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Altered mammary gland development in the p53+/m mouse, a model of accelerated aging

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Abstract

The tumor suppressor p53 is important for inhibiting the development of breast carcinomas. However, little is known about the effects of increased p53 activity on mammary gland development. Therefore, the effect of p53 dosage on mammary gland development was examined by utilizing the p53+/m mouse, a p53 mutant which exhibits increased wild-type p53 activity, increased tumor resistance, a shortened longevity, and a variety of accelerated aging phenotypes. Here we report that p53+/m virgin mice exhibit a defect in mammary gland ductal morphogenesis. Transplants of mammary epithelium into p53+/m recipient mice demonstrate decreased outgrowth of wild-type and p53+/m donor epithelium, suggesting systemic or stromal alterations in the p53+/m mouse. Supporting these data, p53+/m mice display decreased levels of serum IGF-1 and reduced IGF-1 signaling in virgin glands. The induction of pregnancy or treatment of p53+/m mice with estrogen, progesterone, estrogen and progesterone in combination, or IGF-1 stimulates ductal outgrowth, rescuing the p53+/m mammary phenotype. Serial mammary epithelium transplants demonstrate that p53+/m epithelium exhibits decreased transplant capabilities, suggesting early stem cell exhaustion. These data indicate that appropriate levels of p53 activity are important in regulating mammary gland ductal morphogenesis, in part through regulation of the IGF-1 pathway.

Keywords: p53; Mammary gland; Mouse; Ductal morphogenesis; IGF-1

Introduction

In response to cellular stress, the tumor suppressor p53 plays an important role in maintaining genome integrity and inhibiting both cancer formation and progression by regulating the expression of genes responsible for mediating DNA repair, cell cycle arrest, apoptosis, and senescence (Bunz et al., 1998; Chao et al., 2000; Vogelstein et al., 2000). Dysfunctional p53 signaling pathways or p53 mutations are present in over 80% of all human cancers, including breast cancer (Lozano and Elledge, 2000). Proper function and regulation of p53 are important to prevent the development of human breast cancer, as mutations in p53 are found in 30–40% of spontaneous breast carcinomas and female Li–Fraumeni syndrome patients who carry a germline mutation in one p53 allele have an increased incidence of early-onset breast cancer (Akashi and Koeffler, 1998; Coles et al., 1992; Moll et al., 1992). Further work to understand the role of p53 in mammary gland development may provide insight into mechanisms that regulate malignant transformation as well as normal mammary epithelial cell growth.

The loss of p53 has been reported to have little effect on mammary gland development, as p53 null mammary glands were initially shown to be morphologically and functionally comparable to wild-type glands (Donehower et al., 1992; Jacks et al., 1994; Kuperwasser et al., 2000; Purdie et al., 1994). However, loss of p53 in a BALB/c background causes a tran-

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sient delay in involution, indicating that p53 is expressed by the mammary epithelium during early involution, where it mediates apoptosis (Jerry et al., 1998). In the quiescent virgin gland, p53 mRNA levels are high in the mammary epithelium; however, the virgin gland exhibits a decreased p53 response to DNA damage, characterized by cytoplasmic sequestration of p53 (Kuperwasser et al., 2000; Minter et al., 2002). p53 activation is highest during early and mid-pregnancy, correlating with an increase in proliferation and steroid hormones (Becker et al., 2005; Minter et al., 2002). Interestingly, treatment with pregnancy-associated hormones allows nuclear accumulation of p53 following irradiation in virgin glands (Kuperwasser et al., 2000). Limited hormonal exposure of the mammary gland during postpubertal development, through pregnancy or hormonal treatment, has a protective effect against breast cancer in humans and rodents, which is dependent on the presence of wild-type p53 (Medina and Kittrell, 2003). These studies show that p53 activity is dependent upon hormonal status and varies throughout development.

Recent studies have identified the ability of truncated forms of p53 to enhance the activity of full-length wild-type p53, including a mouse model developed in our laboratory, the p53+/m mouse (Tyner et al., 2002). The p53+/m mice express one wild-type p53 allele and a truncated C-terminal p53 mutant allele, referred to as the "m" allele, which consists of p53 exons 7–11, with an Arg to Trp mutation at codon 245 (Tyner et al., 2002). p53+/m mice are highly resistant to tumors, yet display a reduction in median life span and exhibit a variety of accelerated aging phenotypes (Tyner et al., 2002). The p53+/m mice also exhibit alterations in hematopoietic stem cell (HSC) number, proliferation, and engraftment capabilities with age, suggesting that altered p53 levels affect stem cell functionality (Dumble et al., 2007; Gatza et al., 2007). In response to γ-irradiation, p53+/m tissues exhibit increased wild-type p53 protein levels, indicating that the m protein can stabilize wild-type p53 (Tyner et al., 2002; Moore et al., in press). The m allele also increases the activity of wild-type p53, leading to increased transactivation of the p53 target gene p21 and increased resistance to oncogenic transformation in MEFs (Tyner et al., 2002). Although the m protein is not a natural isoform of p53, it is similar to several naturally occurring p53 isoforms, p44 and Δ133p53 (Bourdon et al., 2005). In addition, the similarities between the p53+/m and p44 mouse models indicate that the p53+/m mouse is biologically significant as a model of increased p53 activity (Maier et al., 2004; Tyner et al., 2002).

Since p53 levels play an important role in protection against mammary carcinogenesis, we investigated the effect of increased p53 activity on mammary gland development and stem cell dynamics by utilizing the p53+/m mouse model. Mammary gland stem cells are necessary for mammary gland development and functionality, and alterations in stem cell proliferative capabilities may lead to defects in the gland (Shackleton et al., 2006; Stingl et al., 2006; Woodward et al., 2005). Additionally, IGF-1, which plays a critical role in mediating mammary gland development, has also been implicated in aging (Holzenberger et al., 2003). p53 is known to transcriptionally repress expression of the IGF-1 receptor (IGF-1R) and transcriptionally upregulate IGF-1 signaling inhibitors IGF-BP3 and PTEN, demonstrating a direct role in p53-mediated regulation of the IGF-1 pathway (Buckbinder et al., 1995; Stambolic et al., 2001; Werner et al., 1996). Recently, several mouse models with accelerated aging phenotypes have been shown to exhibit alterations in IGF-1 signaling (Maier et al., 2004; Niedernhofer et al., 2006; Shukla et al., 2006). Collectively, these studies suggest that the p53+/m mouse may exhibit altered IGF-1 signaling, which could affect mammary gland development.

Here we report that p53+/m virgin mice exhibit a defect in mammary gland ductal morphogenesis. Transplants of mammary epithelium into p53+/m recipient mice demonstrate a significant reduction of WT and p53+/m donor epithelium outgrowth, suggesting systemic or stromal alterations in p53+/m mice. In fact, p53+/m mice exhibit a decrease in serum IGF-1 levels and attenuated IGF-1 signaling in the virgin gland. The p53+/m mammary phenotype is rescued by induction of pregnancy, treatment with estrogen, progesterone, estrogen, and progesterone in combination, or LR3-IGF-1. Collectively, these data demonstrate that alterations in p53 activity can have a profound effect in the regulation of mammary gland development.

Materials and Methods

Mice

The mice used in this study were previously reported by our laboratory (Donehower et al., 1992; Tyner et al., 2002), except the TTR-IGF-1 mice (Liao et al., 2006). p53 wild-type (WT) and p53+/m mice backcrossed seven generations into C57BL/6 and p53+/− mice backcrossed 12 generations into C57BL/6 were used for all experiments. The WT and p53+/m mice were generated by crossing WT mice to p53+/m mice and were genotyped by PCR as previously described (Dumble et al., 2007). p53+/− mice were produced by heterozygous crossings and were genotyped by Southern Blot analysis as previously described (Donehower et al., 1992). TTR-IGF-1 heterozygous mice were crossed to BC7-p53+/m mice to generate TTR-IGF-1/+;p53+/m mice and were genotyped by PCR as previously described (Dumble et al., 2007; Liao et al., 2006).

All mice were bred and maintained in a specific pathogen free animal facility at Baylor College of Medicine. The 3-week-old C57BL/6 female recipient mice used in the serial mammary epithelial transplantation experiments were purchased from the Baylor College of Medicine Transgenic Mouse Barrier Facility.

Whole mount preparation

Mammary gland tissue was isolated and whole mounts were prepared as previously described (Medina et al., 2002). The number four inguinal mammary glands were harvested from WT, p53+/−, and p53+/m mice at 8 week virgin, 12 week virgin, 24 week virgin, mid-pregnancy (day 12), and D10 involution (10 days post weaning) and fixed in 10% neutral buffered formalin for at least 24 h. Whole mounts were examined for ductal outgrowth under a dissecting microscope (Olympus SZX12).

In vivo BrdU incorporation

Two hours prior to sacrifice, mice were treated with 7 mg/ml bromodeoxyuridine (BrdU) cell proliferation reagent (Sigma Aldrich, St. Louis, MO, USA) at 0.01 ml/gm of body weight. The number four inguinal mammary glands were harvested from WT, p53+/−, and p53+/m mice at 8 week virgin, 12 week virgin, mid-pregnancy (day 12), and D10 involution and fixed in 4% paraformaldehyde for 2 h followed by 70% ethanol. Fixed tissues were paraffin-embedded and sectioned. Immunostaining for BrdU incorporation was performed using the BrdU *In-Situ* Detection Kit (BD Pharmingen, San Diego, CA, USA). For each