Harderian Gland Pathology in Transgenic Mice Carrying the MMTV/v-Ha-ras Gene

Valerie White,* Eric Sinn,† and Daniel M. Albert*

Twenty-four Harderian glands from 16 transgenic mice carrying a v-Ha-ras gene under the control of the mouse mammary tumor virus promoter were examined histologically upon death of the animals. Eight glands were histologically normal and eleven were hyperplastic. Four additional glands showed predominantly hyperplasia, but also contained foci of dysplasia and in situ carcinoma. One gland contained an adenocarcinoma. The range of pathology observed in the Harderian glands of these transgenic mice was similar to that which arises spontaneously, although hyperplasia, and not adenoma, occurred in these mice as the benign tumor. Invest Ophthalmol Vis Sci 31:577–581, 1990

The Harderian gland is a horseshoe-shaped gland that is found in the medial orbit of most animals with a third eyelid or nictitating membrane. In rodents, it is a well developed tubuloalveolar gland which secretes lipid by a merocrine mechanism and contains dark brown porphyrin granules in the gland lumina. Spontaneously occurring tumors of the gland are frequent; Sheldon et al reported an average of 2.8% incidence in males and 5.1% incidence in females, depending on the strain of mouse observed. Tumor formation also has been induced by chemical carcinogens and radiation. The majority of neoplasms are adenomas of either papillary, cystic papillary, acinar, or cystic type. Occasional adenocarcinomas occur, and compose 5.2% of all tumors, a 0.25% incidence in the study by Sheldon et al. Because of their size, even the benign tumors cause marked exophthalmos, often with an exposure keratitis.

The Ha-ras gene is part of the ras gene family, the oncogene that is implicated most commonly in the genesis of many tumors, both human and animal. Originally isolated from a rat sarcoma virus, the normal cellular homologue has been shown to be situated on human chromosome eleven. The mechanisms by which this gene has been shown to be altered during carcinogenesis include point mutation, increased expression, and loss of one c-Ha-ras allele. c-Ha-ras encodes p21, a protein of 21 kD. This protein is believed to be anchored in the inner plasma membrane of the cell, where it binds GTP and GDP and has GTPase activity. Upon stimulation, the protein is believed to bind GTP; the intrinsic GTPase activity then hydrolyzes GTP to GDP, causing the protein to become inactive. GAP may be the stimulator of the p21–GTP complex or of its target. Point mutations in the gene are believed to alter the conformation of the protein or to reduce its GTPase activity, such that it remains in the active state longer than its wild-type counterpart. It has been postulated that activation leads to cell proliferation by way of the phosphatidylinositol second messenger cascade.

In order to study the effect of a mutated ras gene in vivo, Sinn and co-workers introduced a v-Ha-ras gene under the control of the mouse mammary tumor virus promoter (MMTV) into mouse zygotes, producing several strains of transgenic mice. They found that the MMTV/v-Ha-ras transgene was expressed abundantly in normal mammary tissue and salivary gland; lesser amounts were expressed in Harderian gland, thymus, spleen, lung, and ovary in the female, whereas in the male there were high levels of expression in the seminal vesicle, Harderian gland, and salivary gland and much less expression in mammary fat pad, lung, thymus, and spleen. The major tumors that occurred in these animals were mammary and salivary gland adenocarcinomas and lymphomas. Approximately 20% of the animals developed a bilateral exophthalmos as a result of Harderian gland hyperplasia (although not all glands were examined histologically).

The purpose of the current investigation was to examine the spectrum of Harderian gland pathology...
observed in these animals and to compare it with that occurring in nontransgenic mice.

**Materials and Methods**

Experimental details concerning the generation of the transgenic mice and the results obtained are found in Sinn et al. The current study adhered to the ARVO Resolution on the Use of Animals in Research. Construction of the plasmid pA9 containing MMTV/v-Ha-ras (construction from pBR322, Bethesda Research Laboratories) is described in Huang et al. Briefly, DNA containing the MMTV/v-Ha-ras gene was purified and injected into fertilized eggs (CD-1 x C57BL/6; CD-1 from Charles River, Wilmington, MA, and C57BL/6J from Jackson Laboratories, Bar Harbor, ME) removed from pregnant mice. These were reimplanted into another strain of pseudopregnant mice, which were allowed to give birth normally. Offspring were examined biweekly for tumor formation, and complete autopsies were done at the time of death. Transgenic animals were backcrossed to FVB/N mice (gift from H. Wesphal, National Institutes of Health) and identified by Southern blots with a v-Ha-ras probe and DNA extracted from tails. Tissues for histology were fixed in an equal mixture of 10% formalin and 2.5% glutaraldehyde, processed into paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin.

**Results**

Twenty-four Harderian glands from 16 mice were examined histologically. Eight glands showed normal histology (Figs. 1A, B): small, regularly shaped tubules contained cuboidal cells with cytoplasm filled with small droplets, consistent with lipid that had been extracted during processing. Nuclei contained small nucleoli. Frequent lumina contained dense porphyrin granules.

Eleven glands showed cystic papillary hyperplasia (Figs. 2A, B). These were characterized by involvement of the entire enlarged gland; no rim of normal or atrophic tissue was identified at the periphery of any of these glands. The tumors were composed of cystically dilated tubules, which often contained papillary structures. Cellular morphology was similar to that of the normal gland, although cells were more columnar than cuboidal. Although most nuclei were basally situated, in some cells they were more centrally or apically placed. The eosinophilic cytoplasm was filled with lipid droplets. No mitoses, necrosis, or fibrosis was present in any of these glands. Porphyrin pigments were present in scattered lumina. No invasion of the surrounding orbital tissue was present.

Four glands were predominantly hyperplastic; however, these were unusual in that they showed several small foci of dysplasia or in situ carcinoma as well as, in one case, invasion of the stroma by neoplastic tubules (Fig. 3A, B). The dysplastic cells were contained in glands that were smaller and more irregular in shape than the hyperplastic glands; the cells displayed an increased nuclear-to-cytoplasmic (N/C) ratio, with nuclear hyperchromatism and less abundant cytoplasm than the hyperplastic cells. The invasive glands were much smaller than those that were hyperplastic, and again showed an increased N/C ratio, with a markedly diminished amount of cytoplasm that contained less lipid than the hyperplastic cells. The stroma around the invasive glands was desmoplastic. No mitoses were present in the dysplastic cells; however, several mitoses were seen in the inva-

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**Fig. 1.** (A) Low-power photomicrograph of normal Harderian gland of transgenic mouse (x63). (B) Medium-power photomicrograph of normal Harderian gland. Gland lumina are regularly shaped and contain porphyrin. Cell cytoplasm contains numerous small vacuoles consistent with lipid that had been extracted during processing (x391). (Hematoxylin and eosin)
sive glands. No necrosis was present, and there was no invasion into the adjacent eyeball.

In one gland, nearly all hyperplastic features were destroyed by an invasive adenocarcinoma (Fig. 4A, B). This tumor was composed of small closely packed tubules with tiny lumina, and solid sheets of cells with little or no intervening stroma. Some cells showed an increased N/C ratio and markedly diminished amounts of cytoplasm, which still showed some lipid vacuolation; other cells showed a peripherally placed nucleus that was indented by a single lipid droplet. At the periphery of the tumor, small tubules and single cells invaded a desmoplastic stroma. There was no obvious capsule or circumscription. No mitoses or necrosis were present. Remnants of large, cystically dilated tubules, similar to those seen in hyperplastic glands, were present, suggesting that the carcinoma arose in a hyperplastic gland. Foci of acute and chronic inflammatory cells were scattered throughout the tumor. There was no invasion into the eyeball. No animals died directly from, or were believed to have, metastases from their Harderian gland tumors.

Discussion

This paper illustrates the Harderian gland pathology that occurred when the v-Ha-ras gene driven by the MMTV promoter/enhancer was used to create transgenic mice. The range of pathology was similar to that seen spontaneously in normal mice, although hyperplasias, rather than adenomas, occurred as the benign tumors. We diagnosed hyperplasias based on the involvement of the entire gland or both glands and based on the lack of a circumscribed tumor located within an otherwise normal or atrophic gland. Presumably, the involvement of the entire gland indicates that all of the cells of the transgenic mice carry the oncogene and would respond similarly to a stimulus. In contrast, in the genesis of an adenoma, all of
the neoplastic cells are believed to arise clonally from one initiator cell.

The major and most frequent type of tumor to occur in these transgenic mice was the mammary adenocarcinoma: 84% of females and 22% of males developed this tumor. This frequency contrasts with a 15% incidence of Harderian gland hyperplasia, a nonmalignant condition. The incidence of Harderian gland hyperplasia is still much higher than the spontaneous incidence of Harderian gland tumors in any strain of mouse studied by Sheldon et al, in the largest study of these tumors. During the period of study, no nontransgenic siblings of the transgenic mice developed Harderian gland tumors; this suggests that the v-Ha-ras gene plays an important role in the genesis of these tumors.

Zarbl et al have produced mammary carcinomas in rats by the administration of N-nitroso-N-methylurea, which produces a point mutation in codon 12 of the c-Ha-ras gene. This result suggests that "oncogene activation must be concomitant with, and presumably contributing to, initiation of carcinogenesis." However, animals that have been castrated before administration of this carcinogen have not developed mammary tumors, a finding that indicates a hormonal interaction with the oncogene in this hormonally sensitive organ. Sinn found increased numbers of mammary adenocarcinomas in female transgenic mice, but did not find any difference between the sexes with regard to the incidence of Harderian gland hyperplasia. Sinn’s study is in contrast to that of Sheldon et al, who found that the majority of spontaneous Harderian gland tumors occurred in female mice. Expression of v-Ha-ras mRNA is inducible by the effect of dexamethasone on sequences in the mouse mammary tumor virus promoter in vitro. It is unknown whether this effect is reproduced by cortisol in vivo, and if it were, what the cortisol status of these animals was prior to death. Therefore, organ specificity or hormonal responsiveness may also have an important bearing on tumor formation in these transgenic mice.

Sinn et al found variable expression of v-Ha-ras mRNA in normal-appearing Harderian glands from these transgenic mice. Ras gene expression or mutation has not been studied in normal or tumor-bearing Harderian glands of nontransgenic mice. Currently, it appears that the ras gene may be important in the development of Harderian gland tumors in these transgenic mice. This conclusion is supported by the findings that the incidence of Harderian gland tumors was much higher in these mice than in similar strains of normal mice, and that no nontransgenic siblings developed Harderian gland tumors during the period of study.

Key words: Harderian gland, oncogene, v-Ha-ras, transgenic mouse, orbital neoplasm

References

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