

Biological and genetic properties of the p53 null preneoplastic mammary epithelium¹

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SPECIFIC AIMS

As there is a dearth of molecular and genetic data on ductal hyperplasias in mouse models and the p53 genetically engineered gland represents a specific genetic defect found in a subset of human breast cancers, we undertook a series of experiments to characterize the tumorigenic and genetic properties of the preneoplastic lesion in p53 null mammary gland. In this paper, we report on the histopathology, tumorigenic properties, immortalization status, hormone receptor content, and chromosomal content of a series of serially transplanted ductal premalignant outgrowth lines derived from p53 null mammary epithelium.

PRINCIPAL FINDINGS

1. Ductal hyperplasia and ductal carcinoma in situ are frequent premalignant stages in p53 null mammary epithelium

Of the 14 transplantable outgrowth lines, 2 were derived from p53 heterozygous mice (designated PH) and 12 were derived from p53 null mice (designated PN). Two general types of stable morphological patterns were observed on whole mount and histological analyses. The more frequent pattern was predominately ductal and composed of small ducts with varying density. Few lobules were seen and individual alveoli were scattered and infrequent. This pattern was observed in PN1B, 2–10 and was persistent for all transplant generations of these outgrowth lines. The majority of the ducts were lined by simple cuboidal epithelium resting on a basal layer of myoepithelium (Fig. 1). At different times after transplantation, an apparent progressive epithelial atypia (dysplasia) and hyperplasia were detected in some ducts. This hyperplasia ranged from simple consisting of cells with mild to moderate dysplastic nuclei piled two to three cells thick to extensive, where cells with moderately to severely dysplastic nuclei completely filled the ducts and resembled DCIS (Fig.

1). The second pattern was observed in PN1A and the two PH outgrowth lines. In these lines, outgrowth was predominately ductal 8 wk after transplantation; by 14–16 wk, the ducts were organized as small lobules and the epithelium filled the lumina of the alveolar units.

2. Ductal outgrowth lines are preneoplastic cell populations and exhibit high proliferative indices

The tumor-producing capabilities of the outgrowth lines were assessed for transplant generations 2–10. Some of the lines were highly tumorigenic, characterized by a high tumor incidence and a short tumor latency period, such as PH1, PH2, PN1A, PN3, PN4, and PN8A. Most of these lines were derived from mice aged > 52 wk. The majority of the other lines (PN1B, PN2, PN5, PN7, PN8B, PN9, and PN10) were weakly tumorigenic and characterized by a low tumor incidence and a long tumor latency period. Most of these lines were derived from young mice. Outgrowth line PN6 was intermediate in tumorigenic potential. Exceptions to the relationship between tumorigenic potential and donor age were lines PN1B, PN2, and PN8A. Lines PN1B and PN2 were derived from mice > 52 wk old. Lines PN8B and PN8A diverged from the parent line PN8 at transplant generation 5. The tumorigenic potential of PN8 between TGI–5 was low (12/41–29%) with a long latency period (9–10 months).

The ability of the outgrowth lines to be stably transplanted for 10 generations indicated that these lines were significantly different from normal wild-type mammary cells. The BrdU labeling index provided an assessment of the steady-state proliferative activity in the mammary fat pad; that is, after the outgrowths had

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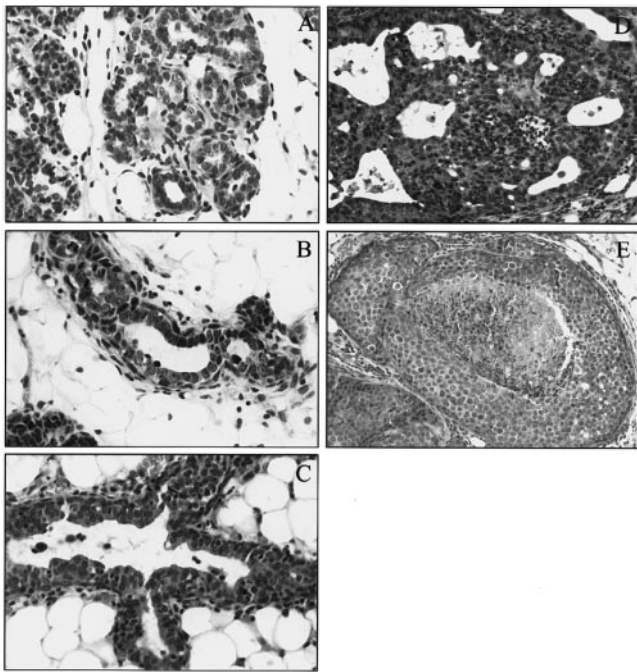


Figure 1. Histopathogenesis of p53 null mammary outgrowths. A) Lobuloalveolar pattern observed in lines PN1A and PH2. B) Ductal outgrowth characteristic of the majority of the outgrowth line. Normal duct. C) Duct showing simple epithelial hyperplasia. D) Small ductal carcinoma in situ with epithelia forming a cribriform pattern. E) Large ductal carcinoma in situ with epithelia forming a comedo pattern.

filled the fat pad and before tumor development. Seven of the lines had a low labeling index of $\leq 2\%$; six lines (PH1, PH2, PN1A, PN3, PN4, and PN8A had a labeling index of greater than 5% ($P < 0.05$ compared to labeling index of the seven other lines.). There was a positive correlation between high labeling index and tumorigenic potential.

The outgrowth lines did not grow when transplanted in the subcutaneous dermis nor did they overgrow neighboring normal mammary duct. There were two exceptions to the latter assay, as the growth of outgrowth lines PH1A and PH8A was not restricted by normal mammary ducts.

Serial transplantation also measured indefinite replicative potential. The p53 wild-type normal mammary

gland has a finite replicative potential. All of the p53 null and heterozygous mammary outgrowth lines exhibited unlimited replicative potential and were considered immortal cell populations. In contrast to the alveolar hyperplasias but in accordance with the pattern observed in other murine and human premalignant lesions, telomerase activity was elevated in all the examined PN lines.

3. p53 null outgrowth lines are estrogen receptor positive and aneuploid

The majority of preneoplastic alveolar outgrowth lines described in the literature are ovarian hormone independent and estrogen receptor negative. The staining patterns and distribution of the receptor positive p53 null cells were similar to that observed in wild-type mammary ducts. The relative frequency of positive cells in the outgrowth lines was scored on a scale of 0–8 and incorporated frequency and intensity of positive cells. The majority of the outgrowth lines were positive with a score comparable to or greater than p53 null normal mammary gland. There were notable exceptions. Outgrowth lines PH1 and PH2 were negative whereas outgrowth lines PN4 and PN8A contained a lower percentage of positive cells (<5% positive). The pattern and staining scores of progesterone receptor were concordant with that of estrogen receptor for all the outgrowth lines.

Mammary tumors that arise from p53 null mammary cells are frequently aneuploid. To determine whether aneuploidy arises in the early stages of neoplasia, we examined chromosome number and aberrations in the stable outgrowth lines. Most lines exhibited a low level of aneuploidy (10–16% cells), but three were diploid by this measure (PH1, PN8A, PN8B). Lines PN3 and PN4 had high levels of aneuploid cells (60 and 30%, respectively). It is surprising that most of the outgrowth lines exhibited only a few cytogenetic aberrations with the exception of the highly aneuploid line PN4.

Table 1 summarizes all the data on the outgrowth lines. An inspection of the data indicates that the only biological properties that correlate with tumorigenic potential were age of the donor mouse and BrdU labeling index.

TABLE 1. Comparison of biological and genetic properties of p53+/- and p53-/- mammary outgrowth lines

Outgrowth line	High TPC ^a						Low TPC							
	PH1	PH2	PN1A	PN3	PN4	PN8A	PN1B	PN2	PN5	PN6	PN7	PN8B	PN9	PN10
Donor age (weeks)	>52	>52	>52	>52	52	9	>52	>52	12	24	9	9	10	10
BrdU labeling index	8.1	9.5	5.9	8.5	6.8	6.0	2.3	1.8	1.5	ND	0.9	ND	2.2	1.0
Hormone receptor (scale of 0–8) ^b	0	0	7	7	2	2	5	8	7	6	7	2	8	6
Percent aneuploid cells	8 ^b	16	10	60	30	6 ^c	14	16	16	10	16	6 ^b	14	12

^a TPC = tumor producing capability.

^b p53 wild-type mammary gland has a score of 5.

^c Diploid chromosome number.

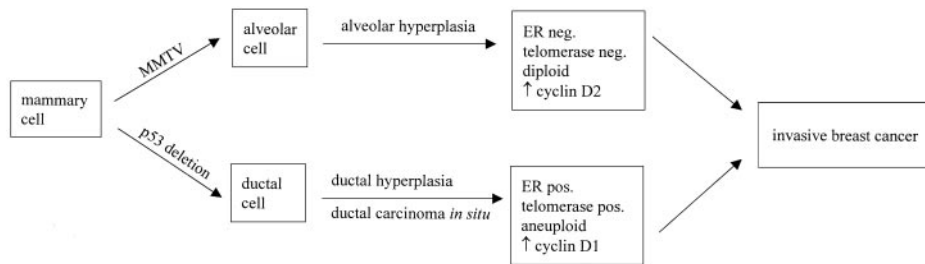


Figure 2. Schematic diagram illustrating two major pathways of pre-neoplastic progression in the murine mammary gland.

CONCLUSIONS

The evidence reported in this paper is a systematic characterization of biological, tumorigenic and cytogenetic properties of suspected preneoplastic gland in p53 null transgenic mice by transplantation analysis. Some of the main characteristics of the mammary tumors and of the preneoplastic lesions arising in the p53 null mammary gland closely mimic subsets of human breast disease. First, the histopathological analysis of the gland indicates that ductal hyperplasia and DCIS are frequent intermediate lesions between the normal duct and invasive cancer. The DCIS represents a late-stage MIN cytologically. The DCIS lesion is not unique to this model, as similar lesions have been described in mice treated with chemical carcinogens, radiation, and in SV40 large T antigen transgenic mice.

Second, the p53 null mammary gland develops immortality at an early stage. p53 null mammary epithelial cells are similar in this respect to the polyoma middle T antigen mammary cells. Immortalization of mammary epithelium is an early event in almost all traditional models of mammary tumorigenesis. The ubiquitous attainment of infinite replicative potential in all outgrowth lines irrespective of their tumorigenic potential suggests that immortalization was concomitant with the absence of p53 gene function. One unique aspect of immortalization of the PN outgrowth lines was the increased expression of telomerase activity. Previous results using MMTV-induced HAN and spontaneous HAN outgrowth lines had demonstrated that immortalization of the mammary alveolar cells was not accompanied by increased telomerase activity. The high telomerase activity detected in the PN outgrowth lines suggest one of two possibilities. Either the telomerase activity is reflective of the type of mammary epithelial cell transformed in the p53 null mammary gland (i.e., duct vs. alveolar cell) or telomerase activity can be regulated by p53 function.

Third, it is evident that the PN cells differ significantly from the alveolar hyperplasias in their estrogen receptor status, as alveolar hyperplasias are estrogen receptor negative and ovarian hormone independent. In the ductal outgrowths and in simple ductal hyperplasias, estrogen receptor status was retained in the majority of the transplantable lines. Our results are similar to those reported recently for the premalignant lesions in SV40 large T antigen transgenic mice where estrogen receptor was lost during tumor progression at

the DCIS stage. But the results are different from the hyperplastic lesions found in the polyoma middle T antigen transgenic mice, where ER was variably expressed and PgR was completely lost. The results in the p53 null mammary epithelial cells mimic a subset of lesions found in human breast disease, where atypical ductal hyperplasias are almost uniformly ER/PgR positive but ~ 25% of DCIS are ER/PgR negative.

Fourth, genetic instability as reflected in aneuploidy was a common event, occurring in most all outgrowth lines. This property distinguishes the p53 null lines from most models of alveolar hyperplasias induced by MMTV or chemical carcinogens. We have shown that 24-wk-old normal appearing mammary gland and hormone stimulated 8-wk-old gland from p53 null mice exhibit aneuploidy. Mammary tumors arising in the p53 null mammary gland are highly aneuploid. It was surprising then that the extent of aneuploidy was low in the majority of outgrowth lines that had been transplanted for eight generations and over a period of 2 years. It is clear that cells in the outgrowths do not accumulate aneuploidy with transplantation. One can interpret the latter result to indicate that excessive aneuploidy is incompatible with cell survival or normal mammary morphogenesis. Support for this idea is presented by Tsukada et al. and Rosen et al., where long-term cultures of nontumorigenic fibroblasts and mammary epithelial cells devoid of p53 gene expression had very low levels of aneuploidy.

Results with the p53 null mammary cells indicate that this model system mimics certain aspects of subsets of human breast cancer not previously observed in traditional models or in the transplanted HAN outgrowth model. Different pathways to murine mammary cancer are illustrated in the schematic diagram (Fig. 2). The presence of aneuploidy and estrogen receptor positive cells in the hyperplastic lesions, acquisition of telomerase activity, and ductal histopathology identify unique properties of this model. We recently found that cyclin D1 protein levels are increased in the ductal hyperplasias, in contrast to alveolar hyperplasias where cyclin D2 protein levels are increased. It is tempting to speculate that differences in molecular and cellular properties between the two types of hyperplasias stem from the nature of the cells initiated by the oncogenic stimulus. In alveolar hyperplasias, the cell initiated by MMTV might be an ER-negative alveolar progenitor cell whereas the cell initiated by p53 gene deletion might be an ER-positive ductal-alveolar progenitor cell. **[F]**