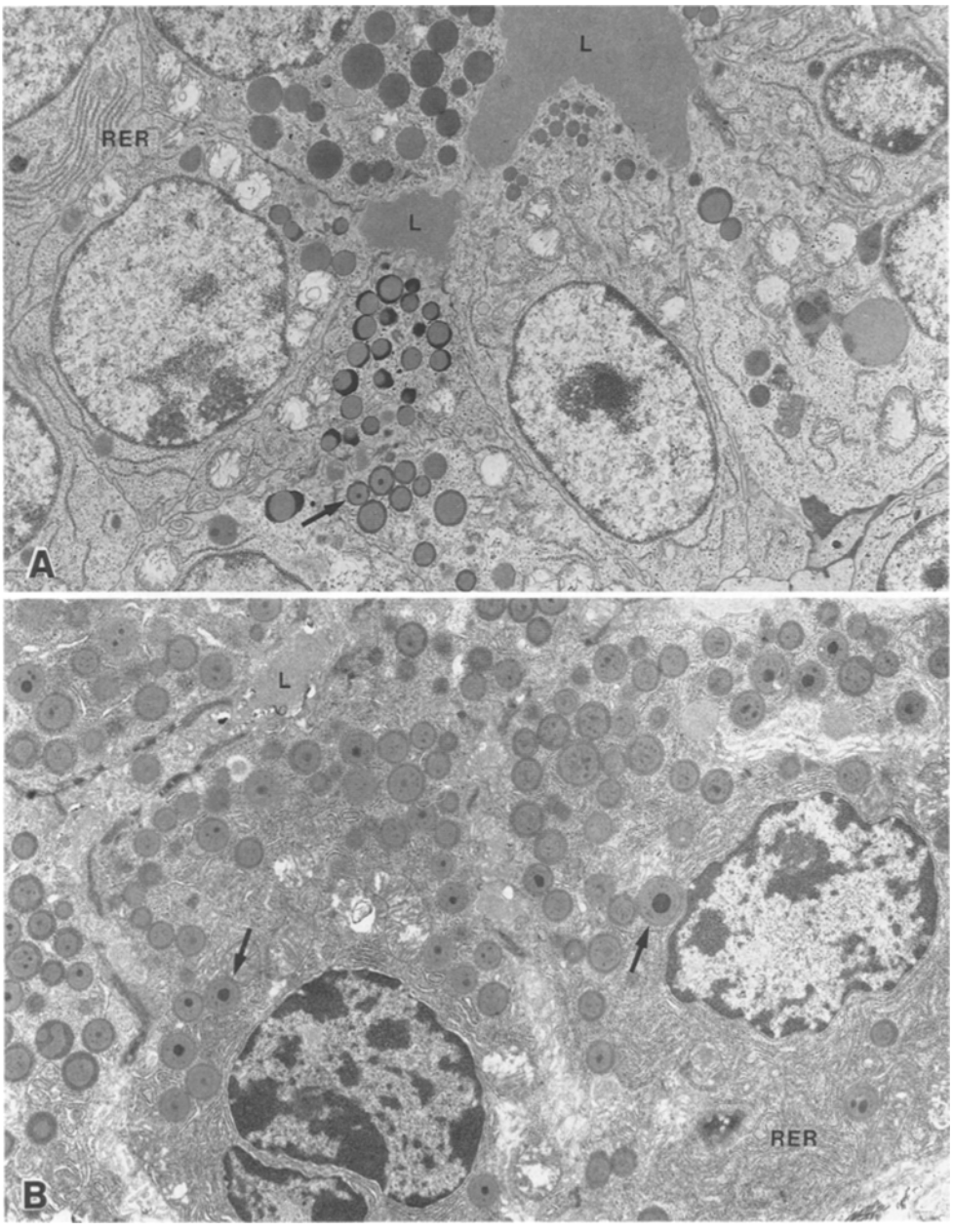

Fig. 6. **A** Parotid gland tumour in a male TG.SH transgenic mouse in which the rough endoplasmic reticulum-containing (*RER*) tumour cells have apical secretory granules with or without densely staining crescentic, circumferential and central (*arrow*) zones. Glandular lumens (*L*) contain homogeneously staining secretory product. **B** Parotid gland from a male TG.SH transgenic mouse without Harderian gland hyperplasia or salivary gland tumours. Note the similarity of the lumen-forming (*L*) acinar cells with many apical secretory granules (with varying internal staining qualities and patterns) and extensive rough endoplasmic reticulum (*RER*) to the ultrastructure of the tumour cells in **A**. Some secretory granules (*arrows*) have similar morphology to those evident in parotid tumours. Uranyl acetate and lead citrate. **A** ×5,800; **B** ×6,900

tumour cell, their arrangement in certain cellular patterns and the localized synthesis of extracellular materials by at least some of the tumour cells (Dardick 1991; Dardick and Nostrand 1987). As a consequence, the tumours not uncommonly present problems in diagnosis and classification. One reason for the limited understanding of the processes that lead to the diverse morphology is the lack of appropriate models systems for basic research.

In human salivary gland neoplasms, *ras* oncogenes have been shown to be expressed (Spandidos et al. 1985). A cell line derived from human salivary gland also expresses the *ras* oncogene protein, p21 (Azuma et al. 1988). Other oncogenes such as c-*erb*B-2 (c-*neu*), however, may also have vital roles in salivary gland pathology (Muller et al. 1988; Semba et al. 1985; Stenman et al. 1991). This emphasizes the essential role that acinic cell carcinoma-producing TG.SH mice and other transgenic animals can have in investigating human salivary gland tumours. Messenger RNA transcripts for *ras* are detectable in a variety of tissues in male TG.SH mice at high levels; this includes salivary and hyperplastic Harderian glands and the seminal vesicles, with lesser amounts in breast, lung, thymus and spleen (Sinn et al. 1987). Despite equally high levels of *ras* expression in the Harderian and salivary glands of female TG-SH mice, salivary gland tumour induction is considerably less frequent than in male mice of this strain (Pattengale et al. 1989; Sinn et al. 1987). Not only is the reverse true in the case of breast tumours (Pattengale et al. 1989; Sinn et al. 1987) but, whereas these are malignant in female TG.SH animals (Pattengale et al. 1989; Sinn et al. 1987), all 7

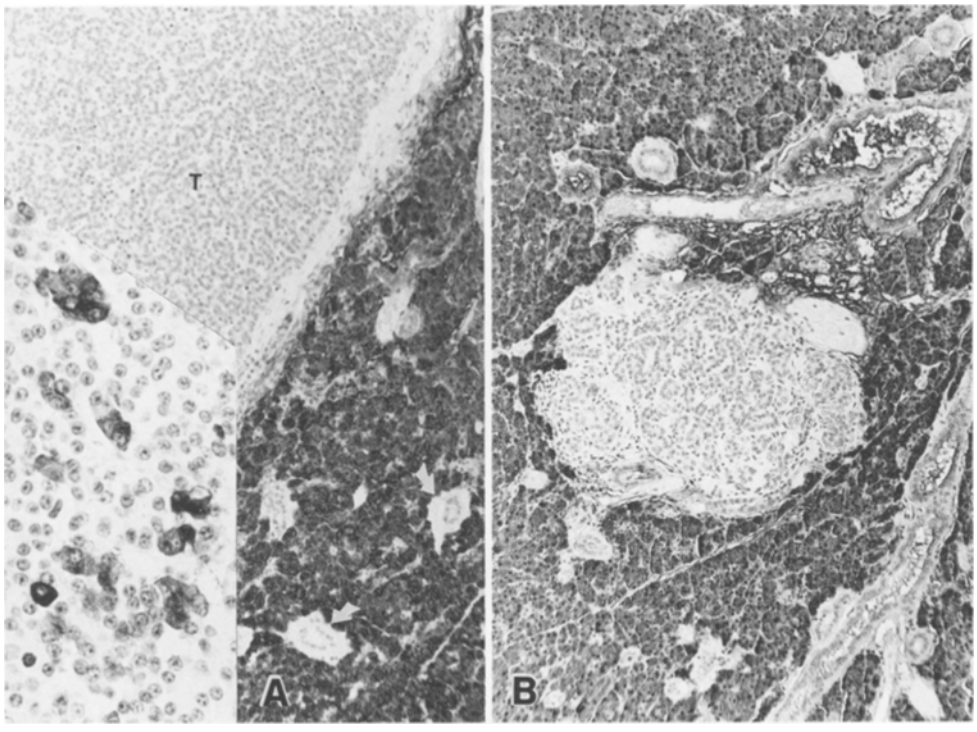

Fig. 7A, B. Parotid gland-containing tumours from male TG.SH transgenic mice immunocytochemically stained using an anti-amylase antibody. **A** Parotid acinar cells are strongly stained, but striated ducts (*arrows*) and the tumour (*T*) are negative. *Inset*: Another parotid tumour stained with anti-amylase antibody shows a few single and small groups of tumour cells with moderate to intense staining. **B** A microscopic focus of parotid gland tumour is negative for amylase while the adjacent acinar cells are strongly decorated. Indirect peroxidase-antiperoxidase technique with haematoxylin counterstain. **A** ×130, and *inset* ×430; **B** ×130

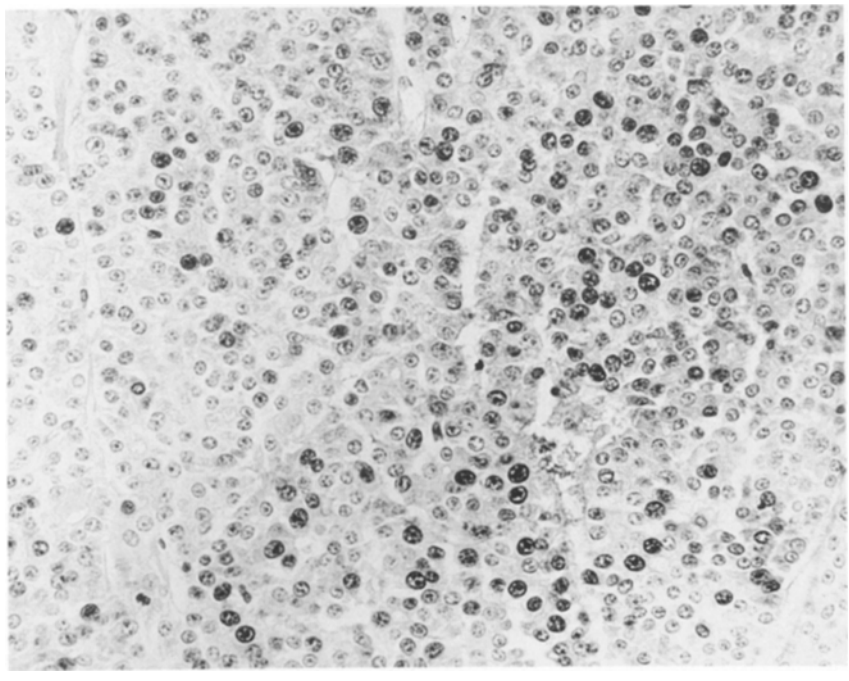

Fig. 8. Parotid gland adenocarcinoma of male TG.SH mouse immunostained with anti-PCNA antibody reveals the considerable number of cycling cells (darkly stained nuclei) that are asymmetrically distributed within the tumor. Indirect peroxidase-antiperoxidase technique with haematoxylin counterstain, ×380

of the breast tumours occuring in males in our series were histologically benign. Significantly, parotid glands bear adenocarcinomas in MMTV/v-Ha-*ras* mice (Pattengale et al. 1989; Sinn et al. 1987) whereas in the *wap-ras* transgenic mouse (Andres et al. 1987; Neilsen et al. 1991), tumours are found exclusively in the submandibular gland. The development of tumours is a multistage process and additional oncogenic events or epigenetic factors, which might include the sex hormones (Cuthbertson and Klintworth 1988; Hanahan 1986; Pattengale et al. 1989; Sinn et al. 1987), are clearly necessary. In humans, distinct biases in female to male distribution occur in certain salivary gland tumours. For example, while Warthin's tumour (Chapnik 1983) and salivary duct carcinoma (Ellis et al. 1991) appears to have a higher frequency in males, canalicular adenoma occurs predominantly in females (Kratochvil 1991). The complexity of expression of transgenes is perhaps illustrated by transgenic mice in which insertion of extra copies of amylase genes – derived from a parotid cDNA library

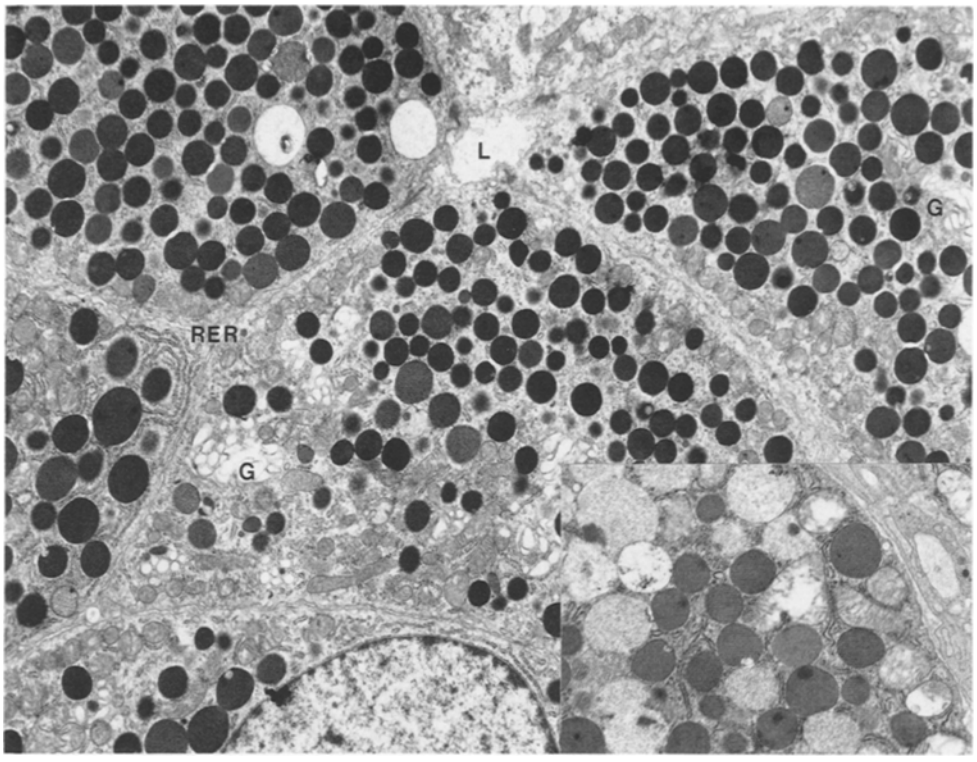

Fig. 9. Human acinic cell carcinoma of parotid gland. Many zymogen-type secretory granules fill the apical regions of tumour cells and some of these granules have eccentric darkly staining regions (*inset*). The tumour cells form a small lumen (*L*) and display Golgi complexes (*G*) and rough endoplasmic reticulum (*RER*). Uranyl acetate and lead citrate, ×8,200 (*inset* ×9,700)

– produce increased expression in liver and, surprisingly, in submandibular gland and brown and white fat, but not in the parotid gland (Jones et al. 1989).

Spontaneous tumours, both benign and malignant of a variety of types, occur in the salivary glands of the mouse at a low incidence (Frith and Heath 1985). The animals (F1 hybrids of CD-1 × C57BL/6 mice) chosen to initiate the transgenic mice from which the TG.SH strain is derived were selected because of the infrequency with which they develop spontaneous tumours (Pattengale et al. 1989). Production and detailed investigations of the TG.SH strain with the *ras* proto-oncogene specifically activated by a mutation in codon 12 (glycine to arginine) and 59 (alanine to threonine) are fully reviewed by Pattengale et al. (1989). As illustrated in the current report, and that of others (Neilsen et al. 1991; Pattengale et al. 1989; Sinn et al. 1987), the incidence of a particular histological type of salivary gland tumour can be increased many fold, and with a relatively short lag-time, in certain strains of transgenic mice. In our series of 73 male TG.SH transgenic mice, 16 or 22% spontaneously developed one or more parotid gland tumours (totalling 30). In the *wap-ras* transgenic mouse model (Neilsen et al. 1991), 22 of 43 males (51%) developed an adenocarcinoma or anaplastic carcinoma of the submandibular gland. One advantage with the TG.SH strain is the phenotypic marker, Harderian gland hyperplasia (noted as unilateral or bilateral exophthalmos), that in all cases precedes salivary and, in some cases, breast tumour induction. Selecting this group ensures a high incidence of acinic cell carcinoma, since 16 of 23 (70%) of TG.SH mice with exophthalmos eventually developed this salivary gland tumour. Such affected mice can be culled in order to obtain salivary glands with early lesions and to facilitate studies of factors and cell types involved in or responsible for tumour formation.

The TG.SH transgenic mouse is unique in its fidelity with respect to morphological and functional aspects of human acinic cell carcinoma. As in the mouse model, acinic cell carcinomas occur preferentially in the human parotid gland (Lewis et al. 1991). Histologically, these tumours in both mouse and man differentiate as acinar units composed of tumour cells complete, with zymogen-like granules and predominance of rough endoplasmic reticulum ultrastructurally. Like human acinic cell carcinomas, the murine version appears to be a low-grade adenocarcinoma. Despite rapid growth and extensive mitotic activity, no metastases to local lymph nodes or distant sites occurred in the transgenic mice in the current series. In a similar fashion, human acinic cell carcinomas are often circumscribed, infrequently present with local metastases, distant metastases are uncommon and usually a late event, and 5, 10 and 20 year survival rates of 90%, 83%, and 67%, respectively, are reported (Lewis et al. 1991). Few animal models for human neoplasia, including the transgenic mice currently available, approximate the human condition to this extent both biologically and morphologically. In humans, there is no information on the aetiology and pathogenesis in the salivary gland variant of acinic cell carcinoma and, for that matter, little relevant to other salivary gland tumours. The TG.SH animal, therefore, offers an unprecedented opportunity to study oncogenic events in acinic cell carcinoma.

Acknowledgements. The authors appreciate the financial support of the Conn Smythe Foundation, Toronto, Ontario and the Charlie Conacher Cancer Research Fund, The Toronto Hospital Foundation, Toronto, Ontario. Technical assistance was provided by Richard Leung, Vienna Ostrynski, Ken Ekem and Michael Stratis. Gross photographs were taken by Shirley Hochstetler of Instructional Media Services, The Toronto Hospital and the figures produced by Allison McKay. Drs. Jim Kenyon and Anne Walker and the technical staff of the Animal Facility of the Max Bell Research Unit, The Toronto Hospital, provided invaluable assistance.

References

Andres A-C, Schönenberger C-A, Groner B, Hennighausen L, LeMeur M, Gerlinger P (1987) Ha-ras oncogene expression directed by a milk protein gene promoter: tissue specificity, hormonal regulation, and tumor induction in transgenic mice. Proc Natl Acad Sci USA 84:1299–1303

Azuma M, Yoshida H, Kawamata H, Yanagawa T, Furumoto N, Sato M (1988) Cellular proliferation and ras oncogene of p21 21,000 expression in relation to the intracellular cyclic adenosine 3′:5′-monophosphate levels of a human salivary gland adenocarcinoma cell line in culture. Cancer Res 48:2898–2903

Celis JE, Celis A (1985) Cell cycle dependent variations in the distribution of the proliferating cell nuclear antigen in cultured cells: subdivision of S phase. Proc Natl Acad Sci USA 82:3262–3266

Chapnik JS (1983) The controversy of Warthin's tumor. Laryngoscope 93:695–716

Cuthbertson RA, Klintworth GK (1988) Transgenic mice – a gold mine for furthering knowledge in pathobiology. Lab Invest 58:484–502

Dardick I (1991) Histogenesis and morphogenesis of salivary gland neoplasms. In: Ellis GL, Auclair PL, Gnepp DR (eds) Surgical pathology of the salivary glands. WB Saunders, Philadelphia, pp 108–128

Dardick I, Nostrand AWP van (1987) Morphogenesis of salivary gland tumors: a prerequisite to improving classification. Pathol Annu 22(pt.1):1–53

Ellis GL, Auclair PL, Gnepp DR, Goode RK (1991) Other malignant epithelial neoplasms. In: Ellis GL, Auclair PL, Gnepp DR (eds) Surgical pathology of the salivary glands. WB Saunders, Philadelphia, pp 455–488

Frith CH, Heath JE (1985) Adenomas, adenocarcinomas, salivary gland, mouse. In: Jones TC, Mohr U, Hunt RD (eds) Monographs on pathology of laboratory animals: digestive system Springer-Verlag, Berlin, pp 190–192

Hanahan D (1986) Oncogenesis in transgenic mice. Oncogenes and growth control. In: Kahn P, Graf T (eds) Springer, Berlin Heidelberg New York, pp 349–363

Hoffler H (1991) Oncogene and receptor expression. In: Seifert G (ed) Cell receptors. Morphological characterization and pathological aspects. Springer, Berlin Heidelberg New York, pp 435–456

Jones JM, Keller SA, Samuelson LC, Osborn L, Rosenberg MP, Meisler MH (1989) A salivary amlyase transgene is efficiently expressed in liver but not in the parotid gland of transgenic mice. Nucleic Acids Res 17:6613–6623

Kratochvil FJ (1991) Canalicular adenoma and basal cell adenoma. In: Ellis GL, Auclair PL, Gnepp DR (eds) Surgical pathology of the salivary glands. Saunders, Philadelphia, pp 202–224

Leder A, Pattengale PK, Kuo A, Stewart TA, Leder P (1986) Consequences of widespread deregulation of the c-*myc* gene in transgenic mice: multiple neoplasms and normal development. Cell 5:485–495

Lewis JE, Olsen KD, Weiland LH (1991) Acinic cell carcinoma: clinicopathologic review. Cancer 67:172–179

Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P (1988) Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-*neu* oncogene. Cell 54:105–115

Neilsen LL, Discafani CM, Gurnani M, Tyler RD (1991) Histopathology of salivary and mammary gland tumors in transgenic mice expressing a human Ha-*ras* oncogene. Cancer Res 51:3762–3767

Pattengale PK, Stewart TA, Leder A, Sinn E, Muller W, Tepler I, Schmidt E, Leder P (1989) Pathology and molecular biology of spontaneous neoplasms occurring in transgenic mice carrying and expressing activated cellular oncogenes. Am J Pathol 135:39–61

Seifert G (1991) Histological typing of salivary gland tumours. World Health Organization international histological classification of tumours, 2nd edn Springer, Berlin Heidelberg New York

Seifert G, Brocheriou C, Cardesa A, Eveson JW (1990) WHO international histological classification of tumours: tentative histological classification of salivary gland tumours. Pathol Res Pract 186:555–581

Semba K, Kamata N, Toyoshima K, Yamamoto T (1985) A v-*erb*B related oncogene, c-*erb*B-2 is distinct from c-*erbB*-1/epidermal growth factor receptor gene and is amplified in a human salivary gland adenocarcinoma. Proc Natl Acad Sci USA 82:6497–6501

Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P (1987) Coexpression of MMTV/v-Ha-*ras* and MMTV/c-*myc* genes in transgenic mice: synergistic action of oncogenes in vivo. Cell 49:465–475

Spandidos DA, Lamothe A, Field JK (1985) Multiple transcriptional activation of cellular oncogenes in human head and neck solid tumors. Anticancer Res 5:221–224

Stenman G, Sandros J, Nordkvist A, Mark J, Sahlin P (1991) Expression of the ERBB2 protein in benign and malignant salivary gland tumors. Genes Chrom Cancer 3:128–135

Stewart TA, Pattengale PK, Leder P (1984) Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/*myc* fusion genes. Cell 38:627–637

Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE (1988) Expression of the int-1 gene in transgenic mice is associated with mammary hyperplasias and adenocarcinomas in male and female mice. Cell 55:619–625