

# p53: Protection against Tumor Growth beyond Effects on Cell Cycle and Apoptosis

Xuyi Wang<sup>1,2</sup>, Evan R. Simpson<sup>1,3</sup>, and Kristy A. Brown<sup>1,2</sup>

The tumor suppressor p53 has established functions in cancer. Specifically, it has been shown to cause cell-cycle arrest and apoptosis in response to DNA damage. It is also one of the most commonly mutated or silenced genes in cancer and for this reason has been extensively studied. Recently, the role of p53 has been shown to go beyond its effects on cell cycle and apoptosis, with effects on metabolism emerging as a key contributor to cancer growth in situations where p53 is lost. Beyond this, the role of p53 in the tumor microenvironment is poorly understood. The pub-

lication by Wang and colleagues demonstrates for the first time that p53 is a key negative regulator of aromatase and, hence, estrogen production in the breast tumor microenvironment. It goes further by demonstrating that an important regulator of aromatase, the obesity-associated and tumor-derived factor prostaglandin E<sub>2</sub>, inhibits p53 in the breast adipose stroma. This review presents these findings in the context of established and emerging roles of p53 and discusses possible implications for the treatment of breast cancer. *Cancer Res*; 75(23); 5001–7. ©2015 AACR.

## Background

### Traditional tumor suppressor functions of p53

The tumor suppressor p53, encoded by the *TP53* gene, is the most commonly silenced or mutated gene in cancer, with 50% to 55% of human cancers having experienced the loss of wild-type p53 activity (reviewed in ref. 1). Under normal conditions, p53 levels are low and, in some cases, undetectable. However, stress signals such as DNA damage, oncogene activation, and hypoxia stabilize p53 protein and induce increased cellular p53 levels by posttranslational modifications such as phosphorylation and acetylation. Activated p53 causes a variety of responses including cell-cycle arrest or apoptosis, thereby providing a critical barrier against tumor development (reviewed in refs. 1, 2). As a transcription factor, activated p53 binds to a number of genes that contain p53-binding sites within their regulatory regions. Bioinformatic studies have found more than 4,000 putative p53-binding sites in the existing human genome. Somatic mutations in *TP53* are common in cancers and are associated with poor prognosis and low response to chemotherapy (3).

Numerous p53 target genes have been identified as downstream effectors of p53 with changes in cell function being dependent on the regulation of several genes (2). For example, p53 mediates cell apoptosis by activating mitochondrial and death receptor-induced apoptotic pathways, both pathways resulting in the induction of caspase signaling, which then

induces apoptosis. The mitochondrial pathway is mainly regulated by p53 effector Bcl-2 proteins such as Bax (4) and PUMA (5). In tumors with wild-type p53, p53 responses can be inhibited by downregulating p53 activity or its effectors' activity. For instance, in estrogen receptor-positive (ER<sup>+</sup>) breast cancers, ER prevents the p53-mediated apoptotic response by directly interacting with p53 (6).

Although most p53 mutations result in inactivation or dysfunction of p53, some mutations in p53 lead to the selective loss of apoptotic functions while retaining the ability to induce cell-cycle arrest. Certain p53 mutations lead to p53 becoming oncogenic through gain-of-function mechanisms (7). Cell-cycle arrest driven by p53 requires the transcription of p21, which is a cyclin-dependent kinase inhibitor, or other p53 target genes such as 14-3-3 $\sigma$  and GADD45 (8). In general, DNA damage or stress will increase levels of p53 protein, which in turn induces p21 transcription and leads to cell-cycle arrest at G<sub>1</sub>, allowing cells to survive until the damage has been repaired or the stress removed (9, 10). The G<sub>1</sub> arrest is primarily regulated by p21, whereas G<sub>2</sub> arrest is stimulated by GADD45, p21, and 14-3-3 $\sigma$  (11). The role of p53 to suppress tumor growth and promote apoptosis via these pathways is well-characterized. However, novel roles for p53 are emerging and these are proving important contributors to the tumor suppressor functions of p53.

### p53 as a metabolic checkpoint

Emerging evidence suggests that p53 is also involved in the regulation of metabolism and cell homeostasis without causing cell-cycle arrest or apoptosis (12). For example, nutrient deficiency leads to the activation of p53 through direct phosphorylation at Ser15 by AMP-activated protein kinase (AMPK), a key regulator of cell metabolism (13). p53 also regulates metabolism and cell homeostasis in normal cells and tissues. Lipin1 is a recently identified p53 target gene that regulates the expression of genes involved in fatty acid oxidation through PPAR $\alpha$  (14). Under nutrient/glucose deprivation conditions, p53 is upregulated by AMPK and stimulates Lipin1 and malonyl-CoA decarboxylase expression, leading to an increase in fatty acid oxidation (14, 15).

<sup>1</sup>Metabolism and Cancer Laboratory, Centres for Cancer Research and Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, Victoria, Australia. <sup>2</sup>Department of Physiology, Monash University, Clayton, Victoria, Australia. <sup>3</sup>Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia.

**Corresponding Author:** Kristy A. Brown, Metabolism and Cancer Laboratory, Hudson Institute of Medical Research, 27–31 Wright Street, Clayton, Victoria 3168, Australia. Phone: 61-3-95944333; Fax: 61-3-95946125; E-mail: kristy.brown@hudson.org.au

**doi:** 10.1158/0008-5472.CAN-15-0563

©2015 American Association for Cancer Research.

This allows cells to use fatty acids as an alternative energy source. Reciprocally, p53 also promotes the expression of AMPK, leading to the negative regulation of mTOR (16). The PI3K/Akt/mTOR pathway can suppress apoptosis and stimulate proinflammatory gene expression, which in turn promotes cancer growth and progression (17). Negative regulation of mTOR is also observed in autophagy, which can be induced by starvation and metabolic stresses.

Oncogenic transformation is often associated with enhanced aerobic glycolysis and reduced oxidative phosphorylation, which is known as the Warburg effect. Aerobic glycolysis allows cancer cells to generate ATP as well as stimulate anaerobic metabolism of intermediates such as  $\alpha$ -ketoglutarate to support cancer cell proliferation and survival (18). Recently, p53 has been shown to inhibit the Warburg effect by reducing glycolysis and enhancing oxidative phosphorylation via upregulation of genes including TIGAR and SCO2 (synthesis of cytochrome oxidase 2), as well as inhibiting the expression of glucose transporters GLUT1, GLUT3, and GLUT4 (19, 20). SCO2 increases mitochondrial respiration and TIGAR inhibits glycolysis and promotes NADPH production and glutathione recycling, whereas repression of glucose transporters blocks the uptake of glucose (19).

In summary, a number of genes are regulated by p53 to maintain metabolism and energy homeostasis in cells/tissues under normal and stressed physiologic conditions. Changes in tumor cell metabolism are now recognized as a hallmark of cancer (21) and p53 is integral to this process.

#### Dysregulated metabolism as a driver of estrogen production in the breast adipose

Adipose tissue is responsible for energy storage and acts as an endocrine organ that regulates metabolism via endocrine, autocrine, and paracrine processes. Dysregulated adipose tissue plays a crucial role in cancers (22). In postmenopausal women, breast adipose tissue becomes the main site for estrogen production; obesity can lead to an increase in estrogen levels by increasing the expression of aromatase, the enzyme that catalyzes the final step in estrogen biosynthesis, and this has been shown in mouse models of obesity and in women (23–25). Adipocytes and adipose stromal cells (ASC) are major components of adipose tissue and ASCs have been shown to contribute to the production of estrogens. Leptin produced from adipocytes can promote ER<sup>+</sup> breast cancer growth (26, 27). However, ASCs appear to be the main site of aromatase expression in the breast (28). Furthermore, obesity is associated with an increase in COX2 expression and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production and has been shown to be a major driver of aromatase expression (24, 25, 29).

We have previously demonstrated that metabolic pathways involving AMPK are key regulators of aromatase in breast ASCs. Specifically, AMPK is phosphorylated and activated by upstream kinase LKB1. AMPK phosphorylates the CREB coactivator CRTC2 and leads to its cytoplasmic sequestration. In obesity and in cancer, PGE<sub>2</sub> stimulates CREB phosphorylation and the downregulation of LKB1/AMPK, leading to the nuclear entry of CRTC2 (Fig. 1A). CRTC2 was found to bind aromatase promoter PI.3/PII and this interaction is significantly increased in the presence of the PGE<sub>2</sub>-mimetic forskolin (FSK) and phorbol 12-myristate 13-acetate (PMA) in breast ASCs. Moreover, overexpression of CRTC2 leads to an increase in promoter PII activity and a further increase is observed in the

presence of FSK/PMA (30). This, in turn, drives the expression of aromatase. We have also recently demonstrated that HIF1 $\alpha$  with known roles in promoting tumor growth and emerging roles in regulating metabolism is stimulated by PGE<sub>2</sub> in ASCs and stimulates the expression of aromatase. Unpublished data from Sasano and colleagues showed enhanced aromatase expression and staining in tightly packed undifferentiated ASCs around tumor cells in patients with breast cancer. This process of undifferentiation and increased stromal cell proliferation is known as desmoplasia, which leads to the formation of a dense fibroblast layer surrounding malignant epithelial cells; it is essential for structural and biochemical support of tumor growth (31, 32).

#### Li-Fraumeni syndrome

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant hereditary syndrome with the majority of affected individuals carrying germ line mutations in the *TP53* gene (33). LFS is characterized by a high susceptibility of developing a number of malignancies, predominantly in childhood and early adult life. Half of individuals with LFS develop at least one LFS-associated cancer by age 30 and 15% to 35% of cancer survivors with LFS will develop multiple primary tumors over their lifetimes (34). It has also been proposed that patients with LFS have deregulated metabolism, including increased oxidative stress and hypoxia, to provide a microenvironment conducive to tumor formation, which can be one complication of dysfunctional p53 for these patients (35). Breast cancer is the most common type of cancer in women with LFS and the majority of tumors are ER<sup>+</sup> (36).

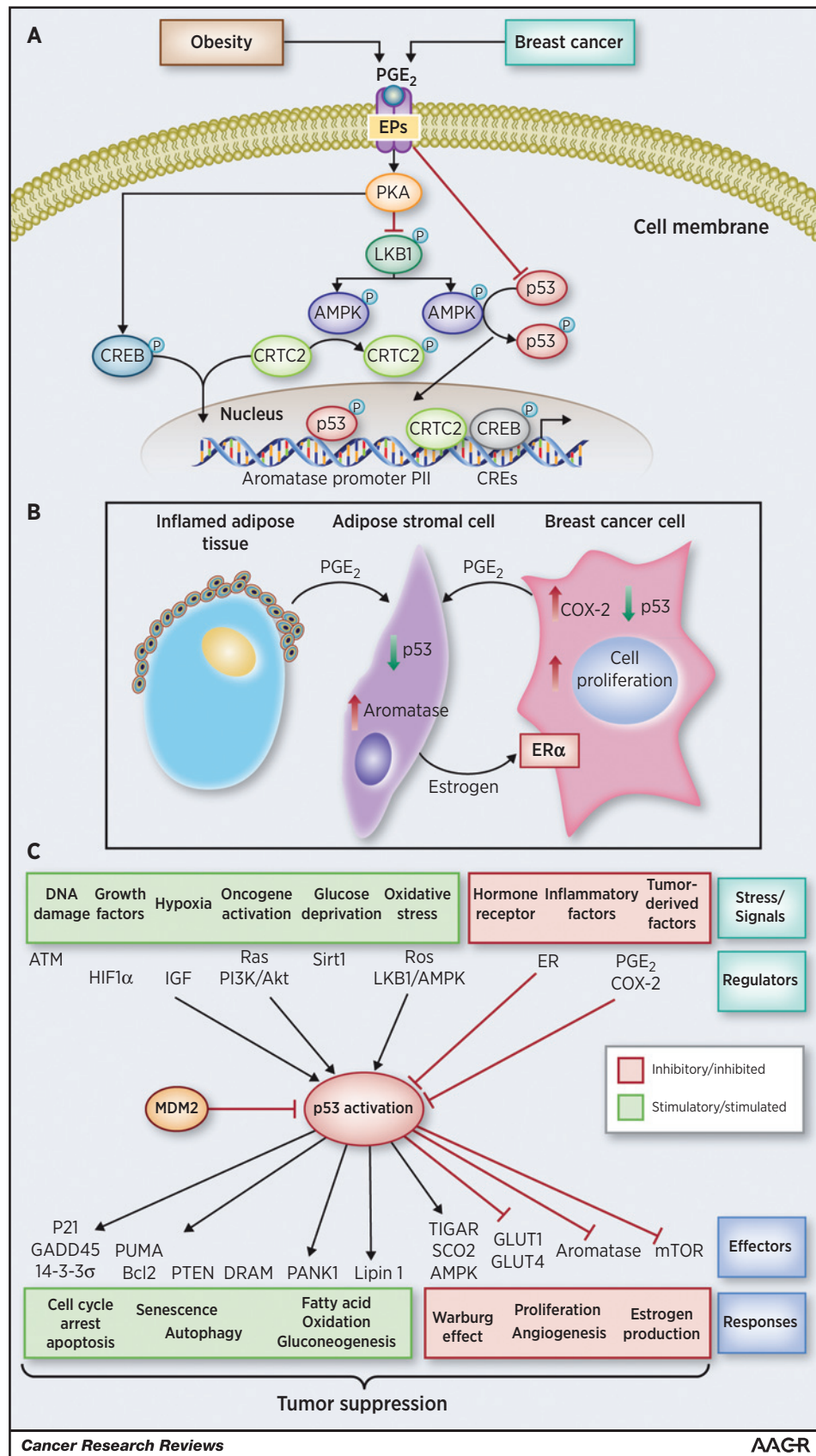
Moll and colleagues previously found that wild-type p53 can accumulate in the cytoplasm of tumor cells in inflammatory breast cancers, leading to functional inactivation of p53 (37). Interestingly, Molinari and colleagues demonstrated that estradiol caused the cytoplasmic accumulation of wild-type p53 in MCF7 breast cancer cells, leading to the G<sub>1</sub>-S transition (38). A number of posttranslational modifications have been described to explain this change in subcellular localization, including phosphorylation (Ser15, T18, S20, T81), acetylation, and monoubiquitination (39). Of interest, AMPK has been shown to phosphorylate p53 at Ser15 (40). Coupled to findings demonstrating that p53 inactivation results in increased estrogen production in mouse mammary epithelial cells (41), we hypothesized that tumor suppressor p53, a downstream target of the LKB1/AMPK pathway, has a role in the regulation of aromatase in human breast ASCs.

## Key Findings

### PGE<sub>2</sub> as a regulator of p53

Prostaglandins regulate cell migration and invasion in cancer, and high prostaglandin levels are associated with many cancers including those of the breast. PGE<sub>2</sub> is a key inflammatory mediator produced in adipose tissue in the context of obesity and breast cancer. Obesity-associated inflammatory foci in the human breast are associated with elevated levels of COX2 and PGE<sub>2</sub> (25). Meanwhile, stabilized p53 binds to its target genes in the nucleus, whereas inactivated and dysfunctional wild-type p53 are accumulated in the cytoplasm. Abnormal subcellular localization of p53 can be an indication of tumorigenic processes in tissues (13, 42, 43). We therefore examined the effect of PGE<sub>2</sub> on p53 in ASCs. Our results show decreased p53 transcript expression, nuclear protein

**Figure 1.** Established and emerging roles of p53 in preventing tumor growth. A, signaling pathways involved in regulating aromatase in response to PGE<sub>2</sub> in ASCs. B, interplay between PGE<sub>2</sub>-p53-aromatase in the breast microenvironment. C, role and regulation of p53 in breast carcinogenesis.



Downloaded from <http://aacrjournals.org/cancerres/article-pdf/75/23/5001/12724245/5001.pdf> by guest on 22 April 2024



expression, and phosphorylation at Ser15 in ASCs (44). The mechanism for transcriptional regulation of p53 remains to be determined, but AMPK is known to activate p53 through phosphorylation at Ser15, and our earlier studies have demonstrated an inhibitory effect of PGE<sub>2</sub> on AMPK, providing a potential mechanism for the decreased protein expression and activity of p53 in the presence of PGE<sub>2</sub> (13, 30). As a result of decreased phosphorylation, p53 nuclear localization is also decreased. This is consistent with findings in clinical samples of breast cancer, where we demonstrate by immunofluorescence that tumor-associated ASCs have lower nuclear p53 intensity and increased perinuclear intensity compared with normal ASCs (44). Perinuclear p53 represents inactive p53 (45). Our findings also demonstrate that PGE<sub>2</sub> causes an increase in ASC proliferation. Considering the important role of p53 in regulating cell-cycle arrest and apoptosis, it is possible that this PGE<sub>2</sub>-mediated increase in cell proliferation is mediated by the downregulation of p53.

#### p53 as a novel regulator of aromatase

Aromatase has tissue-specific promoters and promoters I.3/II are activated by PGE<sub>2</sub>, leading to an increase in aromatase expression and estrogen production in tumor-associated ASCs of postmenopausal women (46). To study the effects of p53 on aromatase expression, we used the small-molecule RITA to stabilize p53 and induce its activity in ASCs. Our study reveals that RITA-stabilized p53 inhibits the PGE<sub>2</sub>-mediated expression of PII-specific aromatase transcripts, as well as aromatase protein expression and activity. We have found that this is due to the direct interaction of p53 within the promoter PII region (Fig. 1B). However, in the presence of PGE<sub>2</sub>, binding of p53 to promoter PII is reduced. We also found a positive correlation between perinuclear p53 (inactive) fluorescence intensity and aromatase fluorescence intensity in ASCs of both tumor-free and tumor-bearing breast tissue. Consistent with this, aromatase fluorescence intensity was found to be higher in ASCs from patients with LFS than in non-LFS individuals. Our study has therefore established a novel role of p53 in regulating aromatase in the context of obesity and breast cancer.

## Implications

### Implications for stromal cell proliferation and metabolism in obesity and breast cancer

Our recent study has revealed a novel role for tumor suppressor p53 in inhibiting the PGE<sub>2</sub>-mediated expression of aromatase, thus preventing breast tumor growth without causing apoptosis or cell-cycle arrest. Under normal conditions, p53 levels are low and they help maintain cell metabolic balance. High body mass index (BMI) is associated with increased risk of postmenopausal breast cancer and with poorer outcome in those with a history of breast cancer (47). Moreover, obesity is a factor that has been associated with both increased risk and poor breast cancer prognosis due to possible local adipose inflammation and increased levels of inflammatory and protumorigenic factors (48). Aromatase inhibitors are currently first-line therapy for hormone receptor-positive postmenopausal breast cancer (49); however, some studies have suggested that aromatase inhibitors may be less effective in obese women and these women may have a poorer prognosis due to greater peripheral aromatase activity and plasma estrogen levels (50).

Obesity leads to the formation of crown-like structures (CLS), which are a histologic feature whereby infiltrating immune cells surround necrotic adipocytes, and promote adipogenesis and inflammation in white adipose tissue (24). Increased numbers of CLS are associated with reduced adipocyte differentiation, impaired adipocyte function, and higher levels of proinflammatory mediators such as TNF $\alpha$ , interleukins, and PGE<sub>2</sub> (25). As previously demonstrated, PGE<sub>2</sub> suppresses LKB1/AMPK signaling in breast ASCs (30). AMPK is a key regulator of metabolism and is known to promote p53 expression and activation. Reduced AMPK activity, in response to PGE<sub>2</sub>, results in the decreased activation of p53, potentially leading to increased proliferation of ASCs. Consistent with these findings, we have also recently shown that obesity-associated inflammatory factors such as TNF $\alpha$  and PGE<sub>2</sub> induce glucose uptake by stimulating the expression of the glucose transporters GLUT1 and GLUT3 in undifferentiated ASCs (51). Increased glucose uptake is a conserved mechanism for the energetic support of actively proliferating cells and for the Warburg effect. Taken together, our findings suggest that inflammatory factors promote metabolic alterations in adipose tissue by inhibiting p53. This would contribute to the accumulation of stromal cells (desmoplastic reaction) and be associated with an estrogen-rich tumor microenvironment supportive of cancer development and growth.

### Implications for interactions among p53, estrogen, and ER in breast cancers

In contrast to ER<sup>-</sup> breast cancers, which frequently harbor mutations in the p53 tumor suppressor, ER<sup>+</sup> breast cancers are predominantly wild-type for p53. In ER<sup>+</sup> breast cancers, the interaction between p53 and ER demonstrated the repressive effect of ER on the p53-mediated apoptotic response induced by DNA damage (6). Overexpressed aromatase results in increased estrogen biosynthesis and breast tumor growth in ER<sup>+</sup> postmenopausal breast cancers. Estrogen and ER $\alpha$  are negative regulators of p53 and are able to inactivate p53 in tumor epithelial cells. In addition, estrogen increases p53-ER $\alpha$  interactions (52). This mechanism may also be relevant to observations in Li-Fraumeni-associated breast cancers, as the majority of germ line TP53-mutated breast cancers are hormone receptor-positive (36). Interestingly, Duong and colleagues found that Mdm2 directly interacts with ER $\alpha$  in a ternary complex with p53 and is involved in the regulation of ER $\alpha$  turnover. RITA treatment caused dissociation of the p53/Mdm2/ER $\alpha$  complex, which leads to a co-stabilization of p53 and ER $\alpha$  proteins in breast cancer cells (53). However, it is disputable that ER $\alpha$  is expressed in adipose stromal cells (54, 55). The lack of ER $\alpha$  expression was observed by Knower and colleagues, whereas Booth and colleagues detected ER $\alpha$  transcript and protein expression in ASCs (55, 56). Therefore, the mechanism of p53, estrogen, and ER $\alpha$  in ASCs needs to be determined.

### Implications for the treatment of estrogen-dependent breast cancer

Because of its established role as a tumor suppressor, an enormous effort has been made to target p53 for the treatment of breast cancer. Current approaches for p53 targeting include wild-type p53 activation and restoration and mutant p53 reactivation. There are several strategies to target p53, including

inhibition of p53 degradation through disrupting the p53–HDM2 interaction, gene therapy that introduces wild-type p53 into cancers, restoration of mutant p53 to wild-type p53, elimination of mutant p53, and p53-based vaccines (reviewed in ref. 57). The conventional strategies for restoration of p53 function in tumors are aimed at protecting wild-type p53 from proteasomal degradation. Most inhibitors of p53–HDM2 interactions have extremely high binding affinities to HDM2. Non-HDM2-targeting p53 modulators have also been considered as potential therapeutics for cancers (58). For instance, p28 (NSC745104) is the first non-HDM2-mediated peptide inhibitor of p53 ubiquitination, and a clinical phase I trial has showed that p28 was tolerated with no significant adverse events, demonstrating a proof-of-concept for this new class of cancer treatment (58). Nutlin and RITA are both considered as potential therapeutics for wild-type p53 cancers. Nutlin-3 is a potent and selective inhibitor of HDM2–p53 interactions by binding to HDM2. In contrast, RITA is a p53 activator that binds to p53 thereby preventing HDM2–p53 interactions and proteasomal degradation (45). A mechanistic study revealed that RITA-stabilized p53 abrogates key oncogenic pathways such as Akt and c-Myc in cancer cells (59). Interestingly, human fibroblasts and lymphocytes were shown to be less sensitive to the proapoptotic effects of RITA than tumor cells, whereas our studies demonstrate that RITA, at these concentrations, inhibits estrogen production in breast ASCs. This suggests that p53 could be targeted to inhibit aromatase and breast cancer cell growth. These HDM2 inhibitors or p53 activators have been suggested to be only effective in wild-type p53 containing cancers. However, our results demonstrate that in breast ASCs, where p53 mutations are rare, stimulation of p53 would inhibit estrogen production and hence provide a novel strategy for the treatment of estrogen-dependent cancer. Reports have also demonstrated p53-independent effects of RITA, and although effects on cell viability were not observed in our studies, others have previously found that 1  $\mu\text{mol/L}$  RITA can cause cell death in p53-null cells (60).

Our results also demonstrate that PGE<sub>2</sub> suppresses p53 expression in ASCs. Whether this also occurs in breast cancer cells remains to be determined; however, previous studies have demonstrated that overexpression of COX2, the rate-limiting step in prostaglandin synthesis, is associated with the repression of p53 target genes in normal human mammary epithelial cells (61). Inhibition of COX2 using nonsteroidal anti-inflammatory drugs is associated with a decreased breast cancer risk and has been proposed as a means of breast-specific aromatase inhibition. Therefore, COX2 inhibitors may be useful for the treatment of estrogen-dependent breast cancers by restoring p53 expression and inhibiting estrogen production and cancer cell growth.

## References

- Vousden KH, Lu X. Live or let die: the cell's response to p53. *Nat Rev Cancer* 2002;2:594–604.
- Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000;408:307–10.
- Coutant C, Rouzier R, Qi Y, Lehmann-Che J, Bianchini G, Iwamoto T, et al. Distinct p53 gene signatures are needed to predict prognosis and response to chemotherapy in ER-positive and ER-negative breast cancers. *Clin Cancer Res* 2011;17:2591–601.
- Bouvard V, Zaitchouk T, Vacher M, Duthu A, Canivet M, Choisy-Rossi C, et al. Tissue and cell-specific expression of the p53-target genes: bax, fas, mdm2 and waf1/p21, before and following ionising irradiation in mice. *Oncogene* 2000;19:649–60.
- Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell* 2001;7:683–94.
- Bailey ST, Shin H, Westerling T, Liu XS, Brown M. Estrogen receptor prevents p53-dependent apoptosis in breast cancer. *Proc Natl Acad Sci U S A* 2012;109:18060–5.
- Rowan S, Ludwig RL, Haupt Y, Bates S, Lu X, Oren M, et al. Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. *EMBO J* 1996;15:827–38.

## Implications for p53 status and ER<sup>+</sup> breast cancer therapies

Aromatase inhibitors and antiestrogens are most common therapies for estrogen-dependent breast cancers. Aromatase inhibitors have been reported to be more effective than the antiestrogen tamoxifen in treating breast cancer. Studies have reported the effect of these therapies on p53 expression and activity. For instance, Ichikawa and colleagues found that the levels of wild-type p53 in tamoxifen-treated breast cancer cells increased in a time-dependent and a dose-dependent manner (62). Another report also demonstrated that aromatase inhibitors letrozole, anastrozole, and 4-hydroxyandrostenedione and antiestrogens tamoxifen and faslodex induced growth suppression and cell-cycle arrest that was associated with upregulation of wild-type p53 protein and mRNA levels (63).

Nearly one third of breast tumors carry mutations in the p53 gene, which are correlated with high histologic grade and rapid progression (64). Recently, studies in whole-genome analysis indicated that p53 mutations were significantly correlated with aromatase inhibitor resistance (65). p53 protein accumulation is found to be associated with aromatase inhibitors resistance, and nuclear accumulation is suggestive of mutations in the *TP53* gene. p53 has been considered as a prognostic biomarker in oncology, with p53 overexpression being associated with a shorter disease-free interval, and both early and late recurrence in ER-positive postmenopausal breast cancer patients treated with aromatase inhibitors (66, 67). Taken together, p53 status has potential prognostic value for estrogen-dependent postmenopausal breast cancer.

## Conclusions

The tumor suppressor function of p53 in breast cancer is multifaceted; it has established roles in stimulating cell-cycle arrest and apoptosis and emerging roles in the control of metabolic function. We now show that p53 is a key regulator of aromatase and estrogen production in the breast adipose tissue. Targeting p53 may therefore be a novel strategy for the treatment of estrogen-dependent breast cancer.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Grant Support

This research was supported by NHMRC (Australia) Project Grant # GNT1005735 (K.A. Brown and E.R. Simpson), by a grant from the National Breast Cancer Foundation (NC-14-011; K.A. Brown), and by the Victorian Government Operational Infrastructure Support Program. K.A. Brown is supported by an NHMRC (Australia) Career Development Award GNT1007714.

Received February 26, 2015; revised June 14, 2015; accepted July 20, 2015; published OnlineFirst November 16, 2015.

8. el-Deiry WS. Regulation of p53 downstream genes. *Semin Cancer Biol* 1998;8:345–57.
9. Vousden KH, Prives C. Blinded by the light: the growing complexity of p53. *Cell* 2009;137:413–31.
10. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993;75:805–16.
11. Stewart ZA, Pietsenpol JA. p53 Signaling and cell cycle checkpoints. *Chem Res Toxicol* 2001;14:243–63.
12. Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Res* 2004;64:2627–33.
13. Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol cell* 2005;18:283–93.
14. Assaily W, Rubinger DA, Wheaton K, Lin Y, Ma W, Xuan W, et al. ROS-mediated p53 induction of Lpin1 regulates fatty acid oxidation in response to nutritional stress. *Mol cell* 2011;44:491–501.
15. Liu Y, He Y, Jin A, Tikunov AP, Zhou L, Tollini LA, et al. Ribosomal protein-Mdm2-p53 pathway coordinates nutrient stress with lipid metabolism by regulating MCD and promoting fatty acid oxidation. *Proc Natl Acad Sci U S A* 2014;111:E2414–22.
16. Moore T, Beltran L, Carbajal S, Strom S, Traag J, Hursting SD, et al. Dietary energy balance modulates signaling through the Akt/mammalian target of rapamycin pathways in multiple epithelial tissues. *Cancer Prev Res* 2008;1:65–76.
17. Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. *Nat Rev Cancer* 2012;12:159–69.
18. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 2008;7:11–20.
19. Madan E, Gogna R, Bhatt M, Pati U, Kuppusamy P, Mahdi AA. Regulation of glucose metabolism by p53: emerging new roles for the tumor suppressor. *Oncotarget* 2011;2:948–57.
20. Wu W, Zhao S. Metabolic changes in cancer: beyond the Warburg effect. *Acta Biochim Biophys Sin* 2013;45:18–26.
21. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
22. Björntorp P. Adipose tissue distribution and function. *Int J Obes* 1991;15:67–81.
23. Baglietto L, English DR, Hopper JL, MacInnis RJ, Morris HA, Tilley WD, et al. Circulating steroid hormone concentrations in postmenopausal women in relation to body size and composition. *Breast Cancer Res Treat* 2009;115:171–9.
24. Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, et al. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. *Cancer Prev Res (Phila)* 2011;4:329–46.
25. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. *Cancer Discov* 2012;2:356–65.
26. Hursting SD, Hursting MJ. Growth signals, inflammation, and vascular perturbations: mechanistic links between obesity, metabolic syndrome, and cancer. *Arterioscler Thromb Vasc Biol* 2012;32:1766–70.
27. Liu E, Samad F, Mueller BM. Local adipocytes enable estrogen-dependent breast cancer growth: Role of leptin and aromatase. *Adipocyte* 2013;2:165–9.
28. Vachon CM, Sasano H, Ghosh K, Brandt KR, Watson DA, Reynolds C, et al. Aromatase immunoreactivity is increased in mammographically dense regions of the breast. *Breast Cancer Res Treat* 2011;125:243–52.
29. Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. *Endocrinology* 1996;137:5739–42.
30. Brown KA, McInnes KJ, Hunger NI, Oakhill JS, Steinberg GR, Simpson ER. Subcellular localization of cyclic AMP-responsive element binding protein-regulated transcription coactivator 2 provides a link between obesity and breast cancer in postmenopausal women. *Cancer Res* 2009;69:5392–9.
31. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005;121:335–48.
32. Angelucci C, Maulucci G, Lama G, Proietti G, Colabianchi A, Papi M, et al. Epithelial-stromal interactions in human breast cancer: effects on adhesion, plasma membrane fluidity and migration speed and directness. *PLoS One* 2012;7:e50804.
33. Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med* 1969;71:747–52.
34. Gonzalez KD, Noltner KA, Buzin CH, Gu D, Wen-Fong CY, Nguyen VQ, et al. Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol* 2009;27:1250–6.
35. Pantziarka P. Li Fraumeni syndrome, cancer and senescence: a new hypothesis. *Cancer Cell Int* 2013;13:35.
36. Masciari S, Dillon DA, Rath M, Robson M, Weitzel JN, Balmana J, et al. Breast cancer phenotype in women with TP53 germline mutations: a Li-Fraumeni syndrome consortium effort. *Breast Cancer Res Treat* 2012;133:1125–30.
37. Moll UM, Riou G, Levine AJ. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. *Proc Natl Acad Sci U S A* 1992;89:7262–6.
38. Molinari AM, Bontempo P, Schiavone EM, Tortora V, Verdicchio MA, Napolitano M, et al. Estradiol induces functional inactivation of p53 by intracellular redistribution. *Cancer Res* 2000;60:2594–7.
39. Gu B, Zhu WG. Surf the post-translational modification network of p53 regulation. *Int J Biol Sci* 2012;8:672–84.
40. Imamura K, Ogura T, Kishimoto A, Kaminishi M, Esumi H. Cell cycle regulation via p53 phosphorylation by a 5'-AMP activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, in a human hepatocellular carcinoma cell line. *Biochem Biophys Res Commun* 2001;287:562–7.
41. Choi HK, Roh SH, Kim HG, Han EH, Jeong HG, Kang KW. Enhanced expression of aromatase in p53-inactivated mammary epithelial cells. *Endocr Relat Cancer* 2008;15:139–47.
42. Gostissa M, Hofmann TG, Will H, Del Sal G. Regulation of p53 functions: let's meet at the nuclear bodies. *Curr Opin Cell Biol* 2003;15:351–7.
43. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science (New York, NY)* 2004;303:844–8.
44. Wang X, Docanto MM, Sasano H, Lo C, Simpson ER, Brown KA. Prostaglandin E2 inhibits p53 in human breast adipose stromal cells: a novel mechanism for the regulation of aromatase in obesity and breast cancer. *Cancer Res* 2015;75:645–55.
45. Momand J, Wu HH, Dasgupta G. MDM2—master regulator of the p53 tumor suppressor protein. *Gene* 2000;242:15–29.
46. Zhou D, Zhou C, Chen S. Gene regulation studies of aromatase expression in breast cancer and adipose stromal cells. *J Steroid Biochem Mol Biol* 1997;61:273–80.
47. James FR, Wootton S, Jackson A, Wiseman M, Copson ER, Cutress RI. Obesity in breast cancer – What is the risk factor? *Eur J Cancer* 2015;51:705–20.
48. Eichholzer M, Richard A, Rohrmann S, Schmid S, Guth U. Overweight, obesity, and breast cancer screening: results from the 2012 Swiss Health Survey. *Eur J Cancer Prev*. 2015 Mar 19. [Epub ahead of print].
49. Lu Q, Nakamura J, Savinov A, Yue W, Weisz J, Dabbs DJ, et al. Expression of aromatase protein and messenger ribonucleic acid in tumor epithelial cells and evidence of functional significance of locally produced estrogen in human breast cancers. *Endocrinology* 1996;137:3061–8.
50. Ioannides SJ, Barlow PL, Elwood JM, Porter D. Effect of obesity on aromatase inhibitor efficacy in postmenopausal, hormone receptor-positive breast cancer: a systematic review. *Breast Cancer Res Treat* 2014;147:237–48.
51. Docanto MM, Ham S, Corbould A, Brown KA. Obesity-associated inflammatory cytokines and prostaglandin E2 stimulate glucose transporter mRNA expression and glucose uptake in primary human adipose stromal cells. *J Interferon & Cytokine Res* 2015;35:600–5.
52. Berger C, Qian Y, Chen X. The p53-estrogen receptor loop in cancer. *Curr Mol Med* 2013;13:1229–40.
53. Duong V, Boulle N, Daujat S, Chauvet J, Bonnet S, Neel H, et al. Differential regulation of estrogen receptor alpha turnover and transactivation by Mdm2 and stress-inducing agents. *Cancer Res* 2007;67:5513–21.

54. Haslam SZ, Nummy KA. The ontogeny and cellular distribution of estrogen receptors in normal mouse mammary gland. *J Steroid Biochem Mol Biol* 1992;42:589-95.
55. Knower KC, Chand AL, Eriksson N, Takagi K, Miki Y, Sasano H, et al. Distinct nuclear receptor expression in stroma adjacent to breast tumors. *Breast Cancer Res Treat* 2013;142:211-23.
56. Booth ME, Hanby AM, Speirs V. Steroid hormone receptor expression in breast cancer stroma. *Breast Cancer Res Treat* 2014;143:605-7.
57. Hong B, van den Heuvel AP, Prabhu VV, Zhang S, El-Deiry WS. Targeting tumor suppressor p53 for cancer therapy: strategies, challenges and opportunities. *Curr Drug Targets* 2014;15:80-9.
58. Warso MA, Richards JM, Mehta D, Christov K, Schaeffer C, Rae Bressler L, et al. A first-in-class, first-in-human, phase I trial of p28, a non-HDM2-mediated peptide inhibitor of p53 ubiquitination in patients with advanced solid tumours. *Br J Cancer* 2013;108:1061-70.
59. Grinkevich VV, Nikulenkov F, Shi Y, Enge M, Bao W, Maljukova A, et al. Ablation of key oncogenic pathways by RITA-reactivated p53 is required for efficient apoptosis. *Cancer Cell* 2009;15:441-53.
60. Weilbacher A, Gutekunst M, Oren M, Aulitzky WE, van der Kuip H. RITA can induce cell death in p53-defective cells independently of p53 function via activation of JNK/SAPK and p38. *Cell Death & Dis* 2014;5:e1318.
61. Choi EM, Heo JI, Oh JY, Kim YM, Ha KS, Kim JI, et al. COX-2 regulates p53 activity and inhibits DNA damage-induced apoptosis. *Biochem Biophys Res Commu* 2005;328:1107-12.
62. Ichikawa A, Ando J, Suda K. G1 arrest and expression of cyclin-dependent kinase inhibitors in tamoxifen-treated MCF-7 human breast cancer cells. *Human Cell* 2008;21:28-37.
63. Thiantanawat A, Long BJ, Brodie AM. Signaling pathways of apoptosis activated by aromatase inhibitors and antiestrogens. *Cancer Res* 2003;63:8037-50.
64. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000;124:966-78.
65. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. *Nature* 2012;486:353-60.
66. Yamamoto M, Hosoda M, Nakano K, Jia S, Hatanaka KC, Takakuwa E, et al. p53 accumulation is a strong predictor of recurrence in estrogen receptor-positive breast cancer patients treated with aromatase inhibitors. *Cancer Sci* 2014;105:81-8.
67. Jia XQ, Hong Q, Cheng JY, Li JW, Wang YJ, Mo M, et al. Accumulation of p53 is prognostic for aromatase inhibitor resistance in early-stage postmenopausal patients with ER-positive breast cancer. *OncoTargets Ther* 2015;8:549-55.