Abstract
Estrogens directly promote the growth of breast cancers that express the estrogen receptor α (ERα). However, the contribution of stromal expression of ERα in the tumor microenvironment to the protumoral effects of estrogen has never been explored. In this study, we evaluated the molecular and cellular mechanisms by which 17β-estradiol (E2) impacts the microenvironment and modulates tumor development of ERα-negative tumors. Using different mouse models of ER-negative cancer cells grafted subcutaneously into syngeneic ovariectomized immunocompetent mice, we found that E2 potentiates tumor growth, increases intratumoral vessel density, and modifies tumor vasculature into a more regularly organized structure, thereby improving vessel stabilization to prevent tumor hypoxia and necrosis. These E2-induced effects were completely abrogated in Erα-deficient mice, showing a critical role of host ERα. Notably, E2 did not accelerate tumor growth when ERα was deficient in Tie2-positive cells, even in mice grafted with wild-type bone marrow. These results were extended by clinical evidence of ERα-positive stromal cell labeling in the microenvironment of human breast cancers. Together, our findings therefore show that E2 promotes the growth of ERα-negative cancer cells through the activation of stromal Erα (extra-hematopoietic Tie-2 positive cells), which normalizes tumor angiogenesis and allows an adaptation of blood supply to tumors, thereby preventing hypoxia and necrosis. These findings significantly deepen mechanistic insights into the impact of E2 on tumor development with potential consequences for cancer treatment. Cancer Res; 72(12); 1–10. ©2012 AACR.

Introduction
Estrogen receptor α (ERα) holds a key position for diagnosis and treatment of breast cancers. Indeed, its expression by breast cancer cells dictates the use of endocrine therapies, such as tamoxifen and aromatase inhibitors, that blocks estrogen activity (1). It is consistent with the fact that estrogen could directly promote the growth of these tumors classified as ERα positive. However, evidence of an increasing number of data argues that tumor stromal microenvironment contributes to malignant development and progression of various cancers (2). ERα is expressed by a large number of tissues and mediates a vast range of the biologic effects of estrogen on the reproduction as well as in other physiologic functions (3–8). Nevertheless, the contribution of tumor microenvironment and particularly of stromal ERα to the protumoral effect of estrogen remains an open question.

Interestingly, various clinical and experimental data support this putative implication. Indeed, breast tumors are classified as ERα-positive even when only 1% of breast cancer cells express ERα, questioning the mechanisms accounting for the efficacy of targeting a so rare cell population. In addition, ovariectomy seems to be efficient to decrease long-term recurrence risk and mortality of breast cancer classified as ERα-positive and also as ERα-negative (9), and particularly of BRCA1 tumors that generally do not express ERα (10, 11). These data point out that, beside cancer cell–associated ERα, additional E-dependent mechanisms interfere with the efficacy of endocrine therapy. This is supported by the fact that tamoxifen response rates were low but still can be found in ER-negative/progesterone receptor–negative tumors (<10%; ref. 12). However, these ERα-negative breast tumors and particularly triple-negative breast cancer are no longer treated with endocrine therapy and exhibit poor prognosis (13). In immunodeficient animal models, pregnancy or 17β-estradiol (E2) supplementation was found to accelerate the growth of human ER-negative breast cancer cells (14, 15). E2 has been shown to impact angiogenesis (4, 14); however, there is still a paucity of information with regard to the specific molecular mechanisms by which E2 could impact tumor microenvironment and angiogenesis. Particularly, the
contribution of host ERα and ERβ remains poorly documented (16, 17). To improve the management of cancer patients and particularly of women, it is mandatory to get mechanistic information with regard to the various ways by which E2 could impact tumor development and progression. This is of particular interest knowing that estrogen exert multiple functions in cells, depending on targeted cell type and organ (6, 18).

The aim of this work is to characterize which ER expressed by the tumor microenvironment and which ER-expressing cells are important for tumor development. Because the innate and adaptive immunity make crucial contributions to tumor development and to the antitumor effects of conventional radiation and chemotherapeutics, we used immunocompetent animals (19, 20).

In models of ERα-negative tumor cell lines injected to syngeneic immunocompetent mice, we report here that E2 induces tumor growth through an increase and improvement of angiogenesis by triggering stromal cells via Tie2-dependent ERα.

**Materials and Methods**

**Cell culture**

Mouse B16K1 (MHC class I–positive B16F10) melanoma cell line was used as previously described (21). Mouse Lewis lung carcinoma cell line (LL2, #CRL-2051), mouse breast tumor cell line 4T1 (#CRL-2539), and mouse endothelial cell line derived by SV40 (#CRL-2181) were purchased and authenticated from American Type Culture Collection, routinely cultured as recommended by manufacturer and used from passages 3 to 8. B16K1, LL2, and 4T1 were last authenticated in February 2012 by Leibniz-Institut DSMZ GmbH.

**Animals**

Female C57BL/6j and Balb/C mice (4 weeks old) were obtained from Charles River Laboratories. ERα–/–, ERβ–/– mice, Tie2-Cre+/–/ERα-flox (Cre+/–) mice, and their control wild-type (WT) littermates ERα+/–, ERβ+/–, and Tie2-Cre–/–/ERα-flox (lox+/–) mice were generated as described previously on C57BL/6j background (22, 23).

**In vivo tumor models**

Four-week-old female mice were ovariectomized (OVX) to prevent endogenous estrogen production. Two weeks before cancer cell injection, mice were implanted subcutaneously with a pellet releasing E2 (Innovative Research of America) or were sham operated (untreated control group). B16K1, LL2, or 4T1 (4 × 10^5 cells suspended in PBS) were injected subcutaneously to both flanks of WT or transgenic C57BL/6j mice (B16K1, LL2) or of Balb/C (4T1) mice.

**Harvesting of tumors**

Before sacrifice, mice were anesthetized and perfused by intravenous injection of fluorescein isothiocyanate (FITC)–conjugated lectin (Vector) that was allowed to circulate for 5 minutes. The tumor vasculature was then fixed by intracardiac perfusion of 4% PFA and then embedded in paraffin or OCT.
E2 promotes ER-negative tumor growth in vivo and increases angiogenesis. In vivo growth curve of B16K1 (A), LL2 (B), and 4T1 (C), untreated (OVX, N = 6) or treated with E2 (+E2, N = 6). D, in vivo growth curve of B16K1 injected to Rag2−/− or WT mice, untreated (OVX, N = 7) or treated with E2 (+E2, N = 8). E, double staining for CD31 endothelial cell marker (red) and lectin-FITC (green) on size match B16K1 tumors; scale bars, 135 µm. F, quantifications of CD31-positive density. G, perfused vessel density as measured by lectin-FITC density. H, percentage of perfused vessels. N = 5 to 8 tumors; optical fields, 8 to 12 per tumor. All results are mean ± SEM. For all statistical analysis: *, P < 0.05; **, P < 0.01; ***, P < 0.001 E2 versus OVX.

E2 increases angiogenesis in ER-negative tumors

As angiogenesis is a key process that sustains tumor growth in the early steps of tumor development and can also be influenced by tumor size (25), the effect of E2 on tumor angiogenesis was explored at different time points to collect tumors that time matched and size matched (Supplementary Fig. S3). Indeed, on day 11 (time match), tumors had a mean volume of 75 or 200 mm3 in mice untreated or treated by E2, respectively. To match the size, tumors of E2-treated mice were thus harvested earlier on day 7 (75 mm3), and tumors in untreated OVX mice were collected later, on day 15 (200 mm3), when they reached a volume of 200 mm3.

Blood vessel density was evaluated through CD31 immunostaining (Fig. 1E) and quantified by computer-assisted image analysis (Fig. 1F). E2 increased 2.1-fold the intratumoral vessel density in tumors of 75 mm3, and this enhancement was maintained in 200 mm3 tumors. To analyze vessel function, vessel perfusion was measured upon FITC-conjugated lectin injection (Fig. 1E and G). Computer-assisted image analysis of double staining for CD31 and lectin (Fig. 1H) revealed that the perfusion rate of tumor blood vessel reached 80%, irrespective of tumor size and E2 impregnation. Altogether, the E2-induced growth of ER-negative cancer cells is associated with increased density of intratumoral perfused vessels.

E2 improves tumor angiogenesis qualitatively

Vessel morphology was evaluated with endothelial cell marker CD34 on size match tumors. Tumor vessels from untreated mice followed serpentine course and looked tortuous with irregular and heterogeneous structures (Fig. 2A, left panels). In contrast, vessels in E2-treated mice progressed from tumor periphery through aligned orientation (Fig. 2A, right panels). Nuclei of adjacent endothelial cells were more distant from each other in E2-treated tumors as compared with the untreated ones and endothelial cells presented an elongated morphology feature of lined endothelial cells monolayer (Fig. 2A, bottom panels, pointed by arrows)

Furthermore, confocal microscopy analysis of lectin–FITC staining of thick (100 µm) tumor sections followed by 2D projection of z-slice images emphasized a major impact of E2 on tumor vessel architecture (Fig. 2B). In untreated group, vessels were uneven and dilated, whereas E2 treatment promoted a dense network of thin vessels evenly distributed with multiple branching points. Standardized computer-assisted image analysis of 3D image constructions from z-slice images of thick tumor sections allowed vessel diameter quantification and 3D architecture evaluation (Fig. 2C). This original method detailed in Supplementary Fig. S1 revealed a heterogeneous distribution of vessel diameters in untreated mice, ranging from 0.1 to more than 20 µm. The mode, the value that occurs most frequently, of vessel diameter distribution was 11.02 ± 1.04 µm and 12.47 ± 0.65 µm for tumors of 75 mm3 (Fig. 2D) and...
200 mm$^3$ (Fig. 2E), respectively. In contrast, E2-treated tumors were characterized by a narrow distribution of vessel diameters remaining below 15 μm. In those E2-treated mice, a highly regular vessel network was observed, in both 75 and 200 mm$^3$ tumors, with a lower mode value \(4.57 \pm 0.20\) \(\mu m\) and \(4.55 \pm 0.23\) \(\mu m\), respectively. Altogether, E2 improves the overall structure of tumor vascular network and regulates its organization.

**E2 improves vessel stabilization and oxygenation of ER-negative tumors**

Coverage of vessels by mural cells is a criterion of vessel stabilization and maturity (26). Double staining for lectin–FITC and the mural cell marker α-smooth muscle actin (αSMA) was quantified by computer-assisted image analysis. Little perivascular labeling was observed at the onset of tumor growth irrespective of the treatment (size match: 75 mm$^3$, Fig. 3A top panels). After 4 days (size match: 200 mm$^3$), E2 increased the...
Stromal ERα Normalized Angiogenesis in ER-Negative Tumor

density of αSMA-positive vessels by 2.9-fold magnitude, where-
as the mural cell coverage of vessel remained unchanged in
untreated animals (Fig. 3A, bottom panels, Fig. 3B and C).
Confocal microscopy (Supplementary Fig. S4) and morphologic
analysis of αSMA staining confirmed that the maturation of
neovessels by mural cells was increased by E2 during tumor
growth. In addition, under E2 treatment, vascular cell prolif-
eration assessed by Ki-67 immunostaining (Supplementary Fig.
S5) was high at the beginning of tumor growth on day 7 and
decreased on day 11. By contrast, the proliferative rate of
vascular cells remained unchanged in untreated group (OVX).

Tumor hypoxia may result from an insufficient number of
patent vessels or from supernumerary nonperfused vessels
(27). Visualization of the hypoxic tumor area using pimonida-
zeole showed that E2 prevented the appearance of large hypoxic
area with time and tumor size (Fig. 3D and E). Quantification in
size match 200 mm^3 B16K1 tumors (Fig. 3F) indicated that
under E2 treatment, necrotic area represented only 0% to 5%
(mean score: 0.83 ± 0.11, N = 12), whereas necrosis reached
10% to 15% (mean score: 3.13 ± 0.46, N = 16) without E2
treatment (Fig. 3G). Later on (tumors of 400 mm^3), necrosis
area increased to 30% to 50% without E2, but remained low
(5%–10%) under E2 supplementation (Supplementary Fig. S6).
Similarly, necrosis in 400 mm^3 4T1 tumors remained between
5% and 10% (mean score: 1.86 ± 0.48, N = 8) under E2
treatment (Fig. 3G), but reached 15% to 20% (mean score:
4.86 ± 0.99, N = 7) in untreated mice. Thus, during the early
steps of tumor growth, E2 promotes angiogenesis quantita-
ively. Then, later on, it favors vessel stabilization by mural cell
recruitment associated with a decrease of vascular cell prolif-
eration, thereby optimizing blood supply as attested by reduc-
tion of both tumor hypoxia and necrosis.

Host ERα is necessary to induce E2-dependent growth of
ER-negative tumors

As ERα mediates most of the vascular effects of E2 (5), the
ability of E2 to stimulate the growth of B16K1 and LL2 was
assessed in ERα−/− mice and in their control ERα+/+ litter-
mates. The protumoral effect of E2 observed, to different
extent, on both B16K1 and LL2 growing in ERα−/− mice,
was completely abrogated when the host was ERα deficient (Fig. 4A
and B). In contrast, E2 still accelerated ER-negative cancer
cell growth in ERβ-deficient mice (Fig. 4C). These findings
supported that host ERα is absolutely required for the E2-
mediated protumoral effect on these ER-negative cancer cells,
whereas ERβ is dispensable.

The effect of E2 on angiogenesis and oxygenation of ER-
negative tumor requires host ERα

No significant difference of tumor vessel density was
observed in ERα−/− mice in response to E2 (Fig. 4D). Quan-
tification from 3D images of lectin–FITC–stained sections
showed that the regular distribution of tumor vessel diameters
dilated by E2 in ERα+/+ mice was completely lost in ERα−/−
mice (Fig. 4E). Clearly, in ERα−/− mice treated or not with
E2, the tumor vessel network remained unchanged, irregular
with large vessels displaying variable diameters up to 20 μm
(Fig. 4F). Moreover, staining for the pimonidazole revealed

Figure 4. ERα is necessary to mediate the E2-dependent increase of
tumor angiogenesis, oxygenation, and growth. In vivo growth curve of
B16K1 (A) or LL2 (B) in ERα−/− mice and of B16K1 in ERα−/− mice (C),
untreated (OVX) or treated with E2. For D–J, B16K1 cells were implanted in
ERα+/− mice and collected at size match (200 mm^3). D, tumor vessel
density quantified by CD31-positive staining, N = 8. Vessel diameter
histograms of lectin–FITC–positive vascular network in B16K1 tumors
from E2-treated ERα+/− versus ERα−/− mice (E) or from untreated (OVX)
versus E2-treated ERα−/− mice (F); N = 4 to 6. G, double staining for PIMO
(green) and DAPI (blue); scale bars, 600 μm. H, quantification of hypoxic
PIMO-positive tumor area, N = 5 to 7, I, hematoxylin coloration revealing
tumor necrosis area; scale bars, 500 μm. J, scored quantification of tumor
necrosis area; N = 10 to 16. All results are mean ± SEM. For all statistical
analysis: NS, no statistical difference (P > 0.05); *, P < 0.05; **, P < 0.01;
***, P < 0.001 E2 versus OVX. BM, bone marrow.
derived cells contributes to vasculogenesis and tumor progression (29, 30), we evaluated the role of hematopoietic Erα. OVX C57BL/6J mice were lethally irradiated and successfully grafted with bone marrow cells from either Erα+/– or Erα–/– mice, then treated or not with E2 (Supplementary Fig. S7). The B16K1 tumor growth was similarly accelerated by E2 in mice grafted with Erα+/- or Erα–/– bone marrow (Fig. 5B), suggesting that bone marrow Erα is dispensable for the protumoral effect of E2. To ascertain that bone marrow Erα is dispensable and that the loss of E2 effect on tumor growth in the Tie2-Cre/+–/– mice is not due to the concomitant Erα inactivation in hematopoietic cells, we reconstituted Tie2-Cre/+–/– mice or their control littermates with Erα+/- bone marrow. In these chimeric mice with Erα–/– expressing cells and Erα+/- hematopoietic cells, E2 do not accelerate the growth of B16K1 tumor cells anymore, although the E2 protumoral effect is well visible in control littermate mice (Fig. 5C and D). These data excluded any major contribution of Erα expressed by hematopoietic cells to the protumoral effect of E2. Thus, Erα of Tie2-positive cells, but not bone marrow-derived cells Erα, is necessary to mediate the effect of E2 on the tumor growth of ER-negative cancer cells.

**E2 modulates angiogenic factor expression in ER-negative tumors**

To further characterize whether intratumoral angiogenic factors can be influenced by E2 treatment, expression of a set of proteins known to regulate angiogenesis was analyzed using an antibody array on whole-protein extracts of size matched tumors. Of all candidates analyzed, levels of basic fibroblast growth factor (bFGF) and of VEGF-D remain unchanged, whereas both VEGF-A and platelet-platelet factor-4 (PF4) were upregulated by E2 (Fig. 6A). In addition, among the VEGF receptors, VEGFR-3, whose expression was very low in untreated mice compared with VEGFR-1 and VEGFR-2, was strongly upregulated in E2-treated tumors. As we showed that angiogenesis contributes to E2-mediated growth of ER-negative cancer cells, we evaluated in vitro whether E2 could modulate the angiogenic expression profile of an endothelial ER-positive cell line, CRL-2181 (Fig. 6B). Among the set of genes analyzed, expression of VEGF-A, VEGF-D, VEGFR-1, sVEGFR-1, thrombospondin-1 (TSP-1), and PECAM was significantly upregulated by E2 treatment. Transcripts of VEGF-B, VEGFR-3, TGFβ1, and VCAM-1 tended to increase under E2 treatment, whereas those of PI GF, Notch1, and Dll4 decreased.

Altogether, these data indicated that E2 could modulate intratumoral angiogenic factor levels and that endothelial expression of some angiogenic factors could be impacted by E2 treatment.

**Erα is expressed by peritumoral microenvironment of human breast cancers**

To assess whether the expression of Erα occurs in vivo in the stroma of human tumors, immunohistochemistry (IHC) on human Erα-positive and Erα-negative breast cancer tissues was conducted. Close to the strong staining of malignant Erα-positive tumor cells (Fig. 7A), a relevant Erα staining was
observed in some stromal cells. Stromal expression, detected in some fusiform-shaped fibroblast-like cells (thin arrows), was also detected in human breast tumors classified as ERα-negative tumors (Fig. 7B and C). In addition, double immunostaining for ERα combined either with CD45 (Fig. 7D) or SMA antibody (Fig. 7E) revealed that ERα-positive stromal cells were not leucocytes and were SMA negative. However, some rare costainings for ERα and CD31 (Fig. 7F) were observed in human breast cancer stroma. These data indicated that ERα immunostaining can be found in stromal cells in the microenvironment of human breast cancers irrespectively of the ERα expression by cancer cells.

Discussion

Until now, several evidences have suggested that E2 affects tumor microenvironment independently of its direct effect on tumor cell growth. However, the cellular and molecular mechanisms driving these interactions remained undetermined in immunocompetent mice. This study describes novel insights showing that E2 acts through ERα expressed by tumor microenvironment to promote tumor growth via an increased and normalized angiogenesis.

The cellular effectors of inflammation are key constituents of the microenvironment. Moreover, E2 is known to increase the production of proinflammatory cytokines, in various cell populations such as CD4+ lymphocytes (31), natural killer cells (32), and macrophages (33). First, we evidence here that the protumoral impact of E2 on ERα-negative tumor cell growth was also present in Rag2−/− immunodeficient mice that lack T and B lymphocytes, indicating that these immune cells do not play a major role in the E2 effect. Second, using chimeric mice reconstituted with bone marrow from ERα−/− or ERα+/+ mice, we show that ERα of bone marrow–derived cells is not required for acceleration of B16K1 tumor growth in C57BL/6J mice by E2. This result diverges from Gupta and colleagues (14) who described that bone marrow cell recruitment was sufficient to mediate the E2-induced growth of human ER-negative tumorigenic breast epithelial cells implanted to immunodeficient mice. Major differences in the experimental protocols, that is, selective ERα ablation in bone marrow of immunocompetent animals (this work) versus coinjection of a mix of immunodeficient bone marrow cells, epithelial tumor cells, and Matrigel into immunodeficient mice (14) probably accounts for the apparent discrepancy.

The present data clearly show that E2 impregnation not only increases vessel density but also improves qualitatively tumor

![Figure 6](https://www.aacrjournals.org) E2 modulates angiogenic factor expression in ER-negative tumors. A, intratumoral protein expression of bFGF, VEGF-A, VEGF-D, PF4, VEGFR-1, VEGFR-2, and VEGFR-3 in size match B16K1 tumors, untreated (OVX) or treated with E2, N = 5. B, quantitative reverse transcriptase PCR analysis of angiogenic genes from EC-CRL-2181 results are expressed as change in mRNA expression in E2-treated endothelial cells (percent of OVX level), N = 4. All results are mean ± SEM. For all statistical analysis: NS, no statistical difference (P > 0.05).

* P < 0.05; ** P < 0.01; *** P < 0.001 E2 versus OVX.

![Figure 7](https://www.aacrjournals.org) Detection of ERα by IHC in the peritumoral microenvironment of human breast cancers. ERα staining of stromal cells (large arrows), fibroblast-like cells (thin arrows), and cancer cells (surrounded by the line) in ERα-positive breast tumors (A) and in ERα-negative breast tumors (B and C). Double staining for ERα (red) and CD45 (D; green), SMA (E; green), or CD31 (F; green). Scale bars = 50 μm, except for panel D in which scale bar = 10 μm.
angiogenesis, by improving vessel structure, organization, and stabilization by mural cell recruitment. This impact of E2 completely relies on host ERα, as tumor growth and vascular network in ERα−/− mice were unresponsive to E2 treatment. In tumors that grew in E2-treated host, the vessel network was dense with multiple branching points and clearly oriented. Vessels were composed of lined endothelial cells presenting flattened and elongated morphology with distant nuclei, their diameter were thin, and even resulting in a more regular shape. E2 also improved their maturation by increasing neovessel coverage by mural cells, and drastically reducing both tumor hypoxia and necrosis. Modulation of intratumoral angiogenic factors is a likely mechanism underlying this improvement of angiogenesis. Upregulation of VEGF-A by E2 was already documented (4), whereas upregulation of VEGFR-3 is of particular interest because it was recently reported to control the rapid conversion of tip cells to stalk cells during angiogenesis (34). At the endothelial cell level, transcription of various genes reported to be related to angiogenesis and vessel maturation was modulated by E2. Indeed, the increase of VEGFR-1 and sVEGFR-1 has been implicated in vessel maturation (26, 35). The antiangiogenic factor TSP-1 was also upregulated in endothelial cells by E2, a modulation already reported in T47-D and MCF-7 cells (36). Knowing the high degree of complexity by which E2 impacts cell physiology, all these data indicate that the balance between pro- and antiangiogenic factors can be modulated by E2 and contributes through paracrine interactions between endothelial cells, cancer cells, and other stromal cells to increase and improve tumor angiogenesis and thus tumor growth.

Altogether, these data show that E2 optimizes blood supply to ER-negative cancer cells through an important modification of tumor vasculature that seems normalized as described by Carmeliet and Jain (26, 37). This concept of normalization was documented by Mazzone and colleagues (26) who observed that tumor vessel normalization improves tumor perfusion and oxygenation and decreases metastasis. Importantly, hypoxia is a negative prognostic factor associated to chemotherapies and radioresistance and is described to promote tumor invasion and metastatic dissemination (37–40). As vessel normalization emerges actually as a potential therapeutic option (25, 37), the stromal ERα-mediated impact of E2 on ER-negative cancer cell growth and angiogenesis is of peculiar interest. Indeed, even if E2 indirectly contributes to promote ER-negative cancer cell growth, the decreased tumor hypoxia and necrosis conferred by E2 could present a therapeutic advantage in terms of metastasis and efficacy of chemotherapies. This idea is supported by the Southwest Oncology Group run trials, which reported that E2 increases response to chemotherapy in young premenopausal women treated for lung cancer, turning the negative protumoral effect of E2 into a positive factor in response to chemotherapy (41).

Using the Tie2 promoter-driven ERα expression, we further showed that ERα expressed by Tie2-positive cells plays a crucial role in the protumoral effect of E2. Tie2 is largely reported to be an endothelial cell marker as it is expressed mostly by endothelial cells lineage (28). However, more recently, it has also been reported in hematopoietic lineage, in pericytes, fibroblasts/myofibroblasts (42–44). Tie2-expressing monocytes have been shown to promote tumor angiogenesis in various mouse tumor models (45). In addition, bone marrow-derived endothelial progenitor cells (EPC) could also have contributed to the formation of tumor endothelium, but it remains highly controversial (29). In this work, E2 elicited similar protumoral effects in chimeric mice irrespective of the presence or absence of ERα in bone marrow–derived cells. Moreover, the protumoral effect of E2 was abrogated in chimeric mice harboring ERα-negative Tie2-expressing cells, but with ERα-positive hematopoietic cells, showing a minor role of E2 on bone marrow–derived EPC in tumor vasculature development. This result corroborates studies showing that tumor endothelium does not predominantly originate from bone marrow cells, particularly in C57BL/6 mice (46, 47). As we showed that the protumoral effect of E2 relies on ERα-dependent promotion and improvement of angiogenesis, this is in line with the crucial role played by endothelial ERα in the various effects exerted by E2 on endothelium, that is, endothelial cell migration and proliferation in vitro, reendothelialization acceleration in vivo (5, 23, 48–50) and with the major contribution of pericytes to vessel stabilization (26, 37, 40). Thus, Tie2-positive cells expressing ERα, but not bone marrow ERα, are necessary to promote the E2-induced growth of ER-negative tumors.

Finally, we clearly detected ERα in human stromal cells surrounding human ER-positive and also ER-negative breast cancers. These stromal ERα-positive cells were negative for CD45 and SMA and were rarely positive for CD31. However, the lack of selective fibroblast markers led the phenotype of the stromal ERα-positive cells difficult to be clearly identified. Until now, this stromal expression of ERα is not routinely evaluated during diagnosis of human breast tumors. Nevertheless, it could be relevant if a correlation between stromal ERα expression, prognosis, and/or treatment response could be addressed in women.

In summary, we show here that ERα of microenvironment plays a crucial role in the in vivo growth of ERα-negative cancer cells under E2 treatment. Indeed, stromal ERα is necessary to induce tumor growth, mediating adaptation of tumor angiogenesis and vessel stabilization that subsequently improves oxygen and nutrients delivery, thereby preventing hypoxia and necrosis. This could have implications in the management of patients, particularly in the diagnosis and the schedule of hormono-, radio-, and chemotherapies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Stromal Estrogen Receptor-α Promotes Tumor Growth by Normalizing an Increased Angiogenesis

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