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Estrogens, regulation of p53 and breast cancer risk: a balancing act

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Abstract The paradoxical effects of ovarian hormones in both the promotion and prevention of breast cancer have been debated for over 30 years. Genetic studies have demonstrated that ovarian hormones act through NF-κB to stimulate proliferation and ductal elongation, whereas the p53 tumor suppressor protein plays a central role in rendering the mammary epithelium resistant to tumorigenesis. Transcriptional profiles now suggest that ovarian hormones stimulate a constellation of genes that interact with NF-κB and p53 to arbitrate the competing demands for proliferation and surveillance. Genes that participate in chromatin remodeling are among the acute transcriptional responses to estrogens and progestins. These genes are proposed to initiate epigenetic programs that influence the balance between proliferation and surveillance, and render the breast epithelium resistant to tumors.

Keywords P53 · Breast cancer · Estrogen receptor alpha · Estrogen receptor beta · Parity · Risk · Epigenetics

The paradoxical effects of ovarian hormones

Hormonal exposures are prominent among the factors determining risk of breast cancer. Associations between early menarche and late menopause as well as hormone replacement therapies suggest that lifetime exposures to ovarian steroids (estrogens and progestins) are associated with increases in the relative risk of breast cancer of 1.2–1.5 [1]. Elevated levels of circulating estrogens are also associated with increasing risk in a dose-dependent fashion among pre-menopausal women [2]. While estrogens can have direct genotoxic effects by alkylating DNA [3], excessive signaling through the estrogen receptor appears to be the primary mechanism for breast carcinogenesis as modest increases in expression of estrogen receptor alpha (ERα) in transgenic mice resulted in mammary hyperplasias [4]. Levels of estrogen and progesterone receptors vary among strains of mice, influencing mammary susceptibility [5, 6], and polymorphisms in the gene encoding ERα in humans (designated *ESR1*) have been linked to increased breast cancer risk [7–9]. Therefore, excessive or inappropriate signaling through estrogen and progesterone receptors are key factors determining the risk of breast cancer.

However, ovarian hormones pose a paradox as estrogens and progestins also mediate the protection from breast cancer afforded by parity. A full-term pregnancy early in reproductive life reduces breast cancer incidence by up to 50% [10, 11]. Estrogen and progesterone are sufficient to mimic the effect of pregnancy in reducing the incidence of carcinogen-induced mammary tumors [12–14]. Thus, estrogens alone as well as in combination with progestins are able to engage pathways that are potent inhibitors of breast cancer.

Activity of the p53 tumor suppressor and breast cancer risk

Tumor suppressor genes underlying heritable breast cancers identify pathways that may mediate the protection

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afforded by parity. The p53 pathway is a prominent candidate as heritable mutations in *TP53* are associated with Li-Fraumeni syndrome, and breast cancer is the most common tumor type among women carrying heterozygous mutations [15, 16]. Mutation of *TP53* is common in sporadic breast cancers [17] and appears to be a necessary collaborating alteration in breast cancers associated with heritable mutations in *BRCA1* [18, 19]. *CHK2* and *HDM2* (the human ortholog of *Mdm2* in mice) are regulators of p53 activity, and polymorphisms in these genes were identified as breast cancer risk alleles [20, 21]. Together, these genetic studies suggested that p53 activity in the mammary epithelium may be limiting and that increased activity of p53 could confer resistance to tumors. Indeed, the apoptotic activity of p53 is reduced in the mammary epithelium of BALB/c-*Trp53*+/− mice, predisposing them to spontaneous tumors. Conversely, the activity of p53 was increased in parous mice and delayed the onset of mammary tumors in BALB/c-*Trp53*+/− mice [22], while in the absence of p53 parity failed to reduce mammary tumors [23, 24]. Thus, sufficiency of p53 activity represents a vulnerable link in the barriers to tumorigenesis in the breast epithelium.

A balancing act

While it is clear that stimulation of mammary tissues with estrogen and progesterone promotes proliferation, p53-mediated apoptosis is also increased [25]. How do these hormones induce opposing actions? Transcriptional profiles of mouse mammary tissues after treatment with estrogen and progesterone provide clues to the basis for the apparent paradox [26]. Using protein–protein interaction databases, we found that interactions with p53 and c-Rel were significantly greater than expect by chance (Fig. 1), suggesting that these are major targets regulated by the hormone treatments. The identification of c-Rel, a subunit of NF-κB, is satisfying because NF-κB is an essential mediator for mammary gland development [27, 28]. Deletion of IKKα (required for activation of NF-κB) in mice phenocopies ERα knockouts [29, 30]. Therefore, NF-κB is presumed to mediate the proliferative effects of estrogens. Although estrogen and progesterone did not alter p53 expression or its basal activity [25], these hormones induced a constellation of genes that potentiates the responsiveness of p53 to genotoxic stimuli [31, 32]. Thus, it appears that the proliferative effects of estrogen and progesterone are coordinated with an increase in genome surveillance.

A subset of the gene products induced by estrogens and progestins interacts with both p53 and NF-κB, and thus is ideally situated to arbitrate the decision between proliferative responses mediated by NF-κB and the surveillance activity of p53 (Fig. 1). Of these, EGR1 is especially intriguing as it acts to both temper transactivation mediated by NF-κB as well as promote the transcriptional activity of p53 [26, 33, 34]. In addition to being increased by estrogen and progesterone, DNA damage activates CArG elements in the *EGR1* promoter, resulting in further increases in expression. Proper function of EGR1 is essential for p53-dependent apoptosis in response to irradiation in mouse embryo fibroblasts (MEFs) [35]. In addition to interactions with p53, transcriptional studies of EGR1-deficient MEFs demonstrated interactions with TGFβ signaling. Furthermore, estrogen and progesterone increase active TGFβ1 in the mammary epithelium [36, 37], which collaborates with p53 to restrain proliferation and potentiate radiation-induced apoptosis [25, 37, 38].

In addition to the acute changes, the mammary epithelium retains a "memory" of hormonal exposures that potentiates p53-dependent apoptosis even after the hormones are withdrawn [22]. Chromatin remodeling enzymes are prominent among the genes induced by estrogen and progesterone, suggesting that epigenetic mechanisms may participate. CARM1 and PRMT1 are histone methylases that bind to p53 and enhance transcriptional activation of p53 target genes [39]. Egr1 also participates directly in methylation of target genes [40] and regulates epigenetic patterning of social behaviors [41]. TGFβ signaling is a target of Egr1 [35] and is among the pathways that have been shown to be increased persistently in parous mammary tissues compared to nulliparous [42]. TGFβ signaling may be critical as p53 collaborates with Smads to form complexes with mSin3A that contribute to gene silencing [43]. While p53 and TGFβ signaling can silence genes, ING1 binds specifically with tri-methylated histone 3 (H3K4me3) on active chromatin [44] and recruit p53 to enhance transcriptional activation of target genes [45]. Therefore, prolonged exposure to estrogen and progesterone during pregnancy stimulates chromatin remodeling proteins that together with p53 orchestrate remodeling of the epigenetic landscape in mammary epithelia to favor a range of protective measures that include p53-mediated apoptosis.

A tale of two receptors

Although circulating levels of estrogen and progesterone are increased during pregnancy, 17β-estradiol appears to be more potent in rendering the mammary epithelium resistant to tumors. Estrogenic compounds have anti-tumor activities in humans [46, 47], and estrogen alone was sufficient to prevent mammary tumors in rodents [48]. Furthermore, the

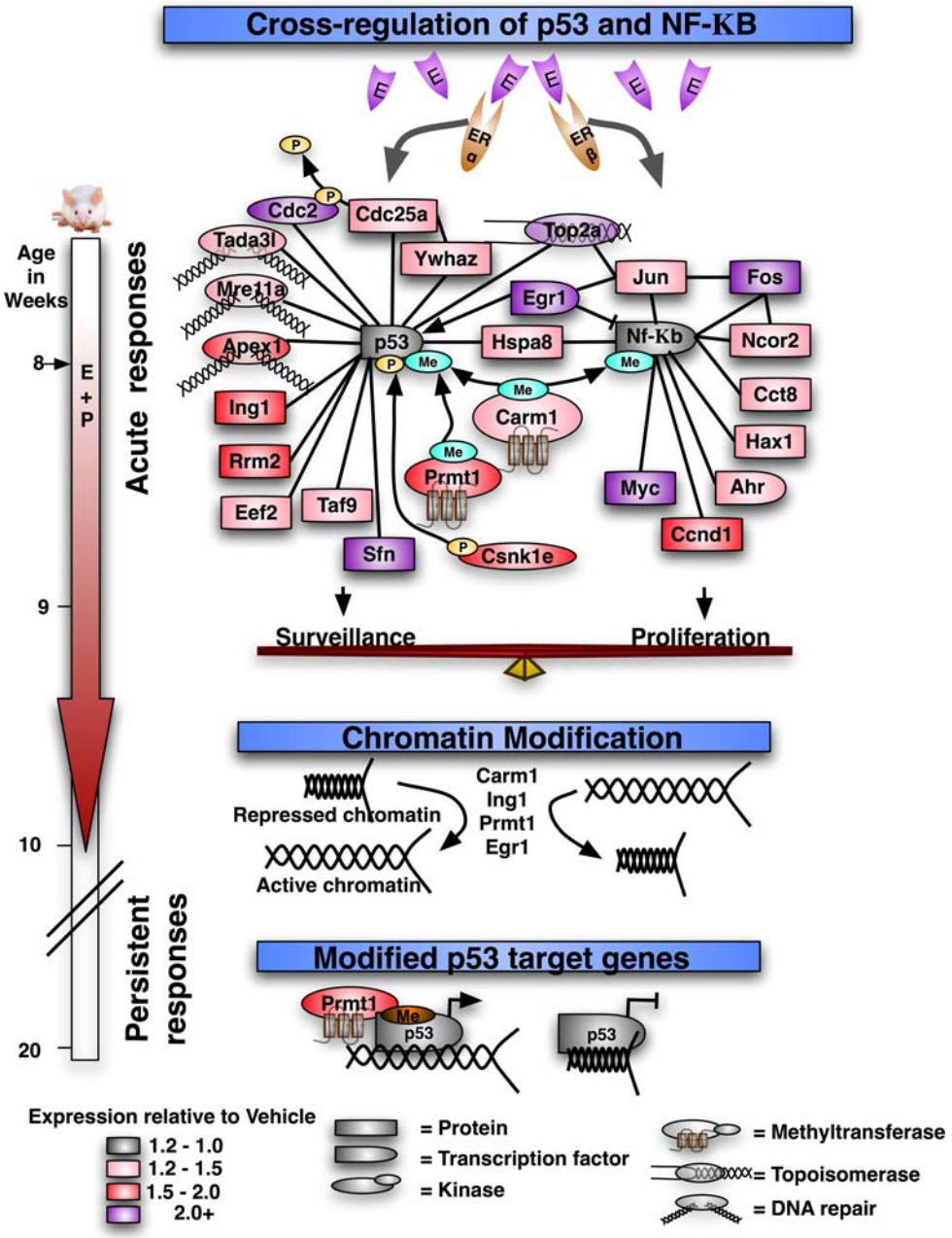

Fig. 1 Balancing the responses to estrogens and progestins. Genes differentially expressed after acute exposure to 17β-estradiol and progesterone (4 days) were identified previously [26]. Among these genes, first-neighbor interactions with p53 and NF-kB were significantly over-represented. Chromatin remodeling enzymes together with p53 may target epigenetic alterations responsible for the persistent increase in p53 activity in parous mammary epithelium. The *colors* indicate the relative levels of mRNA expression in the E + P-treated compared to the vehicle-treated controls (see legend). The interaction database was obtained from NCBI, and the final model is adapted from the visualization using Cytoscape

effect of 17β-estradiol was ~threefold more potent than progesterone in potentiating p53-dependent responses in mice [25]. Two estrogen receptors (ERα and ERβ) mediate the actions of estrogens and are encoded by separate genes designated *ESR1* and *ESR2*, respectively. Both ERα and ERβ share a common structure with major divergence localized to the N-terminal transactivation domain. While ERα and ERβ have similar binding affinities for 17β-estradiol (K_d of 0.1 and 0.4 Nm, respectively), ERβ showed a greater affinity for phytoestrogens such as genestein [49]. Both estrogen receptors transcriptionally activate consensus estrogen-responsive elements in response to physiologic estrogens and phytoestrogens [50]. In cells that express both receptors, heterodimers also efficiently transactivate reporter genes [51]. However, ERα and ERβ also bind AP1 sites, but yield opposing actions with ERα mediating transcriptional activation, whereas ERβ is inhibitory [52]. Therefore, the effects of ERα and ERβ on gene expression depend on the context of promoter elements.

The luminal epithelia of the mammary gland express both ERα and ERβ, and thus heterodimers may predominate. In contrast, the basal epithelial cells express solely

ERβ. Expression of the two estrogen receptors also differs between nulliparous and parous rodents. During pregnancy, the levels of ERβ are increased among multiparous rats compared to nulliparous. This increase is associated with a 50% decrease in BrdU incorporation in the mammary epithelium of parous mice compared to the nulliparous mice [53], suggesting opposing effects of ERα and ERβ.

Attempts to understand the functions of these receptors using cell-based assays have yielded contradictory results with respect to their actions on p53. Using MCF7 cells, exogenous ERα bound the C-terminus of p53 and inhibited transactivation of a PCNA-luciferase reporter [54], while experiments with HeLa cells demonstrated that exogenous ERα bound the N-terminus of p53, sparing it from degradation by HDM2 and enhanced transactivation of a *Cdkn1a*/p21-luciferase reporter [55]. With respect to regulation of endogenous *ESR1*, p53 was shown to both inhibit [56] as well as maintain ERα levels in MCF7 cells [57]. In contrast to these cancer-derived cell lines, introducing exogenous ERα into MCF10A cells resulted in transcriptional activation of a reporter gene, but failed to induce proliferation [58]. Similarly, in normal breast epithelial cells immortalized with telomerase (76N-Tert), treatment with estrogen and progesterone inhibited cell proliferation and was associated with an increase in transactivation by p53 [59]. The inconsistency of the results among experiments may reflect variations in the complement of co-activators among cell lines, which strongly influence the activities of ERα and p53 [60, 61].

A more consistent picture emerges from experiments varying ERβ levels. Transient transfection of ERβ enhanced radiation-induced expression of p21 [62]. Similarly, expression of ERβ inhibited proliferation of MCF7 cells and blocked tumor formation as xenografts [63, 64]. Expression of ERβ also inhibited tumor growth in T47D cells, which express a missense mutant of p53 [65, 66]. Thus, ERβ may have p53-independent anti-proliferative activities. Conditional expression of ERβ in MCF7 cells increased expression of pro-apoptotic target genes [67]. Although ERα and ERβ show similar potency in transactivation of reporter genes and appear to bind to similar target sequences identified by chromatin immunoprecipitation [68, 69], co-expression of ERβ along with ERα resulted in dramatic alterations in transcriptional profiles in breast cancer cell lines [68, 70–73], supporting distinct actions and targets for these receptors. It is notable that EGR1 was increased by threefold in MCF7 cells when ERβ was present [70], which is similar to the induction noted in mouse mammary gland after stimulation with E + P [26]. Therefore, the opposing activities of ERα and ERβ appear to be in an equilibrium that determines the cellular response.

Future implications

Although the mechanisms by which ovarian hormones act to both promote and prevent breast cancer remain incomplete, NF-κB and p53 are likely to be major targets. A division of labor between estrogen receptor subtypes is also suggested, with ERα being essential for proliferation, while ERβ favors genome surveillance via p53. EGR1 offers an attractive target for arbitrating the acute response to genotoxic stress. While the acute responses to estrogen and progesterone remain significant, the effects on epigenetic programs are likely to be more profound in determining the balance among responses and the long-term reduction in the risk of breast cancer. Consequently, rather than simply blocking the actions of estrogen and progesterone, one can envision therapies that harness the effects of these hormones to alter the epigenetic profile so as to shift the equilibrium to favor surveillance and a durable resistance to carcinogenesis.

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