

## Commentary

# Hormonal control of p53 and chemoprevention

D Joseph Jerry, Lisa M Minter, Klaus A Becker and Anneke C Blackburn

Department of Veterinary & Animal Sciences, University of Massachusetts, Amherst, Massachusetts, USA

**Correspondence:** D Joseph Jerry, Department of Veterinary & Animal Sciences, Paige Laboratory, Room 216, 161 Holdsworth Way, University of Massachusetts, Amherst, MA 01003, USA. Tel: + 1 413 545 5335; fax: + 1 413 545 6326; e-mail: [jjerry@vasci.umass.edu](mailto:jjerry@vasci.umass.edu)

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### Abstract

Improvements in the detection and treatment of breast cancer have dramatically altered its clinical course and outcome. However, prevention of breast cancer remains an elusive goal. Parity, age of menarche, and age at menopause are major risk factors drawing attention to the important role of the endocrine system in determining the risk of breast cancer, while heritable breast cancer susceptibility syndromes have implicated tumor suppressor genes as important targets. Recent work demonstrating hormonal modulation of the p53 tumor suppressor pathway draws together these established determinants of risk to provide a model of developmental susceptibility to breast cancer. In this model, the mammary epithelium is rendered susceptible due to impaired p53 activity during specific periods of mammary gland development, but specific endocrine stimuli serve to activate p53 function and to mitigate this risk. The results focus attention on p53 as a molecular target for therapies to reduce the risk of breast cancer.

**Keywords:** chemoprevention, estrogen, p53, progesterone, susceptibility

### Chemoprevention by endocrine factors

The protective effect of pregnancy with respect to breast cancer was documented by Ramazzini over 300 years ago, who noted the higher rates of breast cancer among Catholic nuns. Epidemiological studies have shown that a single full-term pregnancy reduces risk of breast cancer by ~50% [1]. Carcinogen exposures at various times during development of the mammary gland have shown that risk of breast cancer is not static but varies among developmental states [2-4]. The mature nulliparous mammary gland is relatively quiescent with respect to proliferation, but represents a susceptible state. Although p53 protein is expressed in the quiescent mammary epithelium of nulliparous mice, responses to  $\gamma$ -radiation (cell-cycle arrest and apoptosis) were marginal until activated by acute administration of a hormonal regimen mimicking the rise in ovarian steroids that precedes ovulation [5]. In contrast, the proliferation and differentiation associated with preg-

nancy renders the mammary epithelium resistant to development of tumors [4,6].

Sivaraman *et al.* [7] report a sustained activation of p53 function in the mammary epithelium of rats and mice following treatment with a regimen of estrogen and progesterone (E + P) that was effective in inhibiting mammary tumors. In this experiment, postpubertal rats and mice were treated with E + P for 21 days to induce mammary gland development that mimics the changes associated with pregnancy. The hormones were withdrawn to simulate involution of the mammary gland prior to administration of carcinogen.

Accumulation of nuclear p53 protein was detectable after carcinogen treatment in E + P-treated animals, but not in age-matched virgins. Expression of p21/WAF1, which is induced rapidly by p53, was used to assess p53 activity in

the mammary gland [5,8]. Accordingly, elevated levels of p21/WAF1 protein were detected within the mammary epithelium of E + P-treated animals, supporting a functional p53 response to carcinogen treatment. This is consistent with the decrease in proliferation observed in the E + P-treated rats in response to carcinogen treatment [7]. Perphenazine treatment, which induces secretion of prolactin, causing differentiation of the mammary gland but not resistance to tumors [9], failed to activate p53 in this experiment. Differentiation alone is therefore unable to activate p53 or to account for the prophylactic effects of E + P treatment on the mammary epithelium.

These experiments of Sivaraman *et al.* [7] suggest that prophylaxis is mediated by specific hormone receptor pathways (estrogen and progesterone receptors) and that they strengthen the association between p53 activation and chemoprevention.

### **Reconciling the conflicting effects of endocrine factors**

A model of hormone-mediated prophylaxis must also account for the roles of endocrine factors in promoting breast cancer. Estrogen plays an important role in normal proliferation of the mammary epithelium and participates in progression to a neoplastic state. Selective estrogen receptor modifiers offer great promise for chemoprevention [10,11], incriminating estrogen as a primary suspect in breast cancer. However, progesterone and prolactin may act as accomplices, enhancing tumor development [12,13]. Pituitary isografts induce hormone profiles similar to midpregnancy, but result in hyperplasia and increased incidence of mammary tumors [14,15]. The dramatic reduction in risk of breast cancer in women who have undergone ovariectomy serves to confirm the influential role of ovarian steroids in promoting breast cancer [16]. From this perspective, hormonal stimulation is the cause of breast cancer, not the cure!

So how can ovarian steroids mediate both susceptibility to breast cancer and prophylaxis? In the report by Sivaraman *et al.* [7], the authors propose a cell-fate model in which pregnancy hormones restrict the developmental fate of a subset of mammary epithelial cells causing permanent changes in their responses to carcinogen stress, and therefore making them resistant to tumorigenesis. However, prolonged endocrine stimulation can overcome the prophylactic effect of pregnancy [17]. Models must therefore accommodate the reversibility of hormone-mediated resistance to mammary tumors.

Alternatively, hormonal exposure may alter the sensitivity of the mammary epithelium to hormonal stimuli. In support of this notion, the parous mammary gland was shown to be more responsive to hormonal induction of lactose synthetase activity without alteration of either the affinity or

the capacity of estrogen receptors [18,19]. Changes in hormonal sensitivity may reflect alterations in the balance of coregulatory molecules. Like lactose synthetase, persistent activation of p53 in the parous mammary epithelium may result from an alteration in sensitivity rather than a permanent change in cell fate.

The cell-fate model also poses difficulties with respect to development of the mammary gland in subsequent pregnancies. Persistent activation of p53 in the parous mammary gland would appear to limit proliferation as well as to inhibit tumor formation. This is clearly not the case. The inhibitory effects of p53 must therefore be overcome to allow growth and differentiation of the mammary epithelium in subsequent pregnancies. This too is consistent with the fact that hormone-mediated prophylaxis is also reversible [17].

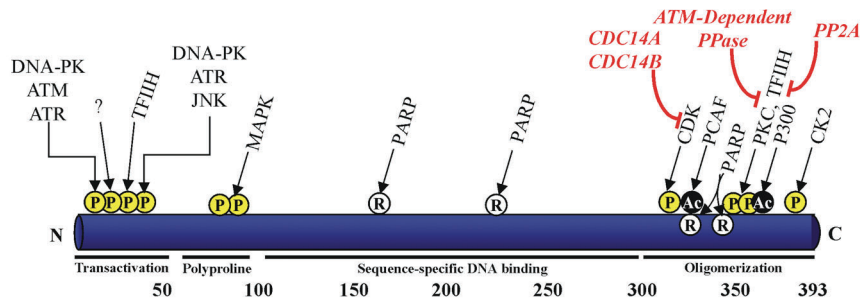
The model of p53-mediated prophylaxis induced by hormonal stimulation is attractive given the role of p53 as a potent inhibitor of cellular proliferation and an inducer of apoptosis. However, a quantitative measure of the effects of the E + P treatments on p53 activity was not undertaken in the report by Sivaraman *et al.* [7]. Demonstration that the protective effect of E + P is abolished in p53-deficient mammary tissue will be required for final proof of this concept. Nonetheless, identification of the hormones required to activate p53 and the endocrine balance necessary to overcome the limits imposed by p53 in the parous gland will offer insights into important signaling pathways and effector genes that regulate p53 activity.

### **Regulators of p53 as targets of chemoprevention**

Expression of p53 mRNA showed dramatic changes across periods of mammary gland development [8], but no direct effect of hormones on levels of p53 mRNA has been established [6]. Nonetheless, alterations in p53 activity were apparent following endocrine manipulations [5]. The effects of hormones on p53 activity may be mediated by interactions with antagonists (e.g. MDM2, MDMX) and agonists (e.g. p19/ARF) of p53 that attenuate or augment its activity.

Post-translational modification has also emerged as a potent means to balance latent and active forms of p53 protein. Recent accounts reveal multiple covalent modifications of p53 protein (phosphorylation, acetylation, ribosylation, sumoylation) that alter its stability and its subcellular localization, as well as affecting its ability to bind DNA and transcriptionally activate target genes [20]. The host of enzymes that catalyze these reactions provides a rich set of targets on which endocrine factors may impinge to regulate p53 function (Fig. 1). These enzymatic activities also provide targets for development of small molecules to alter the activity of p53 protein.

**Figure 1**



Post-translational modifications that alter activity of the p53 protein. Enzymes that have been shown to modify specific amino acid residues of p53 are shown. Enzymes that inhibit the covalent modifications are indicated in red. P, phosphorylation; R, ribosylation; Ac, acetylation.

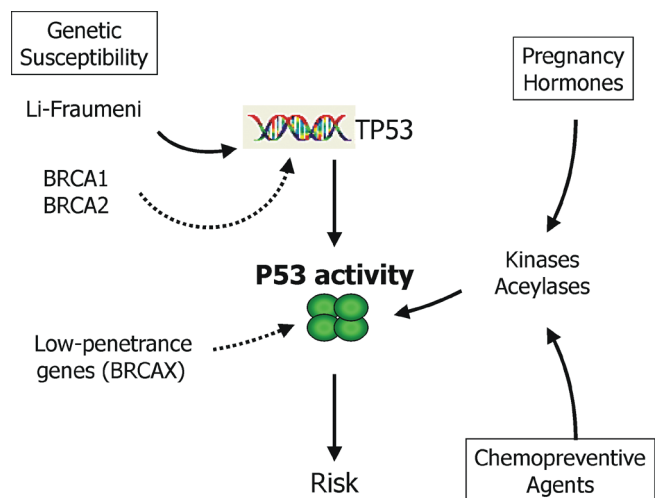
### Susceptibility, genetics and environment

Susceptibility to breast cancer is determined not only by environmental factors, such as reproductive history, but also by genetic factors. Again, p53 appears to play a central role in heritable breast cancer susceptibility syndromes. Li-Fraumeni syndrome is associated with heterozygous germline mutations in the gene encoding the p53 protein (*TP53*). Although families harboring heterozygous mutations in *TP53* suffer from a wide spectrum of tumors, breast cancer is the most prevalent tumor type in women, suggesting a critical role for p53 in the breast epithelium.

Heritable mutations in *BRCA1* also result in a sensitivity to breast cancer that is accompanied by instability and frequent loss of *TP53* [21,22], again implicating disruption of p53 function as a seminal event in breast tumorigenesis. The absence of male breast cancer in both Li-Fraumeni syndrome and *BRCA1* families and the effective reduction in breast cancer risk by endocrine ablation in carriers of *BRCA1* [23] suggest an interaction between the endocrine environment and these susceptibility genes. Therefore, factors that affect either the integrity of the *TP53* gene or its activity may serve to modify the risk (Fig. 2).

The phenotypic variation among women who are carriers of known breast cancer susceptibility alleles suggests the presence of low-penetrance genetic modifiers. These low-penetrance modifiers may play significant roles in what is presently termed 'sporadic' breast cancer, as well as in the nearly 20% of heritable breast cancer that cannot be accounted for by the known high-penetrance breast cancer susceptibility genes [24]. The genes that modify p53 function provide attractive candidates for low-penetrance modifiers as well as targets for hormonal modulation to augment or to diminish risk [25]. In this view, p53 may serve to integrate the effects of genetic susceptibility and environmental exposures to determine risk (Fig. 2).

**Figure 2**



Pathways affecting the risk of breast cancer. The prophylactic effect of pregnancy hormones appears to act directly on the p53 protein to alter its activity. Genetic susceptibility mediated by high-penetrance cancer susceptibility genes appears to affect the integrity of the p53 gene, whereas low-penetrance modifiers may serve to alter the activity of the p53 protein. The kinases and acetylases that regulate p53 activity provide targets for chemopreventive agents.

### Era of hope

As the molecular details determining susceptibility of the breast epithelium emerge, they will foster new enthusiasm for chemoprevention. Elucidation of critical pathways will make it possible to more accurately identify individuals at risk and to tailor interventions. These pathways will also provide markers that can be evaluated at intermediate time points to accelerate testing of new agents for efficacy. The experiments reported by Sivaraman *et al.* suggest that p53 activity may provide one such marker. The enzymes

that activate p53 by altering its post-translational modifications provide targets for novel chemopreventive agents. Small molecules stabilizing the active conformation of p53 are an alternative to prophylactic hormonal treatments that have already shown promise [26]. Although the complex interplay between hormones and heritable risk will continue to pose challenges, the emerging pathways renew hope for interventions aimed at preventing breast cancer.

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