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27-Hydroxycholesterol Promotes Cell-Autonomous, ER-Positive Breast Cancer Growth

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SUMMARY

To date, estrogen is the only known endogenous estrogen receptor (ER) ligand that promotes ER+ breast tumor growth. We report that the cholesterol metabolite 27-hydroxycholesterol (27HC) stimulates MCF-7 cell xenograft growth in mice. More importantly, in ER+ breast cancer patients, 27HC content in normal breast tissue is increased compared to that in cancer-free controls, and tumor 27HC content is further elevated. Increased tumor 27HC is correlated with diminished expression of CYP7B1, the 27HC metabolizing enzyme, and reduced expression of CYP7B1 in tumors is associated with poorer patient survival. Moreover, 27HC is produced by MCF-7 cells, and it stimulates cell-autonomous, ER-dependent, and GDNF-RET-dependent cell proliferation. Thus, 27HC is a locally modulated, non-aromatized ER ligand that promotes ER+ breast tumor growth.

INTRODUCTION

Breast cancer is second most common malignancy in women behind skin cancer, with 1 million new cases diagnosed worldwide each year (McPherson et al., 2000). Estrogen receptor (ER) α-induced signal transduction controls the growth of a majority of breast cancers (Jensen and Jordan, 2003), and the risk of ER+ breast cancer is greatest in postmenopausal women (Patel et al., 2007). Endocrine-based therapies against ER+ breast cancers antagonize ER function (e.g., with synthetic selective estrogen receptor modulators [SERMs] including tamoxifen) or inhibit estrogen biosynthesis (e.g., with aromatase inhibitors) (Patel et al., 2007). However, initial resistance to aromatase inhibition is frequent, with early response rates of only 20%–50%, and there is also acquired resistance. As such, there may be important estrogen-independent, ER-mediated processes promoting ER+ tumor growth that are unhindered by aromatase inhibition (Chen et al., 2006).

We previously identified the cholesterol metabolite 27-hydroxycholesterol (27HC) as an endogenous SERM (DuSell et al., 2008; Umetani et al., 2007). In the present work, we determined how 27HC impacts ER+ breast cancer in vivo in mice and ER+ breast cancer risk in women. In addition, we addressed the following questions: (1) What in vivo mechanisms govern 27HC levels in breast tumors? (2) What are the roles of sterol 27-hydroxylase (CYP27A1) and oxysterol 7α-hydroxylase (CYP7B1), which synthesize and metabolize 27HC, respectively (Russell, 2003)? and (3) How does 27HC stimulate ER+ breast cancer cell growth?

RESULTS

27HC Promotes ER+ Breast Tumor Growth

The capacity of 27HC to stimulate ER+ breast cancer cell proliferation was evaluated in MCF-7 cells by quantifying bromodeoxyuridine (BrdU) or ^{3}H-thymidine incorporation. With an effect comparable to E_2, 27HC promoted MCF-7 cell growth (Figure 1A). In healthy humans, plasma 27HC concentration is 0.22–0.60 μM and 50%–90% of 27HC is esterified (Dzeletovic et al., 1995; Li-Hawkins et al., 2000; Umetani et al., 2007); thus, unesterified plasma levels approximate 10^{-8}M, and 10^{-8}M was the threshold concentration for activation of MCF-7 cell proliferation (Figure 1B). The impact of other oxysterols was also evaluated (Figure S1), and MCF-7 cell proliferation was modestly stimulated by 25-hydroxycholesterol, which alters ER function but not as potently as 27HC and was previously shown to activate ERα-mediated signaling in cancer cells (Umetani et al., 2007; Lappano et al., 2011). 22R-hydroxycholesterol, which inhibits E_2 activation of either ERα or ERβ, and

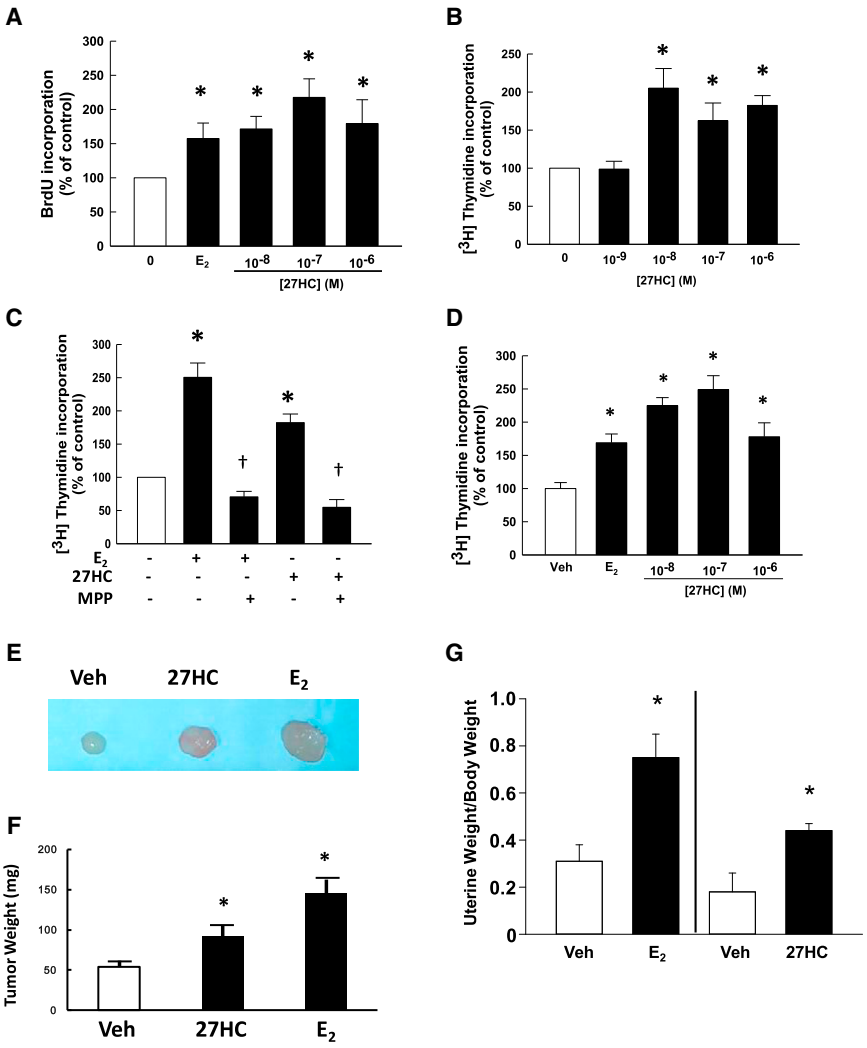


Figure 1. 27HC Promotes MCF-7 Cell and Ishikawa Cell Proliferation, and In Vivo 27HC Stimulates MCF-7 Cell Xenograft Growth and an Uterotrophic Response

(A–D) Cell proliferation was evaluated by quantifying BrdU (A) or ^{3}H-thymidine incorporation (B–D), n = 4–8. (A) Growth responses of MCF-7 cells to E_2 (10^{-8}M) or 27HC treatment (10^{-8} to 10^{-6}M) for 24 hr were compared. (B) The dose response of MCF-7 cells to 27HC (10^{-9} to 10^{-6}M, for 24 hr) was determined. (C) The requirement for ERα in the growth response of MCF-7 cells to E_2 (10^{-8}M) or 27HC (10^{-6}M) was evaluated in cells treated with methyl-piperidino-pyrazole (MPP, 10 μM) for 24 hr. (D) Growth responses of Ishikawa cells to E_2 (10^{-8}M) or 27HC treatment (10^{-8} to 10^{-6}M) for 24 hr were compared.

(E) MCF-7 cell xenograft growth was compared in SCID mice treated with vehicle, 27HC, or E_2 for 28 days. Representative tumors are shown.

(F) Tumor weights at end of study (n = 9–10).

(G) Uterine weight/body weight ratio in mice treated for 28 days with vehicle, E_2, or 27HC (n = 6).

*p < 0.05 versus vehicle, †p < 0.05 versus no MPP. All values shown are mean ± SEM. See also Figures S1, S2, and S3.

7-ketocholesterol, which does not bind to ER (Umetani et al., 2007), did not promote MCF-7 cell proliferation. 27HC also stimulated proliferation in three other ER+ breast cancer cell lines, HCC1428, T47D, and ZR75, indicating that the response is not unique to MCF-7 cells (Figure S2). MCF-7 cells express both ERα and liver X receptors (LXR) (DuSell et al., 2008; El Roz et al., 2012), and 27HC is a ligand for both receptors (Janowski et al., 1999; Umetani et al., 2007). To evaluate whether LXR activation stimulates MCF-7 cell growth, the impact of the LXR agonist T1317 was determined. In contrast to 27HC (Figures 1A and 1B), the LXR agonist T1317 caused a decline in MCF-7 cell proliferation (Figure S3). This finding mirrors prior observations that, whereas ER activation stimulates ERα+ breast cancer cell growth, LXR activation is inhibitory (El Roz et al., 2012; Vedin et al., 2009). A requirement for ERα in 27HC action on MCF-7 cells was then demonstrated by the finding that both E_2- and 27HC-induced cell proliferation was prevented by the selective ERα antagonist methyl-piperidino-pyrazole (MPP, 10 μM) (Figure 1C) (Sun et al., 2002). These results expand upon our prior work on 27HC and MCF-7 cell proliferation, which did not reveal the operative receptor or growth-related responses to less than 10^{-6} M 27HC (DuSell et al., 2008); it is now apparent that at physiologic levels the oxysterol stimulates MCF-7 cell growth via ERα.

In addition to MCF-7 and other ER+ breast cancer cell lines, studies were performed with the human endometrial Ishikawa cell line that provides a cell-culture model of endometrial cancer (Vollmer, 2003). With an effect equal to that of E_2, 27HC stimulated Ishikawa cell growth (Figure 1D). Thus, the proliferative response to 27HC is not unique to breast cancer, and the growth of another sex-steroid-responsive malignancy is also enhanced by the oxysterol.

To determine how 27HC impacts ER+ breast tumor growth in vivo, MCF-7 cell xenografts were studied in ovariectomized female SCID mice. Following tumor establishment with E_2, mice received vehicle, 27HC, or E_2 for 4 weeks, and tumors were harvested (Figure 1E). Tumor weight was increased 73% by 27HC and 173% by E_2 (Figure 1F). Thus, 27HC stimulates ER+ breast tumor growth in vivo.

To evaluate an additional estrogen-sensitive tissue, the effects of 27HC on the uterus were determined in ovariectomized mice (Figure 1G). E_2 and 27HC caused equivalent 2.4- and 2.6-fold increases in uterine weight, providing further evidence that 27HC is a growth-promoting ER ligand in vivo.

27HC Is Abundant in Normal Breast Tissue and in ER+ Tumors from Cancer Patients

The potential impact of 27HC on ER+ breast cancer in women was then investigated. Women with ERα(+) breast cancer and

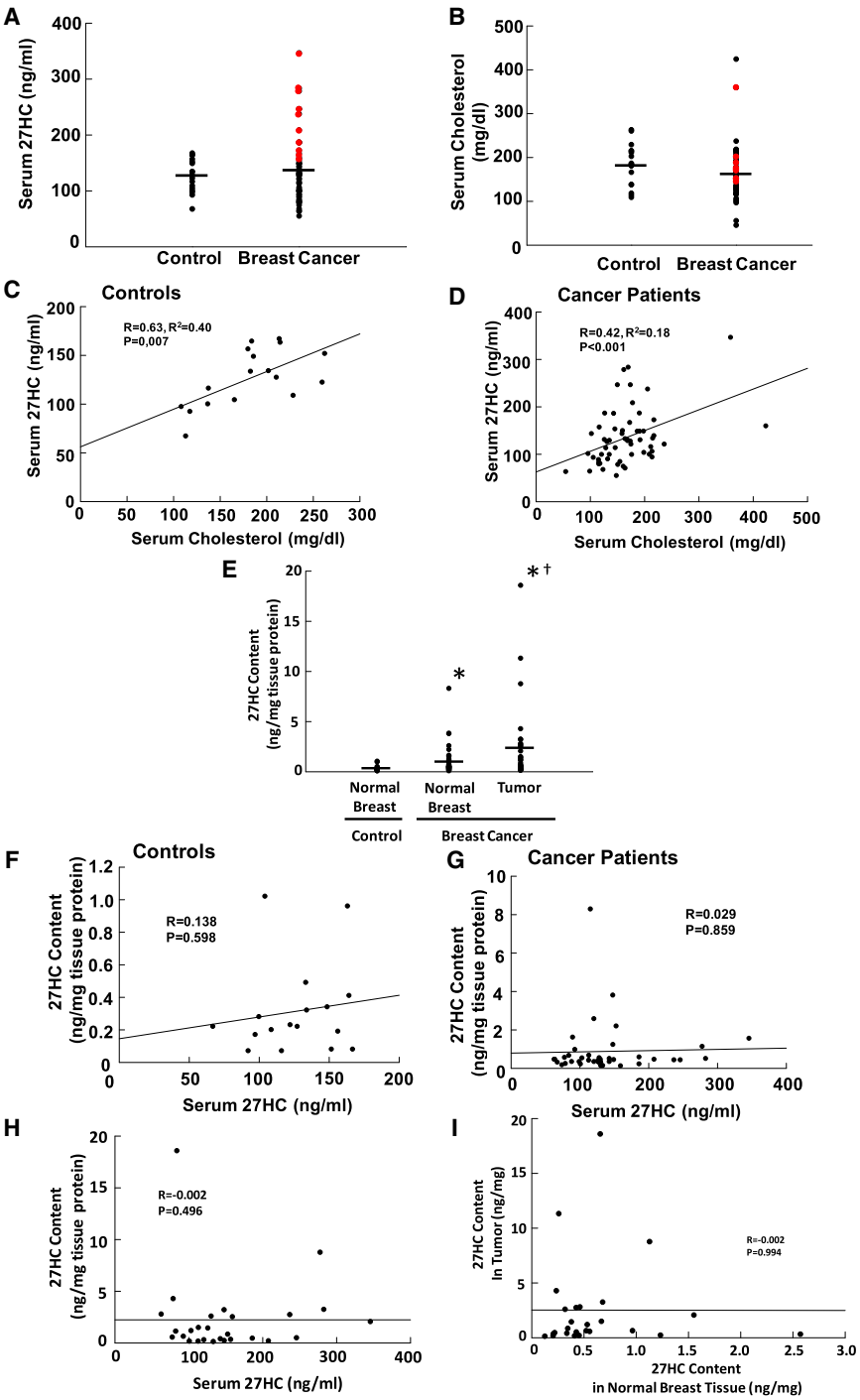


Figure 2. 27HC Content Is Increased in Normal Breast Tissue and Tumors from ER+ Breast Cancer Patients, and It Is Locally Modulated

(A and B) Serum 27HC (A) and total cholesterol concentration (B) in control and breast cancer patients (n = 17 and 58, respectively). Values for ten cancer patients with serum 27HC greater than 2SD above the mean value for controls are shown in red.

(C and D) Relationship of serum 27HC to serum cholesterol concentration in controls (C, n = 17) and cancer patients (D, n = 58).

(E) 27HC content in normal breast tissue from controls (n = 17) and cancer patients (n = 48) and in tumors (n = 32). *p < 0.05 versus control, †p < 0.05 versus cancer patient normal breast.

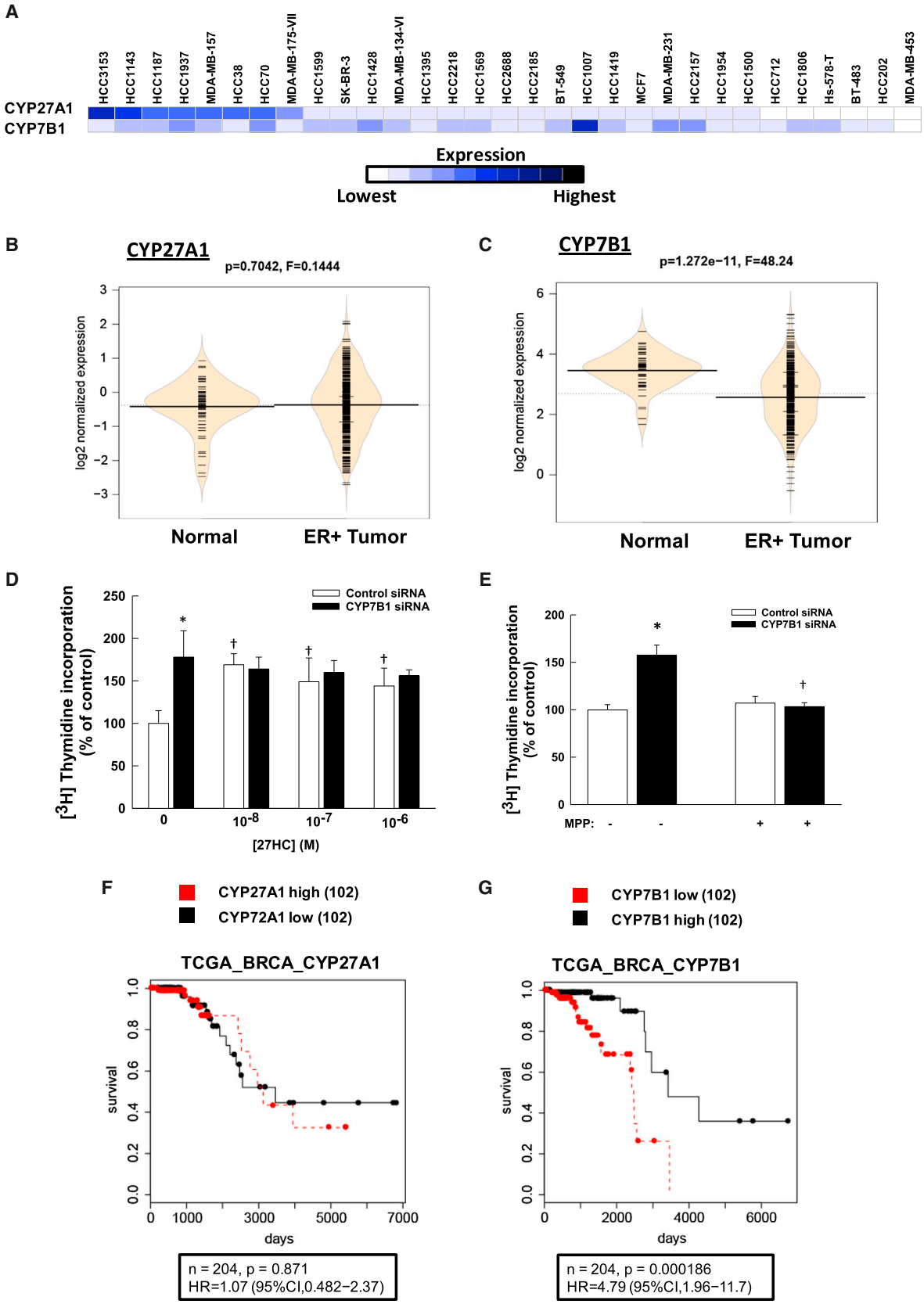
(F and G) Relationship of normal breast 27HC content to serum 27HC in controls (F, n = 17) and cancer patients (G, n = 40).

(H and I) Relationship of tumor 27HC content to serum 27HC (H) or normal breast 27HC content (I) in cancer patients (n = 27).

See also Figure S4.

age- and race-matched cancer-free subjects were recruited from the UT Southwestern Center for Breast Care (n = 66 and 18, respectively). Clinical parameters for the two groups are shown in Table S1. At presentation, mean serum 27HC levels were comparable in the two groups (Figure 2A). Interestingly, there was a broader range of serum 27HC in cancer patients, with ten subjects having levels greater than 2SD above the mean for controls (red values in Figures 2A and 2B). Mean total serum cholesterol levels were also similar in the two study groups (Figure 2B). Notably, of the ten cancer patients with elevated circulating 27HC compared to controls, only one had hypercholesterolemia. Because 27HC is a cholesterol metabolite transported in the same lipoprotein particles as cholesterol, there was a predictable positive association between serum 27HC and cholesterol in both controls and cancer patients (Figures 2C and 2D). However, whereas the amount of variability in serum 27HC that was related to cholesterol in controls was 40%, it was only 18% in cancer patients. These findings suggest that processes governing 27HC synthesis from cholesterol or 27HC metabolism may be altered in a subset of women with ER+ breast cancer.

27HC was then quantified in normal breast tissue and in tumors (Figure 2E). There were insufficient amounts of samples to also measure estrogen content. Compared to controls, there was 3-fold greater 27HC in normal breast tissue from cancer patients, and tumor content was further elevated by 2.3-fold. Possible relationships between breast tissue 27HC content and either serum 27HC (Figures 2F and 2G) or serum cholesterol were assessed (Figures S4A and S4B), and none was found. In addition, in cancer patients no relationships were observed between tumor 27HC content and either serum 27HC or normal breast tissue 27HC (Figures 2H



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and 2I). These findings indicate that 27HC abundance in the breast and in ER+ tumors is not governed by circulating levels of the oxysterol or its substrate, but instead by local mechanisms.

CYP7B1 Expression Is Diminished in ER+ Tumors and Predictive of Overall Survival

To determine if breast cancer cells possess the molecular machinery required to regulate 27HC abundance, CYP27A1 and CYP7B1 expression were evaluated in 31 breast cancer cell lines (Figure 3A). CYP27A1 and CYP7B1 transcripts were detected in the majority of the cell lines, at varying levels. Having determined that neither circulating concentrations of 27HC nor its precursor influence 27HC levels in ER+ breast tumors (Figure 2), we next compared CYP27A1 and CYP7B1 expression in 406 ER+ tumors versus 63 normal breast tissue samples in The Cancer Genome Atlas (TCGA) (2012). CYP27A1 expression was similar in normal breast and ER+ tumors (Figure 3B). In contrast, CYP7B1 expression was decreased by 50% in ER+ tumors compared with normal breast tissue (Figure 3C). As such, the elevation in 27HC found in ER+ tumors is not related to an increase in the synthesizing enzyme CYP27A1 but instead to a loss of 27HC metabolism by CYP7B1.

We next determined how a decline in the ability to metabolize 27HC impacts ER+ breast cancer cell growth (Figure 3D). In the absence of exogenous 27HC, small interfering RNA (siRNA) knockdown of CYP7B1 caused a 78% increase in MCF-7 cell proliferation. Furthermore, whereas 27HC treatment of control cells stimulated growth to levels observed with CYP7B1 deletion, there was no additional proliferation with 27HC in cells deficient in CYP7B1. These findings indicate that ER+ breast cancer cells synthesize 27HC, which promotes replication in a cell-autonomous manner, and that such replication is enhanced if CYP7B1 is deficient. Further evidence of 27HC-induced cell-autonomous growth is provided by the finding that the increase in MCF-7 cell proliferation with CYP7B1 knockdown is prevented by ERα antagonism with MPP (Figure 3E). In intact tumors, stromal cells may be an additional local source of 27HC, because macrophages express the 27HC-synthesizing enzyme CYP27A1 and generate 27HC (Hansson et al., 2003).

Having found that relative CYP7B1 expression influences ER+ breast cancer cell replication, how tumor CYP7B1 expression impacts overall patient outcome was queried using the TCGA data set. Kaplan-Meier curves were generated for cancer patients in the lowest versus highest quartiles for tumor CYP7B1 or CYP27A1 expression. Whereas overall survival curves were similar for patients with low versus high tumor CYP27A1 abundance (Figure 3F), survival was markedly poorer for patients with low versus high tumor CYP7B1 expression (Figure 3G). Multivariate Cox regression modeling further revealed that low CYP7B1 expression continues to be associated with poor overall survival outcome (HR = 7.28 and p = 0.00178) even after adjusting for the effects of age, tumor size, nodal status, and perioperative therapy (Table S2). Thus, the prognosis for ER+ breast cancer is predicted by processes in the tumor that govern 27HC metabolism.

27HC Target Genes Promote ER+ Breast Cancer Growth

Now knowing that diminished CYP7B1 expression enhances ER+ breast cancer cell proliferation, and that in parallel it adversely impacts clinical outcome, potentially operative target genes of 27HC were identified by comparing gene expression in CYP7B1-low versus CYP7B1-high tumors in TCGA. Using a false discovery rate of <0.001, 3,233 genes were downregulated in CYP7B1-low versus CYP7B1-high tumors, and 1,026 genes were upregulated (Figure 4A). Considering only probes with high expression level variance (fold change >2 or <1/2), there were 569 downregulated genes and 14 upregulated genes in CYP7B1-low versus CYP7B1-high tumors (Figure 4B). Gene set enrichment analysis further revealed that genes associated with breast cancer and other malignancies are highly enriched in CYP7B1-low breast tumors (Table S3).

The 14 upregulated genes in CYP7B1-low versus CYP7B1-high tumors (Table S4), which are therefore upregulated under conditions in which 27HC is elevated, include genes implicated in ER-driven cancers and ERα itself. 27HC modulation of four of these genes was tested in MCF-7 cells. C6orf211 is an open reading frame immediately upstream of the ERα gene whose silencing in MCF-7 blunts proliferation (Dunbier et al., 2011). PARD6B (par-6 partitioning defective 6 homolog-β) is a SRC-3 and ER target gene implicated in cell transformation (Labhart et al., 2005; Qiu et al., 2000). The receptor tyrosine kinase RET and its coreceptor GFRα1 (GDNF family α-receptor-1) are upregulated in a subset of ER+ breast cancers, and GDNF-RET signaling is a major determinant of the response to and development of resistance to aromatase inhibition (Morandi et al., 2011). Mirroring E_2 action, 27HC caused 1.9- to 2.3-fold increases in C6orf211, PARD6B, GFRα1, and RET expression (Figures 4C–4F). The impact of 27HC on GDNF expression was also determined, and it increased expression of the ligand by 2.4-fold (Figure 4G). In contrast to 27HC and consistent with a lack of stimulation of MCF-7 cell proliferation, LXR activation by T1317 did not upregulate GFRα1 or RET expression in MCF-7 cells, and GDNF expression was increased by only

Figure 3. Breast Cancer Cells Express CYP27A1 and CYP7B1 and Display Cell-Autonomous 27HC-Driven, ERα-Dependent Growth, and CYP7B1 Expression Is Diminished in ER+ Tumors and Predictive of Overall Survival

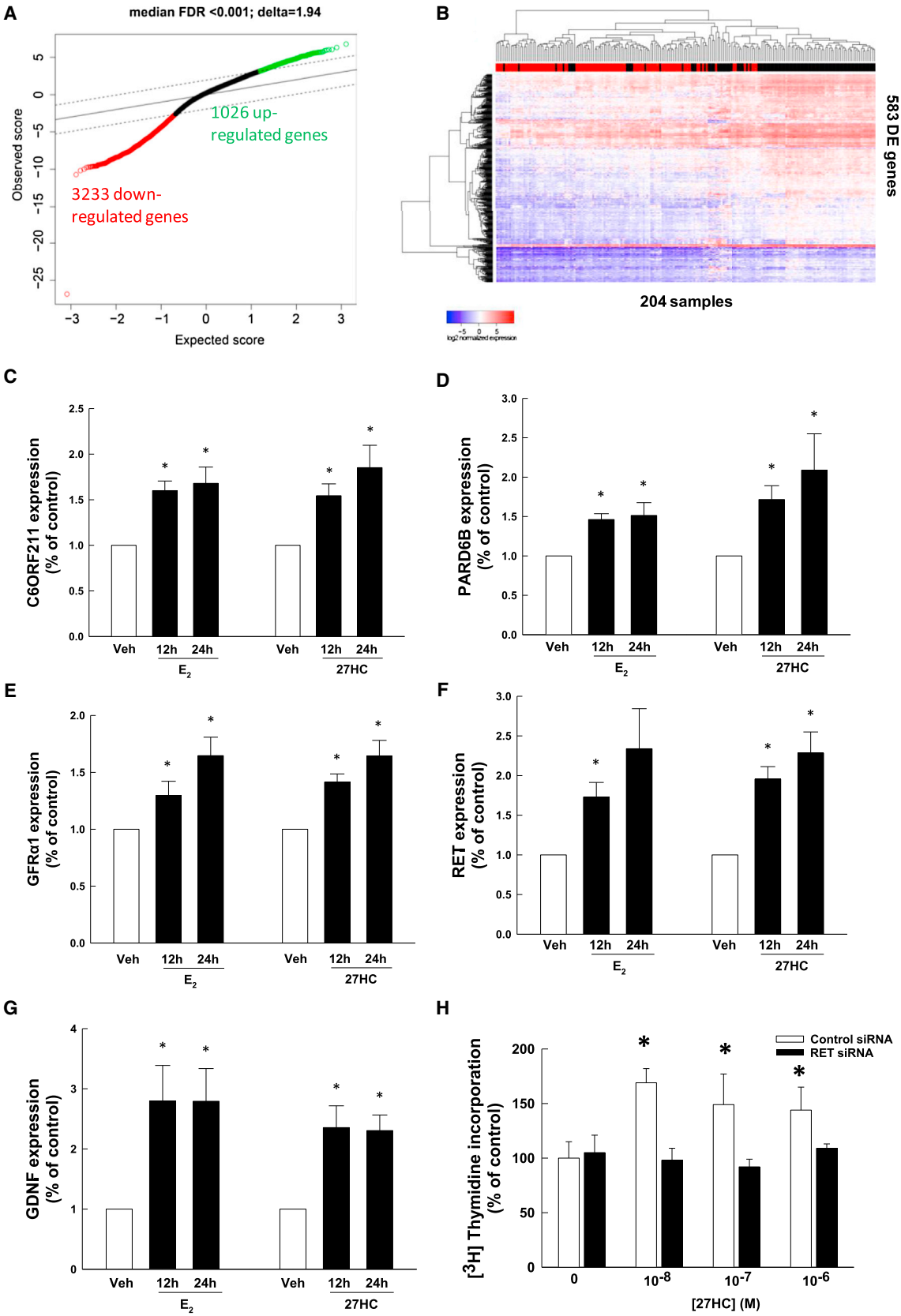
(A) CYP27A1 and CYP7B1 transcript abundance was evaluated in 31 breast cancer cell lines. Heatmap shows absolute expression, and low versus high expression is defined relative to the median expression level of all genes on the array.

(B and C) Expression of CYP27A1 and CYP7B1 were compared in 63 normal breast samples and 406 ER+ breast tumors in The Cancer Genome Atlas (TCGA). In the bean plots shown, each short black line is a sample, the long black line is the mean, and the shape of the bean reveals the distribution of the samples.

(D and E) Cell proliferation was compared in control siRNA-transfected MCF-7 cells and in cells transfected with siRNA targeting CYP7B1, in the absence or presence of 27HC (10^{-8} to 10^{-6}M) for 24 hr (D), or in the absence or presence of MPP (10 μM) for 24 hr (E). In (D) and (E), values are mean ± SEM, n = 4, *p < 0.05 versus control siRNA, †p < 0.05 versus no 27HC or no MPP.

(F and G) Kaplan-Meier curves for TCGA ER+ breast cancer patients in the highest versus lowest quartiles for tumor CYP27A1 (F) or CYP7B1 (G).

See also Figure S6A.



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20% (Figures S5A–S5C). Participation of GDNF-RET signaling in 27HC-induced cell proliferation was then evaluated by silencing RET (Figure 4H). Whereas 27HC stimulated proliferation in control cells, it had no impact on RET-depleted cells. Thus, numerous genes are modulated by 27HC in ER+ breast cancer, these include components of GDNF-RET signaling, and the latter are critically involved in 27HC-induced cancer cell proliferation.

DISCUSSION

To date estrogen has been the only known endogenous ER ligand that promotes ER+ breast tumor growth. Because resistance to aromatase inhibition is common and often evident at treatment initiation (Chen et al., 2006), other ER-mediated mechanisms may be operative in disease pathogenesis. We have discovered that the cholesterol metabolite 27HC stimulates MCF-7 cell xenograft growth in mice. In parallel, we have shown that, in ER+ breast cancer patients, 27HC content in normal breast tissue is increased compared to cancer-free controls, and that tumor 27HC abundance is further elevated. We have also determined in women that neither normal breast nor tumor 27HC content are influenced by circulating levels of 27HC or its precursor, and that increases in tumor 27HC are instead related to diminished expression of the 27HC metabolizing enzyme CYP7B1. These collective findings reveal that 27HC is a locally modulated, nonaromatized ER ligand that promotes ER+ breast cancer growth.

There are three clinical conditions in which 27HC abundance is elevated. The first is during the postmenopausal period. Serum 27HC levels increase in women after menopause (Burkard et al., 2007), and this may be related to estrogen deprivation because in mice E_2 upregulates hepatic CYP7B1 expression in an ERα-dependent manner without impacting CYP27A1 (Yamamoto et al., 2006), and it lowers serum 27HC (T.I. and M.U., unpublished data). 27HC abundance is also predictably elevated in the setting of hypercholesterolemia (Brown and Jessup, 1999; Umetani et al., 2007), and with obesity, which is frequently a comorbidity with hypercholesterolemia (Lerman et al., 2005; Reaven, 2005). In mice, a high-fat, high-cholesterol western diet elevates serum 27HC by 2- to 3-fold (T.I. and M.U., unpublished data). In women, both dyslipidemia and obesity raise breast cancer risk and severity, with obesity particularly having an adverse impact in postmenopausal women (Bianchini et al., 2002; Calle et al., 2003; Furberg et al., 2004; Michalaki et al., 2005), and breast cancer promotion by hypercholesterolemia or obesity has been demonstrated in animal models (Llaverias et al., 2011; Nunez et al., 2008). Notably, epidemiologic studies indicate that in women obesity affects only ER+ breast cancers (Althuis et al., 2004), and similarly in mice only ER+ breast cancer models display greater tumor growth with obesity (Cleary et al., 2010; Gu et al., 2011; Nunez et al., 2008). Although the basis by which menopause, hypercholesterolemia and obesity promote ER+ breast cancer is multifaceted (Cleary et al., 2010; Gu et al., 2011), 27HC should now be considered as a potentially critical pathogenetic factor.

Our discovery of 27HC as the second endogenous ER ligand that promotes ER+ breast tumor growth has important clinical implications. Because estrogen upregulates CYP7B1 (Tang et al., 2008; Yamamoto et al., 2006) and lowers 27HC as noted above, either tamoxifen treatment or aromatase inhibition may increase the abundance of the second oncogenic ER ligand. In addition, 27HC is a nonaromatized molecule whose synthesis is unaffected by aromatase inhibition. As such, assessments of tumor CYP7B1 expression or 27HC content may provide a potentially critical means to personalize hormone-based therapy. Furthermore, lowering 27HC abundance may represent a key strategy for combating a subset of ER+ breast cancers. Just as important is the finding that low tumor CYP7B1 is associated with poorer ER+ breast cancer patient survival, such that the evaluation of tumor CYP7B1 or 27HC abundance may also have prognostic value.

Now knowing that CYP7B1 influences ER+ breast cancer pathogenesis and prognosis, studies of potential somatic mutations of the gene are warranted. At the same time, because endometrial cancer cell growth is also promoted by 27HC, the oxysterol and its regulatory enzymes deserve interrogation in other steroid hormone-responsive cancers. Through such efforts the cancer-promoting capacity of 27HC will be better understood, and this may lead to further optimization of the prevention and treatment of ER-dependent cancers.

EXPERIMENTAL PROCEDURES

Detailed experimental information is provided in the Supplemental Experimental Procedures.

Cell Culture, Gene Silencing, and Assessments of Gene Expression

Studies were performed in MCF-7, HCC1428, T47D, ZR75-1, and Ishikawa cells. In MCF-7, CYP7B1 or RET were deleted using siRNA-based strategies. Potential 27HC or LXR target genes were evaluated by quantitative RT-PCR. In all cell-culture studies, findings were confirmed in three independent experiments.

Cell Proliferation

Cell proliferation was assessed by quantifying ^{3}H-thymidine or BrdU incorporation. The role of ERα in cell proliferation was queried using the highly selective antagonist MPP (Sun et al., 2002).

Orthotopic Breast Tumor Xenograft Model

Experiments approved by the Institutional Animal Care and Use Committee at UT Southwestern were performed in female SCID mice as previously

Figure 4. 27HC Modulates Gene Expression in ER+ Breast Cancer and Thereby Promotes Cancer Cell Growth

(A and B) Gene expression was compared in TCGA ER+ breast tumors in the highest versus lowest quartiles for tumor CYP7B1 expression (n = 102/group). (A) Using SAMR analysis and a false discovery rate of <0.001, 3,233 genes were downregulated in CYP7B1-low versus CYP7B1-high tumors, and 1,026 genes were upregulated. (B) Heatmap of the 583 differentially expressed (DE) genes with high expression level variance between the two CYP7B1 expression groups. CYP7B1 low and high groups are noted above the heatmap in black and red, respectively.

(C–G) Effect of E_2 (10^{-9}M) versus 27HC (10^{-6}M) on MCF-7 cell expression of C6orf211 (C), PARD6B (D), GFRα1 (E), RET (F), and GDNF (G).

(H) The effect of 27HC (10^{-8} to 10^{-6}M for 24 hr) on MCF-7 cell proliferation was compared in cells transfected with control siRNA versus siRNA targeting RET.

In (C)–(H), values shown are mean ± SEM, n = 8, *p < 0.05 versus vehicle. See also Figures S5A–S5C and S6B.

described (Chambliss et al., 2010). Following MCF-7 xenograft establishment with E_2, mice received every-other-day injections of vehicle, 27HC (100 μg), or E_2 (6 μg) for 28 days, and tumors were harvested and weighed.

Control and Breast Cancer Patient Enrollment

Human benign breast tissue and primary breast tumors were obtained from the UT Southwestern Tissue Repository under institutional-review-board-approved studies.

27HC and Cholesterol Quantification

27HC was quantified in serum and tissue samples using high-pressure liquid chromatography-mass spectrometry (McDonald et al., 2012), and total serum cholesterol was measured as previously described (Zhang et al., 2012).

Breast Cancer Cell Line Gene Expression

CYP27A1 and CYP7B1 expression were evaluated in breast cancer cell lines using Affymetrix GeneChips (http://www.affymetrix.com).

Tumor Gene Expression and Prognosis

Using The Cancer Genome Atlas (TCGA) (http://www.cancergenome.nih.gov), expression levels of CYP27A1, and CYP7B1 were compared in normal breast samples and ER+ breast tumors. In tumor samples, differences in gene expression and in overall patient outcome were then compared in the highest versus lowest CYP27A1 or CYP7B1 expression quartiles.

Statistical Analysis

Comparisons were made between multiple groups by analysis of variance (ANOVA) with Neuman-Keuls post hoc testing. When indicated, nonparametric ANOVA (Kruskal-Wallace) and post hoc Dunn testing was performed. Significance was defined as $p < 0.05$. All values shown are mean ± SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.10.006.

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