Journal of Mammary Gland Biology and Neoplasia, Vol. 4, No. 1, 1999

The Comparative Pathology of Human and Mouse Mammary Glands

Robert D. Cardiff[1,2,3] and Sefton R. Wellings[1]

The mouse has emerged as a primary animal model for human breast cancer because the mammary glands of the two species are very similar in structure and function. In this regard the TDLU[4] and LA have similar morphology. The mouse, infected by MMTV, develops "spontaneous" tumors with specific but limited tumor phenotypes. The advent of genetic manipulation has created transgenic mice that develop hyperplasias and tumors morphologically and cytochemically comparable to lesions in humans. Even experienced pathologists have difficulty distinguishing between lesions from the two species, and the morphological similarities support the utility of the mouse model in understanding human breast cancer. In this essay we review our experience with the histopathology of human and mouse mammary disease by comparing the normal gland with hyperplastic, dysplastic and neoplastic lesions of traditional and transgenic origin.

KEY WORDS: Comparative histopathology; mouse; human; mammary gland; breast cancer; breast diseases; transgenic.

INTRODUCTION

The mammary gland is both a source of food and a site of disease. Breast diseases have practical implications in domestic animals, and breast cancer, in particular, has attracted much scientific attention as a major health concern for humans. Although mammary tumors have been reported in humans and many mammals (1), rodents have emerged as the primary biological model for human breast cancer (2–4). The comparative histopathology of the human and rat mammary gland has been reviewed (2). Since genomic manipulation has become an increasingly powerful experimental tool for scientists examining mouse mammary tissues, this technology will facilitate the discovery of and cure for the ultimate root cause of cancer (4). This essay reviews the comparative histopathology of the mouse and human mammary gland with an emphasis on breast cancer, particularly in relation

[1] Department of Pathology and The Center for Comparative Medicine, University of California, Davis, California 95616.
[2] Department of Medical Pathology, 3453 Tupper Hall, University of California, One Shields Ave., Davis, California 95616.
[3] To whom correspondence should be addressed. e-mail: rdcardiff@ucdavis.edu

[4] **Abbreviations:** TDLU, terminal ductal-lobular unit; LTR, long terminal repeat; DCIS, ductal carcinoma *in situ*; HAN, hyperplastic alveolar nodule; ALB, a typical lobule type B; LCIS, lobular carcinoma *in situ*; ALA, atypical lobule type A; MMTV, mouse mammary tumor virus; LA, lobuloalveolar; PyV-MT, Polyoma virus middle T; WAP, whey acid protein.

[5] *Note:* Some of the illustrations are digital images and some are drawn from either whole mounts of subgross preparations stained with hematoxylin at low pH (so that only nuclei are colored); or from routine histology slides stained with hematoxylin and eosin. The drawings permit representation of the full three-dimensional field that is often impossible with the limited depth of focus with a camera. All drawn illustrations closely represent the subject as observed in the morphological preparations. The magnifications are shown as units of length marked directly on the drawings. The terms "acinus" and "alveolus" are synonymous. The medium for the drawings is pen, black ink, and gray tone watercolor wash. The drawings were scanned into Adobe Photoshop using a AGFA ARCUS II photoscanner. The histopathology images were taken with a Kontronic camera and digitized using Adobe Photoshop.

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to our experience with transgenic mice bearing specific combinations of interacting mouse and human genes.

This review is based primarily on our personal observations through many years of surgical and experimental breast pathology and on the examination of gross and/or microscopic samples from over 6000 genetically manipulated mice and controls at the Transgenic Histopathology Laboratory of the University of California, Davis, School of Medicine (*http://www-mp.ucdavis.edu/tgmice/firststop.html*). The collection, preparation, interpretation and archiving of the transgenic collection was initially done to assist the research of several collaborators. Subsequently, the colleague base has expanded by word of mouth to include samples from over 75 laboratories. Our services are open to everyone and the details for the submission of samples can be found at URL: *http://www-mp.ucdavis.edu/tgmice/HistoLab/Histolab.html.*

The U.C.D. transgenic archive contains mammary gland samples from over 2500 mice with over 175 transgenes and/or targeted gene disruptions (i.e., knock outs) sent to us by 137 investigators from 37 different laboratories. This collection has allowed the comparison of mammary glands from wild type and genetically manipulated mice from different laboratories. The collection has also provided the opportunity to examine and interpret samples under relatively uniform conditions of fixation, processing and staining. The uniformity of the sample preparations enhances our ability to assess the significance of minor variations in histopathology. The comparative histopathology of normal mammary gland, hyperplasias, dysplasias and tumors will be briefly reviewed, emphasizing the marked similarities of the preneoplastic progression leading to cancer in the two species (5–8).

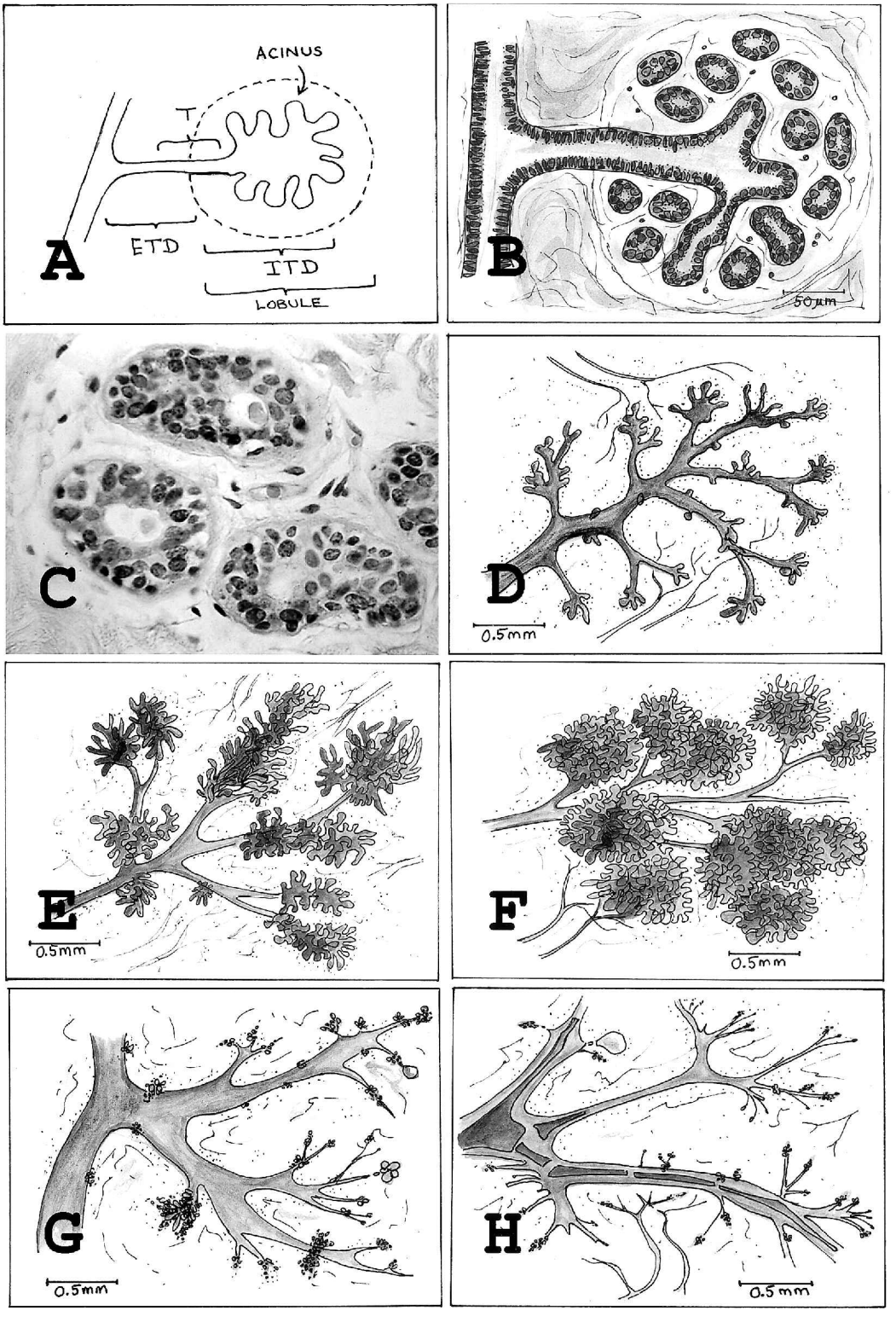
GROWTH AND DEVELOPMENT OF THE MAMMARY GLAND

The mammary glands develop from ectodermal thickenings called the milk line that extend on both sides of the body from neck to inguinal region in the developing fetus (9). As development proceeds, milk buds form as discrete thickenings along the milk line. Whereas, humans have one pair of nipples and glands, rats have six pairs, and most mouse strains five pairs. In the mouse, the number one fat pad and gland are located in the neck region, adjacent to the salivary glands. The number 2 and 3 mammary glands are situated on the chest wall, separated by a thin layer of pectoralis muscle. The number 4 gland is located on the abdominal wall, and is the easiest to identify, access, and dissect, and is therefore used for transplantation experiments. The number 5 gland is located in the inguinal region, and overlaps the proximal portion of gland number 4.

In contrast, the human mammary gland is generally limited to two breasts on the anterior chest wall. However, supernumerary nipples and glands may form anywhere along the milk line, most commonly in axilla or vulva (10).

The mammary gland epithelium originates from the milk bud that forms the primitive nipple and extends into the mammary fat pad as a series of branching ducts (9). The growth rate is relatively slow

Fig. 1. *(opposite)* The basic functional unit of the human breast, the TDLU. The illustrations show the structure of the TDLU in various stages of the life cycle in early menarche (Fig. 1D), fully developed nulliparous (Fig. 1E), fully developed parous (Fig. 1F), early menopausal (Fig. 1G), and elderly (Fig. 1H). (A) Diagram of terminal ductal lobular unit, or TDLU. The dotted line is the interface between the loose intralobular connective tissue, and the dense extralobular connective tissue. ETD is extralobular terminal duct, ITD is intralobular terminal duct (the axial space of the lobule) and T is the "target zone". T is the site of approximately twenty percent of the smallest, and presumed earliest, epithelial proliferations hypothesized to be precancerous. Most epithelial proliferations of presumed precancerous significance arise within the lobule itself. (B) Drawing of the histology of a small normal human mammary lobule in the "resting state". Note that at least two layers of cells are visible: myoepithelial cells (dense small nuclei at the periphery of the acini) and epithelial cells, most of which are luminal in location. (C) Digital microscopic image of a section through a lobule showing details of acinar organization. Note the two layers of epithelium and the difference in the density of the intralobular and extralobular connective tissue. The peripheral cells with clear cytoplasm and elongate nuclei are myoepithelial. The cells at the luminal surface are secretory. (D) Drawing of a subgross preparation of portion of mammary gland of a 15-year-old human female. Menarche occurred at age 13. Note the duct system with terminal branching structures suggesting the locations of future lobules. No lobules or acini are present, however. Compare this photo with the subgross of the mouse gland treated with KGF: (Figs. 2B and 2D). (E) Drawing of a subgross preparation of portion of mammary gland of a 22-year-old nulliparous woman. Note the well developed lobules with acini. (F) Drawing of a subgross preparation of portion of mammary gland of a 30-year-old parous woman. Note the well developed lobules with acini. (G) Drawing of a subgross preparation of portion of mammary gland of a 55-year-old parous postmenopausal woman. Note the duct dilatation and atrophy of lobules. (H) Drawing of a subgross preparation of portion of mammary gland of 80-year-old woman. Note the marked atrophy of lobules, and the presence of trapped secretion within the dilated duct system.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before puberty and then accelerates rapidly to eventually fill the mammary fat pad. The human has a more complex segmented structure starting with the nipple that contains the outlet for five to ten lactiferous ducts (10). Ducts extend in a branching radial pattern to form discrete triangular lobes each with a completely separate ductal system and collagenous stroma, and each is completely separate from adjacent lobes. This complete separation of lobes justifies anatomically the use of lumpectomies and segmental (lobar) resections of neoplastic lesions in the human (9).

In contrast, the rodent mammary gland generally terminates in a single lactiferous duct or sinus that forms five to 10 secondary ducts (9) that grow into the mammary fat pad in a more linear pattern than in humans.

The mammary gland in all species grows largely from the branching terminal end buds that contain undifferentiated progenitor or "stem" cells (11). New end buds with the potential to form lobules also form by lateral branching from mature ducts which also contain stem cells. Thus, terminal branches give rise to functional (milk producing) lobules of the mammary gland.

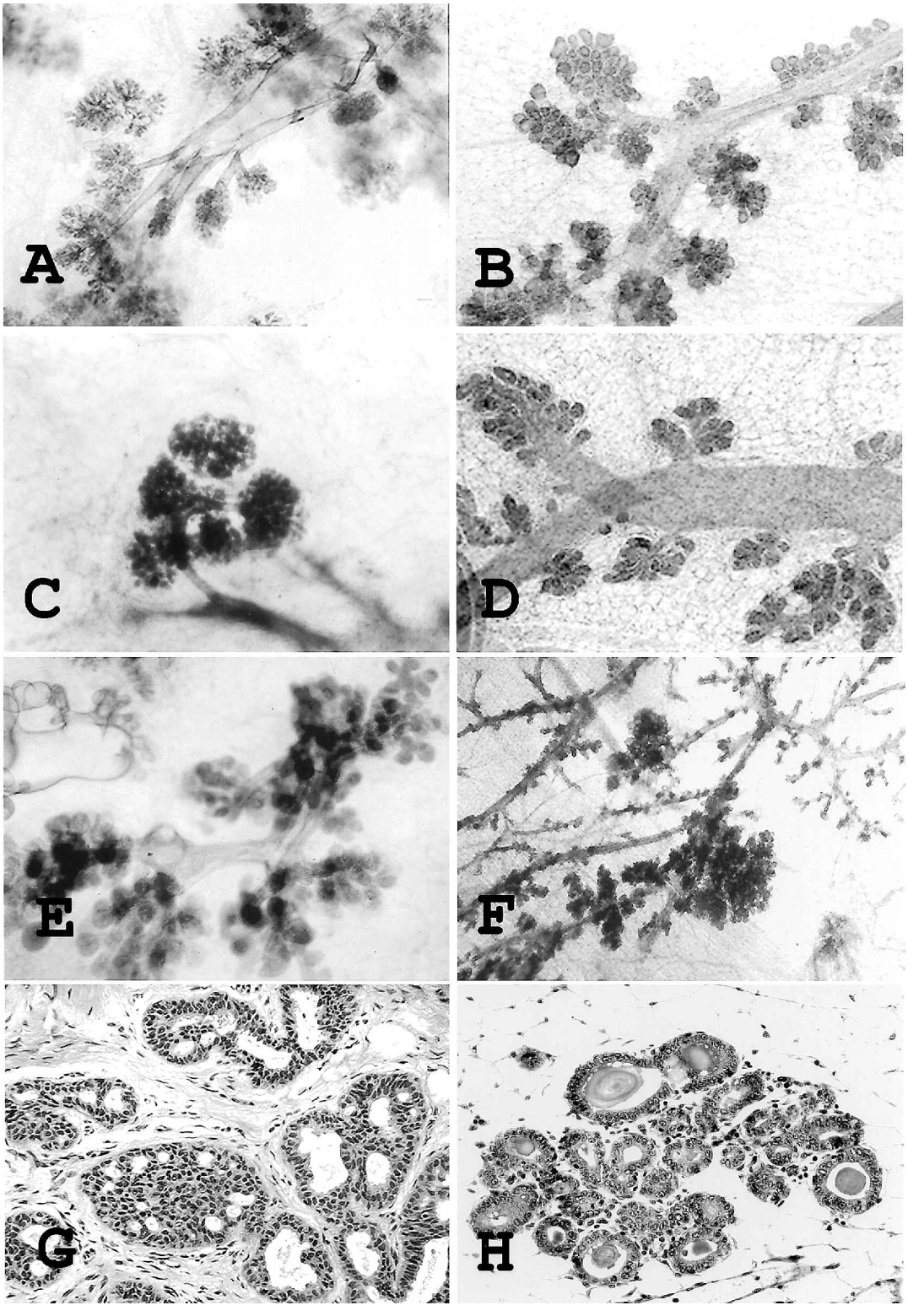
The functional portion of the human mammary gland is called the "terminal ductal lobular unit" (TDLU) (Figs. 1 and 2) (2,12). The major functional units of the mouse mammary gland are termed the lobulo-alveolar (LA) units or the terminal end buds and the side buds (Figs. 2 and 3) (2,12). After development is completed, these structures are the major hormone sensitive areas of the mammary epithelium in all species (13–15). They are also the site of origin for most mammary cancers (16,17) because they contain the major proliferative stem cell populations thought to be most sensitive to somatic cell mutation (2).

Both TDLU and LA have similar epithelial cells. Luminal cells, "stem cells" and myoepithelial cells have been identified in most species using a variety of histochemical techniques (18–20) (Fig. 1C), leading to the recognition of important similarities between the human and murine mammary glands (7,21,22) (Fig. 2). Both the TDLU and the LA, being hormone responsive, are dynamically active throughout the human menstrual or murine estrus cycle (14,15). Both are the ultimate source of milk production.

Human and murine mammary glands also have a number morphologic differences. The human TDLU comprises a small unit resembling a cluster of grapes at the end of a stem, encased in a loose intra-lobular connective tissue surrounded by denser extralobular connective tissue (Figs. 1 and 2) (2,16). The TDLU responds to estrogen and progesterone by synthesizing DNA during the luteal phase (14). With the repetitive cycling (polyestrus) of the human menstruation, the TDLU develops early and regresses in the post-menopausal years (Fig. 3). Women without complete post-menopausal regression appear to be more prone to cancer (Fig. 1G and 1H) (2,6).

In contrast to the human gland, the terminus of the virgin mouse mammary gland is generally composed of an unbranched or minimally branched ductule with a single terminal end bud that may be blunt or globular, depending upon the state of endocrine activation (Figs. 2 and 3) (2). The estrus cycle of the mouse differs from the human with a short 4 to 6 day cycle, no menstruation and a nonfunctional corpus luteum which is activated only in pregnancy or pseudopregnancy (1,23). The mouse does develop LA structures when stimulated by growth factors, by mammotrophic hormones, progesterone and pregnancy (1,12,23) (Fig. 2D and 2F). Estrogen also plays a role in LA formation, either directly, or indirectly, because it is an activator of progesterone receptors (13–15,21). Persistent LA units can also be found in some strains of nulliparous, Mouse Mammary Tumor Virus (MMTV)-infected mice (24). The subgross appearance of these fully developed murine LA units is identical to human TDLU (Fig. 2) and closely resembles lobuloalveolar units of the rat (2). Although the murine LA unit

Fig. 2. *(opposite)* Digital images of human (Figs. 2A, 2C, 2E, and 2G) compared with mouse (Figs. 2B, 2D, 2F, and 2H) mammary glands. The fully developed human TDLU (Figs. 2A and 2C) and ALA (Figs. 2E and 2G) are compared to the mouse LA (Figs. 2B and 2D) and HAN (Figs. 2F and 2H). (A) Subgross image of a human mammary duct with multiple TDLUs. (B) Subgross image of a mouse mammary duct with multiple LA units. This animal has been stimulated with KGF Keratinocyte (growth factor) prior to sacrifice (courtesy of Ruth Lang). (C) Subgross image of human TDLUs, emphasizing the grape-like appearance. (D) Subgross mouse LA units attached to a dilated duct This animal has been stimulated with KGF prior to sacrifice (courtesy of Ruth Lang). (E) Subgross a complex of human ALAs. Note the very dense staining pattern due to the tightly packed cells (See Fig. 3). (F) Subgross of several mouse HANs. This animal was infected with the mouse mammary tumor virus. (G) Microscopic appearance of a human ALA. Note the irregular cellular organization of the acini in comparison to the normal TDLU (Fig. 1C) and the multiple layers of cells. (H) Microscopic image of a mouse HAN. Note the densely crowded acinar structures with some atypia.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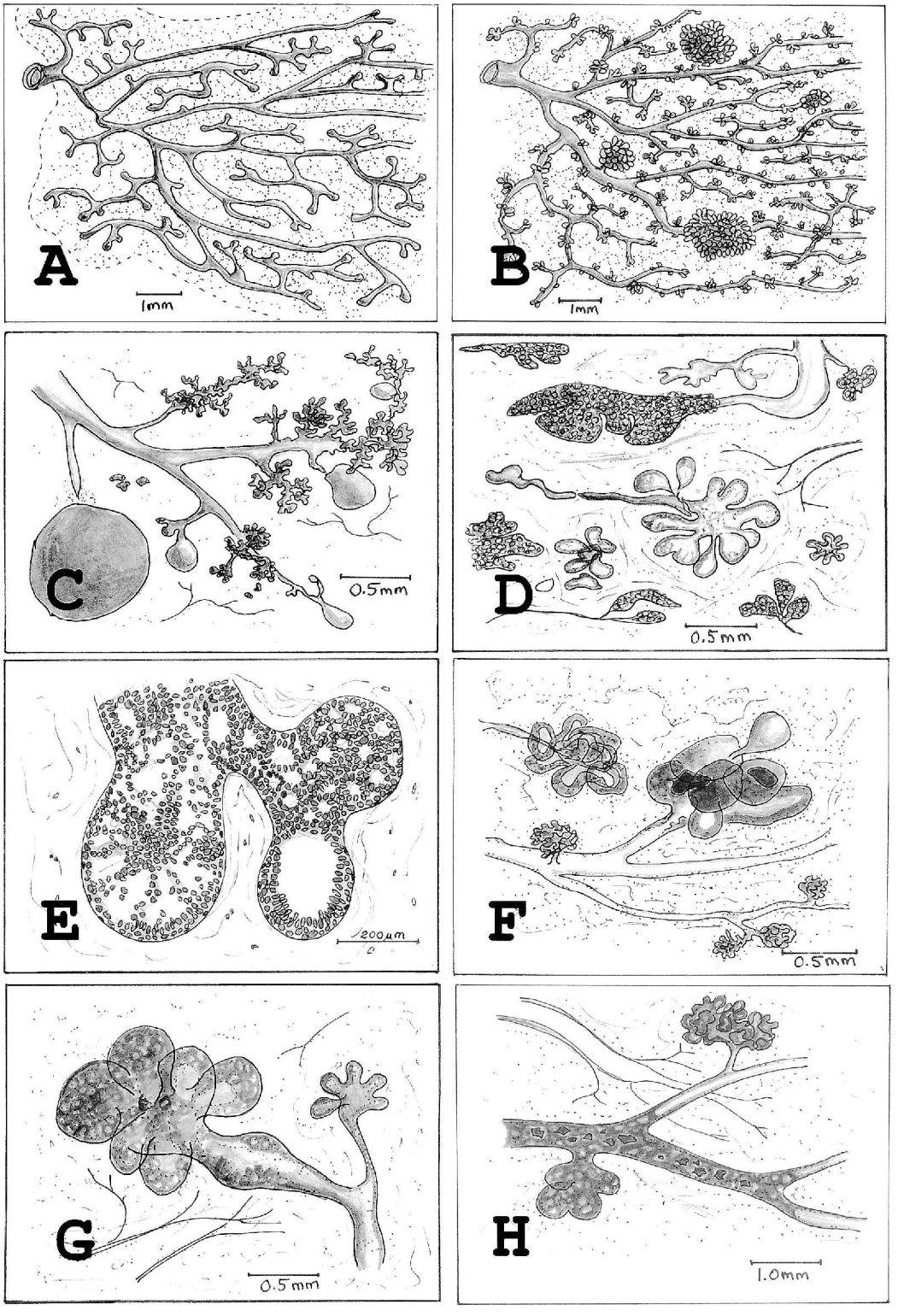
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has no obvious intralobular "loose" connective tissue, comparable stromal factors have been identified (25,26). Compared to the human and other large mammals, the mouse periductal connective tissue is relatively scanty and other differences in the composition of the stroma have been elucidated (26). However, this difference may reflect differences in size; [i.e., structure and function of the stroma in the human gland might be related to its relative volume (26)]. Consequently, the differences do lead to variations in pathobiology.

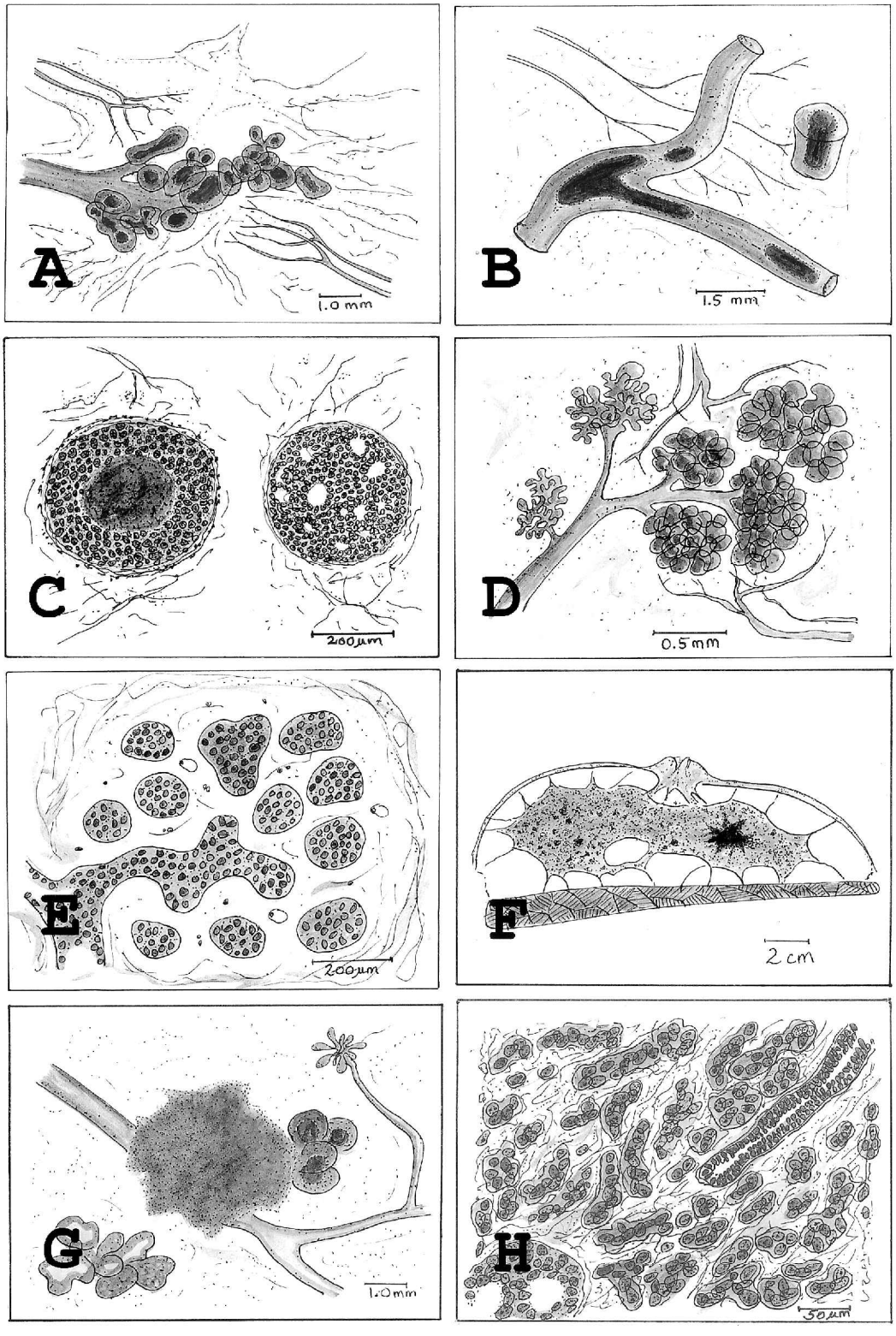
During pregnancy, the mammary gland of all species forms side buds that either branch or form alveoli, and the process progresses to fill the entire fat pad with alveolar structures. The morphologic details of prelactation and lactation have been described elsewhere (27). After weaning, the mammary gland regresses with the death of most of the functional cells. The early phases of regression are characterized by widespread apoptosis with fragmentation of nuclei, formation of membranous surface blebs, disintegration of cytoplasmic organelles and formation of "apoptotic bodies" (28,29). Involution generally occurs unevenly; (i.e., at different rates in different parts of the gland). The fully regressed mammary gland may retain some of this unevenness as well as vestiges of the preceding pregnancy. For example, the mouse mammary gland retains the branches that distinguish it from a virgin gland (Figs. 1–3). The termini end in short, pointed ducts, giving the gland a spiculated appearance resembling thorns. In the human, the ebb and flow of normal hormonal fluctuations act on what seems to be an unevenness in tissue response. This is a hypothetical cause of the nodular and proliferative changes of fibrocystic disease, some components of which lead to biologically threatening hyperplasias and dysplasias of preneoplastic significance (Figs. 3 and 4).

PATHOLOGY OF THE MAMMARY GLAND

Abnormal Development

A variety of developmental abnormalities of the mammary gland have been described either in association with spontaneous or natural events or in association with experimental manipulation (4,30,31). In

Fig. 3. *(opposite)* Pen and ink line drawings illustrate the normal and abnormal whole mount morphology of the mouse mammary gland (Figs. 3A and 3B) and the morphologic progression in the human mammary gland that is believed to lead to breast cancer (Figs. 3C–3H). (A) Whole mount of portion of mammary gland number four of a virgin C3H mouse, 6 weeks old. Note duct system with end buds but no alveoli. (B) Whole mount of a portion of the mammary gland of a parous, nonpregant C3H mouse. Note four hyperplastic alveolar nodules (HAN). Parous mammary gland has scattered alveoli not present in the gland of the virgin animal (also see Fig. 2F). (C) Subgross preparation of mammary gland of a 52-year-old woman with irregular menses. Note small lobules and cysts resulting from obstruction of extralobular terminal ducts (by inflammation), causing distention of the lobules with secretion. The lobules "unfold", eventually producing single isolated distended cysts. (D) Subgross preparation of mammary gland of a woman in her 4th decade showing two atypical lobules, type A, (ALA) in the mid-lower center of the illustration. The surrounding tissue has well developed lobules, a duct (convoluted structure, cross section, lower right) and a dilated lobular formation of uncertain type (upper right). The ALA consist of greatly enlarged lobules with enlarged acini lined by thickened epithelium with visible intraluminal papillary projections and cribriform pattern. Cytological atypia which might be present in ALAs is not predictable from examination of the subgross preparation. Bright field microscopy of stained histology slides is required. The ALAs shown here might have been diagnosed as "blunt duct adenosis", a term now seldom used. (E) Histology slide stained with H and E reveals three different degrees of cytological atypia within adjacent portions of an ALA similar to the one in Fig. 2G. The enlarged acinus to lower right has low grade atypia featuring a relatively simple epithelial lining of uniform thickness with cuboidal and low columnar luminal cells overlying myoepithelial cells (small dense bean-shaped nuclei) and presumed germinal stem cells (larger pale nuclei). The acinus to the left has a diffuse formation of epithelial cells which partially fill the lumen and form a more solid zone centrally, an appearance often seen in intermediate grade atypia. The acinus at upper right has well developed cribriform (micropapillary) pattern characteristic of high grade atypia, bordering on carcinoma-*insitu*. (F) Subgross preparation of mammary gland of a premenopausal woman in her fifth decade. Note two huge ALAs in the upper half of the drawing. The acini of these lobular ALAs are partially "unfolded" to form fewer numbers of larger acini than would be observed in normal lobules. Some acini are solidly filled with epithelium, one shows cribriform pattern, and another (to right) reveals dark dense trapped secretion (or possibly necrosis?) in the center of the lumen. Note two background (normal) lobules that arise directly from larger ducts. These are referred to as "duct lobules". Carcinoma-*in situ* arising in duct lobules quickly enters the duct system, obliterating its origin, possibly leading to the false conclusion that the atypical cell population is arising in the duct. (G) Subgross preparation of mammary gland of a premenopausal woman in her 5th decade. The larger of the two ALAs features cribriform pattern in the acini and intralobular terminal duct, and micropapillary formations in the vicinity of the junction between extralobular terminal duct and intralobular terminal duct ("target zone"). Routine histology is required to distinguish between high grade atypia and carcinoma-*in situ*. (H) Subgross preparation of mammary gland of a premenopausal woman in her 5th decade. Carcinoma-in-situ of the cribriform (micropapillary) type occupies the lobule at lower left and the adjacent duct system. Note the very dark angular granules among the carcinoma cells of the duct. These granules are calcified and suggest sand grains ("crushed stone") on X-ray mammograms.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general, the transgenes driven by the MMTV Long Terminal Repeat (LTR) promoter express relatively low levels of the transgenes (32). The whey acidic protein (WAP) promoter is also commonly used as a mammary specific promotor. Since its induction occurs primarily in estrus and pregnancy, abnormalities in growth and development of the mammary gland were not expected. However, expression of notch4 under control of the whey promoter resulted in defects in lobuloalveolar development and lactation (33) while TGFβ1 expression results in premature senescence (34). Most other experiments with these promoters have not revealed developmental abnormalities. However, an increasing number of studies with knockout mice have revealed defective development of the mammary tree. For example, *src* knock out virgin mice have dilated ducts with limited branching (35), as do *cyclin D1* knock outs (36).

Other abnormalities in mammary development have been described in transgenic strains (3,4). Targeted overexpression of very powerful transgenes can result in developmental anomalies in the virgin mammary gland. For example, the Ornitz RV system using the *int-2* transgene resulted in dilated, short ducts with abnormal mammary buds (37). The *Polyoma middle T* (*PyV-MT*) transgene promoted by either the MMTV LTR or C(3) resulted in the very rapid development of focal mammary lesions in virgin mice (38,39). In addition, we have observed inappropriate development of lobules in virgin mice expressing both *TGFα* and *neu* transgenes (40). The *TGFα* transgene behind a WAP or MMTV promotor results in cystic ducts and extensive dilated lobules (41,42). Some mutants of the *PyV-MT* transgene result in shortened ducts with dilated, cystic end buds (43).

Some transgenic strains have defects in lactation (4), most of which are unrecorded or unpublished. Seen in whole mount preparations, transgene expression often results in incomplete development of the mammary tree. The mouse with activated *src* is one such example with normal development of the mammary tree but with defective lactation (44). Lactational defects have been described in other mice with transgenes such as notch4 and TGFβ (34,45).

Other transgenic animals have diffuse, persistent hyperplasia following pregnancy (4). For example, the *cyclin D1* females have fully lactating mammary glands 6 to 9 months following weaning (46). The mammary epithelium has defective regression, an apparent defect in apoptosis. Hypersecretion has been observed in TGFα mice (42).

Focal Hyperplasia and Dysplasia

In most cases neoplastic progression originates from a clonal, focal epithelial proliferation possessing preneoplastic potential (16,17,22,47–49). The primary preneoplastic lesions stand out from the general background as discrete morphological foci usually visible with the unaided eye, or with the low magnifications of the dissecting microscope (Figs. 2–4). The biological

Fig. 4. *(opposite)* Ink drawings illustrate the proposed neoplastic progression from ALA (Fig. 4A) through DCIS (Figs. 4A–4C), and LCIS (Figs. 4D and 4E) to invasive carcinoma (Figs. 4F–4H). (A) Subgross preparation of mammary gland of a premenopausal woman in her 5th decade, illustrating a lobule (ALA) completely replaced and distended by carcinoma-in-situ of the classical ductal cytological type (DCIS). Geographically isolated formations such as this argue strongly for origin of DCIS in lobules rather than in ducts, as formerly believed. Note the dark central necrosis in the acini. (B) Subgross preparation of mammary gland of a menopausal woman in her 6th decade. No lobules are visible. The duct system is solidly filled with DCIS. Note the very dark central necrosis. The necrotic cellular remnants are sufficiently calcified to appear as "castings" on X-ray mammograms. The ducts illustrated traverse the full thickness of the 2–3 mm thick mammary slice. (C) Histological differences between DCIS of the comedo pattern with central necrosis (left) and DCIS of the cribriform (micropapillary) pattern (right). (D) Subgross preparation of mammary gland of menopausal woman in her 6th decade. Normal lobules to the left. The four enlarged lobules in the right half of the drawing are typical of some cases of lobular carcinoma-*in situ* (LCIS). The affected lobules are enlarged with acini more or less uniformly distended by cells. (E) Histology preparation of LCIS shows acini distended with atypical cells which also occupy portions of the adjacent duct. (F) Subgross slice of human breast through the nipple region reveals a stellate cellular density measuring about 2 cm in diameter. This is typical of scirrhous carcinoma (classical infiltrating duct carcinoma). The carcinoma is located in the center of the mammary layer and has not yet attached itself to the overlying skin or the underlying pectoralis fascia. The mammary layer shows the classical "dysplastic pattern" of dense fibrous tissue in which are embedded numerous lobules, many of which are likely to be pathological. The fibrous dysplastic tissue appears as "ground glass" on X-ray mammograms. (G) Subgross preparation of a small 3–4 mm infiltrating duct scirrhous carcinoma. Three acini, possibly representing remnants of the lobule (ALA) of origin are seen to the right of the infiltrating carcinoma. An ALA is observed at lower left. (H) High power histology of infiltrating duct carcinoma. The upper right shows a normal duct for comparison. The lower left has cribriform pattern of carcinoma which may still be *in situ*. The infiltrating nests of large cancer cells have enlarged hyperchromatic and pleomorphic nuclei.

potential of these visible lesions can be assessed by isolation and transplantation of the individual lesions to the cleared mammary fat pad (4,8,50). However, there is also experimental evidence of "inapparent" hyperplastic cells of possible preneoplastic potential (51). These studies suggest that the neoplastic cells preexist in the population of cells in normal epithelial formations, but are suppressed by the microenvironment. Thus, while larger focal preneoplastic lesions can be visually identified in the background, genetically transformed cells still might occur as individual cells or small groups of cells that remain "inapparent" to the pathologist.

Numerous descriptions of the mammary gland pathology in human and other species have been published. Most have been described as focal abnormalities associated with increases of endogenous or exogenous hormones (4,21,30,31,50). Many articles review the literature, but few truly compare the histopathology of the lesions.

Over half of the women in western countries have some type of focal lumps or bumps in their breasts (46–48). The more severe forms of these lesions are commonly referred to as "fibrocystic disease" of the breast but may also be called mammary dysplasia and other terms. Since proliferative forms of fibrocystic disease are frequently associated with breast cancer, these lesions have been the subject of intense study (6–8,22,47–49). The question is, which, if any, of the many lesions of the human breast are "premalignant".

Based on extensive study of subgross pathology, thirty-six types of focal lesions were identified in the human mammary gland (6). The most significant lesions consisted of proliferating epithelial lesions originating in the TDLU (6–8). A continuum of progressive atypical subgross morphology with cytologic changes in the epithelium was found that could be correlated with the history of neoplasia in either the contralateral or the ipsilateral breast (2,6,7,16,17) (Figs. 2–4). Two subtypes of atypical lobules have been described, "atypical lobules type A" (ALA) (Fig. 2) and "atypical lobule type B" (ALB) (Fig. 4D). ALA are more common and appear to be related to "ductal carcinoma" (Fig. 4). ALB are less frequent and appear in relation to the rarer "lobular carcinoma". Both ALA and ALB are considered to be the preneoplastic equivalent of the mouse HAN (Hyperplastic Alveolar Nodule) (6,7,16,17) (Figs. 2 and 3).

The two types of human lesions with demonstrable cytologic continuums linking benign hyperplasias to malignant populations of cells appear to originate in the TDLU. This observation goes against the common understanding of the origin of "ductal" carcinoma of the breast from the larger or more proximal ducts. Although met with considerable skepticism by the surgical pathologists of the day, the concept that the proliferative lesions of the human TDLU are related to breast cancer is now accepted (22,47,48).

The concept of neoplastic progression in human mammary epithelium was based on analogy with the mouse model (6,7) (Figs. 2–4). Efforts to verify the tumorigenic potential of the human ALA or ALB by a number of indirect techniques have yielded equivocal evidence of their neoplastic potential (7,52,53). Without the benefit of experimental proof provided by autologous transplant in the mouse system, investigators have been limited to morphological and statistical arguments (52,53). The biologic similarities between mouse and human breast cancer have been highlighted in our other reviews (4,6,16,17).

The types of spontaneous, focal mammary lesions found in laboratory mice are much more limited than in humans. The most common focal pathologic changes occur in the mammary gland of MMTV-infected mice. The virus is an important influence on mammary growth and LA differentiation (24). Morphometric studies have suggested that MMTV-infected, virginal mammary glands have more extensive LA development than mammary glands from noninfected animals. Feral mice that lack endogenous or exogenous MMTV have very little LA development and virtually no focal lesions (54).

Two classic focal lesions appear in the mammary glands of MMTV infected female mice: the HAN and the hormone dependent tumor or plaque. The HAN is found in most MMTV-infected females. It appears as a small (1–5mm) nodule frequently outlined by yellowish pigment (Figs. 2F and 2H). The HAN stands out from the background following withdrawal of hormone stimulation. Under the microscope, the HAN appears as a focus of closely crowded acini lined by a single layer of epithelium without significant cytological dysplasia (Fig. 2H). In contrast, the human ALA and ALB tend to fill the acini and have much more marked cytological atypia (Figs. 2G and 3F to 3G).

The mouse HAN can be transplanted into the mammary fat pad cleared of gland where it grows to fill the empty fat pad with hyperplastic outgrowth (50). The hyperplastic tissue is immortal and can be serially transplanted proving its altered biologic potential. In contrast, transplants of normal ducts grow into normal ductal tissue and can be transplanted for only a limited

number of generations (55). The hyperplastic outgrowths develop focal proliferations that eventually emerge as autonomous tumors (50). Transplants of normal ducts do not form tumors (55). This type of experiment provides formal proof that the classical HAN has malignant potential, a proof lacking in human mammary biology.

The plaque is another MMTV-induced focal epithelial mass that appears during pregnancy or experimental hormonal stimulation, but regresses upon withdrawal of the stimulation. The plaque reappears with repeated cycles of stimulation and will eventually become a hormone independent tumor (56). Histologically, the plaque is a series of radiating solid ducts surrounded by dense connective tissue (56). The so-called pregnancy adenomas of women are proliferations of milk producing glands. They usually regress after lactation but do not resemble the histology of the murine plaque.

The mouse mammary gland may also have a variety of other neoplastic and non neoplastic focal abnormalities that are probably not associated with MMTV. The inflammatory nodule and the squamous nodule are the two most frequent, and in our experience do not progress to cancer in the gland-cleared fat pad. Chemical carcinogens have been extensively studied. Chemically-induced dysplasias in rodents tend to be papillary ductal lesions often with squamous metaplasia.

As increasing numbers of transgenic mammary glands become available for study, investigators have discovered similarities between nonmalignant dysplasias of the mouse and the human gland (41,42,57) (Table I). Various cystic lesions are commonly found in both species (41,42). Epithelial proliferations of ducts and lobules (4), ductal carcinoma-*in situ* (57), and sclerosing adenosis (44) have been described in transgenic mouse glands. Although rare, we have seen several classic fibroadenomas in the mouse gland. Fibroadenomas are very common in rats and dogs (1,2). The transgenic lesions that most closely resemble the complex patterns of human fibrocystic disease occur in retired breeders carrying the *myc* transgene (58).

In our experience, many mammary-targeted transgenes produce focal hyperplastic glandular lesions. Although some resemble the HAN, most transgenic foci have much more pleomorphic and more hyperchromatic nuclei than the virus-induced lesions. The morphology of these lesions more closely resemble the human ALA. In addition, the transgenic lesions tend to be associated with more intense fibrosis and chronic inflammation than the virus-induced lesions. However, a systematic comparison has not been carried out.

Table I. Classification of Transgenic Hyperplasia and Dysplasia

Hyperplasia/dysplasia type	Prototype transgenes
Diffuse	
A. Ductal	*int*-2
B. Tubular	TGFα
C. Residual lactation (nonregression)	cyclin D_1
D. Acinar	*int*-1 (*wnt*-1)
E. Lobular	*int*-3 (*notch*)
Focal	
Hyperplastic alveolar nodule	*src*
Squamous nodule	cyclin D_1
Inflammatory nodule	casein kinase II
Sclerosing adenosis	*src*
Cystic hyperplasia	TGFα
Periductal fibrosis	stromelysin
Papillary atypia	cyclin D_1

The biological potential of various lesions found in non-tumorous portions of transgenic mouse mammary glands mice has rarely been tested in transplantation experiments. Although some focal lesions are morphologically suspicious, very few experiments document their possible premalignant or malignant behavior (42). When suspicious lesions have been observed in transgenic mammary glands, we have suggested to various colleagues that their mice may develop tumors later. In some cases, the animals do subsequently develop tumors, implying that the lesions may be premalignant. However, the explosive development of multiple tumors in some transgenic strains supports the notion that some of these smaller, early lesions are already malignant (38). Further, cystic lesions from TGFα mice did not survive in serial passage and frequently became neoplastic upon transplantation (42). Until more such transplantation experiments are completed, the focal lesions found in the transgenic mice are simply suspect and without proven biological potential.

TUMORS

Mammary tumors have been described in a wide variety of mammals (1) and the rat has commonly

been used as a model for breast studies (2). Spontaneous tumors occurring in rats tend to be a proliferation of both connective and glandular tissues, generally classified as fibroadenomas (2). However, carcinogen induced tumors have various levels of differentiation. Most are pure adenocarcinomas, but adenosarcomas and sarcomas are also frequent (2). Cats develop undifferentiated breast cancers (1,59) and tumors have been described in a number of zoo animals including primates (1). A mammary tumor from a Rhesus monkey was the original source of the Type D retrovirus (60). However, MMTV remains the only tumor virus that has a proven relationship to breast cancer, and it is to be emphasized that MMTV is linked only to mammary tumors in mice.

Some investigators did not appreciate the relevance of the murine systems because "spontaneous" mouse mammary tumors do not resemble most human breast cancers either morphologically or biologically (1) (Fig. 5). However, the emergence of transgenic biology has provided remarkable evidence that mouse tumors can be produced by the same genes implicated in human breast cancer. Further, the human transgenes in mice may result in tumors that closely resemble the human cancers (Fig. 6).

Spontaneous mouse mammary tumors were thoroughly described by Dr. Thelma Dunn (61). Her classic description of spontaneous tumors found in the wild type mouse is still useful. Approximately 90% of all MMTV-induced tumors are either micro-acinar (Type A), ductal (Type B) or the distinctive Type C (Figs. 5 and 6). Type C is composed of small cysts lined by a single layer of cuboidal epithelium and encircled by a layer of spindle (myoepithelial?) cells. (Figs. 5 and 6). Although primarily observed in spontaneous, MMTV-associated tumors, they can now be found in selected transgenic strains without virus infection. Interestingly, the transgenic strains with the classical "Dunn type" tumors overexpress genes related to those activated by MMTV. For example, the *FGF-3* transgenics and the *FGF-r1* transgenics produce lesions that resemble the MMTV-induced plaques with int-2 activation (62). The *wnt-1* and *wnt 10b* transgenics produce tumors that closely resemble the Dunn type A tumor (63) (Figs. 5A and 5C). The tumor phenotypes described by Dunn are rarely found in humans.

In spite of the similarities shown in Fig. 6, most mammary tumors in transgenic mice (Table II) do not resemble the spontaneous mammary tumors described by Dunn. (Figs. 5 and 6) (Table II) (5,64). The tumor phenotypes found in mice bearing *myc, ras*, and *neu* are examples of the distinctive patterns seen in transgenic animals (Fig. 5). They have been found in only in mice with *myc, ras*, or *neu*(5). These three tumor phenotypes are associated with the same transgene in animals from different laboratories and different constructs. Therefore, the tumor phenotype is reflective of the transgene (5). *Myc* is the most dominant transgene (4,5), and any combination of *myc* with another transgene results in tumors with large, blue cells when stained with hematoxylin and eosin. *Myc* and *neu*, or *myc* and *ras* result in tumors with the *myc* phenotype (5). This has proven true with an increasing number of transgene and knock out combinations with *myc*. All transgenic or knock out animals that are also associated with a *myc* transgene produce *myc* type tumors.

The solid nodular mammary tumor phenotype consisting of intermediate cells is consistently associated with the *neu* transgene (4,5,64). The *neu* phenotype appears as the dominant phenotype in most bigenic crosses, other than *neu* crossed with *myc*. Further, various types of mutationally activated or inactivated *neu* result in tumors with the *neu* phenotype (65–67). This is of particular interest since the *neu* transgene is the murine homologue to *c-erb-B2* or *Her-2* associated with many breast cancers in humans (68) (Fig. 6).

Most importantly, the carcinomas arising in transgenic animals are histologically comparable to the human (Fig. 6). For example, human ductal carcinoma-*in situ* (DCIS) is characterized by solid masses of polygonal cells with uniform nuclei growing along a duct (Figs. 4A to 4C and 6B, 6C). They frequently have central necrosis that is referred to as the "comedo" pattern (Fig. 4C). The DCIS pattern is very frequently associated with amplification or overexpression of *c-erb-B2* (*HER*-2) (Fig. 6D) (68). Interestingly, when this oncogene, or variations of the *c-erb-B2*, is expressed in transgenic mice behind a MMTV promoter, they produce characteristic tumors that overexpress *c-erb-B2* and are histologically and cytologically very similar to the DCIS (57,66,67) (Figs. 6C and 6D).

Scirrhous carcinomas, the most common human breast cancer, can be found in association with TDLU (Fig. 4). Scirrhous carcinoma is characterized by cords of malignant cells in a dense connective tissue stroma (Fig. 6E). The dense connective tissue imparts the rock hard (scirrhous) character of the tumor. We have never seen a tumor of this type amongst the MMTV-induced and carcinogen-induced mouse mammary tumors.

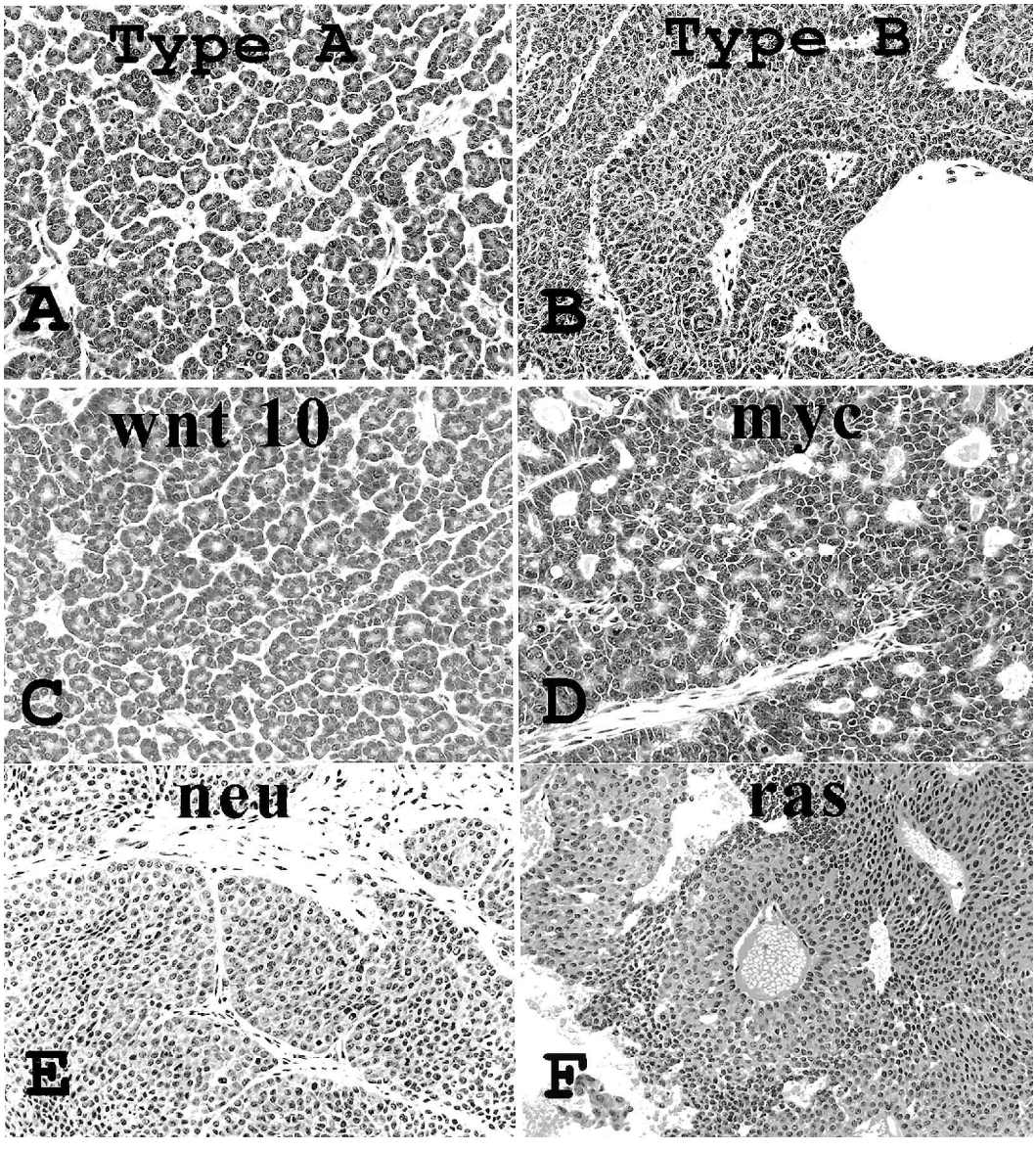
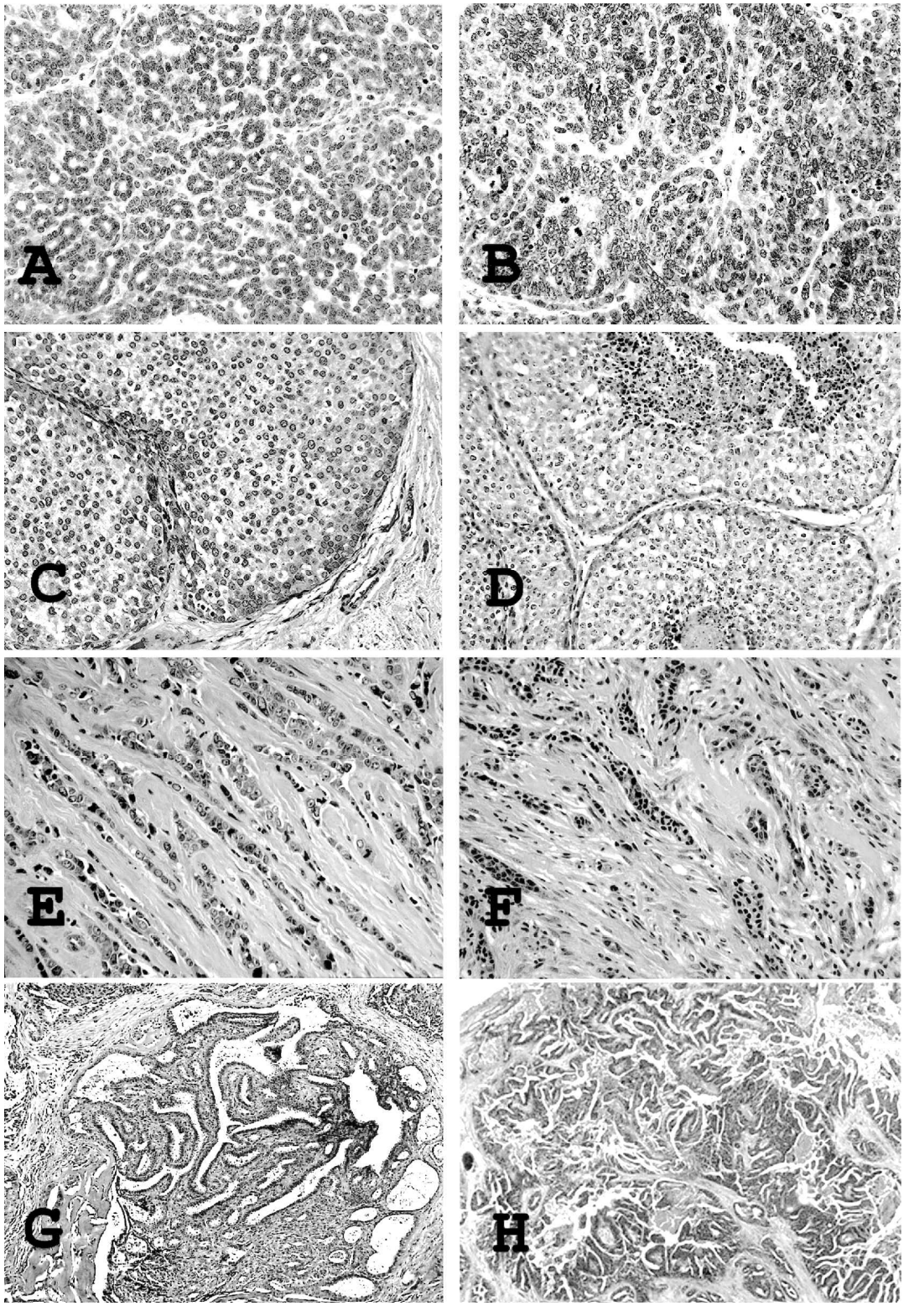


Fig. 5. Digital images of microscopic slides illustrate the typical MMTV-induced "spontaneous" mouse mammary tumors (Figs. 5A and 5B) as described by Dr. Thelma Dunn with comparisons with transgene associated mammary tumors (Figs. 5C–5F). (A) Microscopic image of a Dunn Type A tumor. Note the proliferation of small acinar structures and loose connective tissue stroma. (B) Microscopic image of a Dunn Type B tumor. Note the more solid appearance of the proliferating cells with a peripheral palisade of tumor cells surrounding the cords of cells. Some glands and glandular cysts may be present. (C) Microscopic image of a tumor from a mouse with the *wnt 10b* transgene. This tumor is identical to Dunn Type A tumor (Fig. 5A). (D) Microscopic image of a tumor from a mouse with the *myc* transgene. Note that the tumor forms irregular glands lined by cells with large pleomorphic nuclei and very dark (amphophilic) cytoplasm. (E) Microscopic image of a tumor from a mouse with the *neu* transgene. Note that this has a cell type intermediate between the *myc* and *ras* tumors and a nodular, solid pattern. (F) Microscopic image of a tumor from a mouse with the *ras* transgene. Note that these tumor cells are small and have a dense (red) cytoplasm. The tumor has a papillary appearance that resembles the transitional cell of the human bladder.


A
B
C
D
E
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H

Table II. Classification of Transgenic Tumors

Tumor type	Prototype transgenes
Papillary transitional cell carcinoma (small cell)	*ras*
Adenocarcinoma	
a. papillary	*PyV-MT*
b. tubular	TGFα
c. acinar (large cell)	*myc*
d. alveolar	*int*-1
e. large glands	*met*-1
Nodular carcinoma (intermediate cell)	*neu*
Scirrhous carcinoma	*src*
Adenosquamous	cyclin D_1
Undifferentiated/NOS	many strains

However, such tumors are commonly found in mice with *PyV-MT* or activated c-*src* transgenes (35,38,44,64) (Fig. 6F). The morphology of the human and mouse tumors is virtually identical. Similar tumors are less frequently found in mice with transgenes that directly or indirectly interact with c-*src*.

Papillary tumors are produced by a variety of transgenes including the *PyV-MT* and the *met-1* transgenes (35,38,64,69). These tumors resemble the less common human papillary breast cancer (Figs. 6G and 6H). Acinar tumors of humans are rare but resemble those from the *wnt1* transgene. These and other examples provide some insight into the important and consistent similarities between human and mouse breast cancer.

However, the biology of murine and human tumors differ in important aspects. For example, while 50% of human breast cancers are hormone sensitive, mouse tumors are generally not hormone dependent (21). Further, few mice are anovulatory when they develop mammary tumors while most human breast cancers occur in the post-menopausal age group (70) The comparative pattern of metastasis also merits comment. Human breast carcinomas traditionally spread to the local lymph nodes and then to systemic sites. Lung, bone, brain and liver are the most common organs involved. However, some studies suggest that as many as 20% of women with stage 1 (small cancers confined to the breast) disease have evidence of breast cancer cells in their bone marrow or circulation. Since more than 90% of women with stage 1 breast cancer survive, most of the cells found in the circulation are probably not viable for long.

Metastases from spontaneous mouse mammary tumors have been systematically studied in only a few strains. However, the metastatic rate can be as high as 80% (71). It is noteworthy that metastases to regional lymph nodes are rarely seen and that most metastases are found in pulmonary vessels (71). Although mouse mammary tumor models of lymph node metastases have been developed, most tumor cells harvested and injected into the tail vein colonize the lung and not other organs (72–75).

Interpretation of mouse tumor biology must be also tempered by the degree of laboratory mouse inbreeding. The laboratory mouse is so inbred that our experience with an entire mouse strain is, in some respects, equivalent to seeing a single human breast cancer patient. In retrospect, the vast majority of MMTV-induced mouse mammary tumors are associated with activation of only three genes, *int-1, int-2*, and *int-3* which are not found activated in a significant number of human breast cancers (76). However, there has been almost no effort on the part of the scientific community to correlate gene expression with tumor phenotype. Based on our experience with mammary tumors in mice, we wonder whether the rare human tumor expressing *int-1, int-2*, or *int-3* could have a relatively distinctive, but unrecorded, phenotype. It appears true that when the human oncogenes are effec-

Fig. 6. *(opposite)* Digital images of microscopic slides illustrate the histology of typical MMTV-induced "spontaneous" mouse mammary tumors as described by Dr. Thelma Dunn (Figs. 6A and 6B). The human breast cancers (Figs. 6C, 6E, and 6G) and comparable mouse mammary tumors induced by transgenes (Figs. 6D, 6F, and 6H) have very similar patterns but differ from the Dunn type tumors. (A) Microscopic image of a mouse Dunn Type A tumor. (B) Microscopic image of a mouse Dunn Type B tumor. (C) Microscopic image of a human DCIS showing the solid pattern (see Figs. 4A–4C). (D) Microscopic image of a tumor from a mouse with the *neu* transgene showing the terminal ducts filled with cells with some central necrosis (comedo pattern) (see Fig. 4C). (E) Microscopic image of a human scirrhous carcinoma. Note the narrow cords of tumor cells in a dense connective tissue. The evolution of this type of tumor is illustrated in Fig. 4). (F) Microscopic image of a mouse scirrhous carcinoma in a mouse with the *src* transgene. Compare this pattern with the human scirrhous carcinoma (Fig. 6E) (~400X). (G) Microscopic image of a human papillary carcinoma. Note that the neoplastic epithelium covers a fibrovascular stalk. (H) Microscopic image of a papillary tumor from a mouse with the casein kinase II transgene. Compare the pattern with the human papillary carcinoma (Fig. 6G).

tively transferred to the mouse, the mouse develops breast diseases that are quite similar to the human. As documented here, DCIS and scirrhous carcinomas are induced by the same genes in mice and women.

The insertion of genes associated with human breast disease into mice has resulted in the development of benign and malignant lesions that closely resemble the human counterpart. These transgenic models for breast cancer have broadened our horizons by allowing scientists to study the effects of specific genes in the mammary gland and to facilitate the molecular analysis of this important disease process.

CONCLUSIONS

The comparative histopathology of the mammary gland requires a comparison of glands from different species. Unfortunately, few investigators have the requisite training. Although the mammary glands of different mammals have differences in structure and hormonal environment, the basic biology and histology of mammary glands are remarkably similar. The major pathologic changes appear to be concentrated in the terminal hormone sensitive region of the gland, the LA or TDLU.

The spontaneous breast lesions found in feral and laboratory mice are primarily influenced by insertion mutagenesis of MMTV. Whereas, the virus appears to activate a limited number of oncogenes leading to characteristic histopathology, these genes are rarely activated in the human breast. Consequently, the histopathology in human breast disease does not resemble the "spontaneous" tumor of the mouse. In contrast, insertion and expression of a transgene, or the knockout of a gene produces tumors that closely resemble their human counterparts. The histologic and cytologic phenotype are dependent on the specific transgene expressed in the mammary gland. Neoplastic progression breast lesions in the mouse is suggested by the progressive morphologic and biologic changes. These changes can be correlated with molecular lesions. Given this remarkable morphological resemblance, the human breast should also reflect the progressive biologic and molecular changes found in the mouse lesions.

ACKNOWLEDGMENTS

The authors are indebted to Mr. Robert J. Munn whose advice and assistance with the images has been invaluable and Ms. Judy Walls who has provided the outstanding histotechnology. A special thank you goes to Dr. David W Morris and his graduate student Ms. Ruth Lang who provided the images of the mouse mammary development under KGF stimulation. We appreciate the many valuable insights and editorial suggestions provided by Dr. Earl T. Sawai, Dr. Stephen W. Barthold, and Dr. Murray B. Gardner. We are also indebted to the following collaborators who have so generously shared their materials with the University of California, Davis Transgenic Histopathology Laboratory and who have shared their experimental insights with us: Drs. Wm. Muller, P. Leder, A. Leder, F. Lee, E. Sinn, N. Tulchin, R. Coffey, E. Schmidt, M. Bissell, C. Sympson, R. Nandi, R. Guzman, H. Varmus, J. Liang, D. Morris, T. Vogt, D. Seldin, R. Skoda, M. Shen, D. Ornitz, R. Tepper, L. Young, T. Wang,, L. Zuckerberg, E. Lees, C. Guy, M. Webster, M. Schaller, T. Parsons, S. Muthuswamy, A. Elson, D. Deng, A. Luster, A. Arnold, P. Barry, K. Ravid, A. Cheung, P. Siegel, K. Siminovitch, P. Soriano, Z. Werb, T. Lane, L. Chodosh, R. Lang, and many others.

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