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Advances in Brief

p27^{Kip1} Induces Quiescence and Growth Factor Insensitivity in Tamoxifen-treated Breast Cancer Cells[1]

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Abstract

Tamoxifen, a selective estrogen-receptor modulator, is effective in the treatment and prevention of breast cancer, but therapeutic resistance is common. Pure steroidal antiestrogens are efficacious in tamoxifen-resistant disease and, unlike tamoxifen, arrest cells in a state of quiescence from which they cannot reenter the cell cycle after growth factor stimulation. We now show that in hydroxytamoxifen-treated cells, transduction of the cell cycle inhibitor p27^{Kip1} induces quiescence and insensitivity to growth stimulation by insulin/insulin-like growth factor I and epidermal growth factor/transforming growth factor α. Furthermore, reinitiation of cell cycle progression by insulin/insulin-like growth factor I in hydroxytamoxifen-arrested cells involves dissociation of the corepressors nuclear receptor corepressor (N-CoR) and silencing mediator for retinoid and thyroid hormone receptor (SMRT) from nuclear estrogen receptor α and redistribution to the cytoplasm, a process that is inhibited by mitogen-activated protein/extracellular signal-regulated kinase, but not phosphatidylinositol 3′-kinase, inhibitors. These data suggest that agents that up-regulate p27^{Kip1} or inhibit growth factor signaling via the extracellular signal-regulated kinases should be tested as therapeutic strategies in tamoxifen-resistant breast cancer.

Introduction

Antiestrogens, including SERMs[4] (*e.g.*, tamoxifen) and pure estrogen antagonists, are potent inhibitors of proliferation in ER-positive breast cancer cells, where they antagonize the mitogenic effects of estrogen (1). Tamoxifen is the endocrine therapy of choice for the treatment of hormone-sensitive breast cancer and has also shown efficacy in some prevention trials (2). However, the effectiveness of tamoxifen is restricted by the frequent development of cellular resistance (2, 3). The mechanisms underlying tamoxifen resistance are not well understood (1–4), but there is increasing evidence that up-regulation of tyrosine kinase receptors, particularly the *erb*B receptors, and their signaling pathways are important in endocrine resistance (3–5). Structurally distinct steroidal antiestrogens, of which ICI 182780 is one example, have been developed that lack agonist activity and have greater long-term effectiveness than tamoxifen in inhibiting breast cancer cell growth *in vitro* and *in vivo* (6, 7). Because a proportion of tamoxifen-resistant breast cancers retain sensitivity to steroidal antiestrogens (7), differences in the mechanisms of action of the two classes of antiestrogen could potentially include novel pathways that contribute to resistance.

The molecular mechanisms of action of ICI 182780 as a growth inhibitory agent *in vitro* have recently been clarified. Treatment of estrogen-responsive MCF-7 breast cancer cells induces down-regulation of the G_1-specific cyclin, cyclin D1 (8), as a direct consequence of inhibition of expression of the proto-oncogene c-Myc (9). This initiates a cascade of molecular events, including the redistribution of the CDK inhibitor p21$^{WAF1/CIP1}$ from cyclin D1-Cdk4 complexes to cyclin E-Cdk2 complexes, inhibition of cyclin E-Cdk2 activity, and the consequent accumulation of the CDK inhibitor p27^{Kip1} (10, 11). A distinguishing feature of ICI 182780-mediated growth arrest is induction of quiescence (10). Quiescence (G_0) is a physiological state that is distinct from G_1 in terms of responses such as the time required for cell cycle reentry after mitogenic stimulation and the ability to initiate DNA synthesis. Molecular differences between G_0 and G_1 include hyperphosphorylation of the transcription factor E2F4 and its association with the pocket protein p130 during G_0 to form p130-E2F4 complexes that are thought to mediate much of the transcriptional repression of genes specifically down-regulated in G_0 (12). Consequently, the accumulation of p130 and its association with E2F4 after treatment with ICI 182870 are indicative of arrest in G_0 (10). The ICI 182780-induced quiescent state is associated with insensitivity to mitogenic growth factors (13). In contrast, SERM treatment is less effective in the presence of growth factors, raising the possibility of a different state of growth arrest (14, 15). Recruitment of the corepressors N-CoR and SMRT to the ER has been suggested as one potential mechanism by which tamoxifen inhibits cell proliferation (16). However, it is unclear how corepressor utilization by tamoxifen might contribute to growth factor sensitivity and modulation of antiestrogen sensitivity. It is also unclear whether corepressor utilization acts in concert with other events, such as p27^{Kip1} accumulation, or whether these are distinct pathways used by different classes of antiestrogens. Because insights into the molecular mechanisms whereby SERMs and pure antiestrogens induce growth arrest may shed light on cellular pathways that contribute to antiestrogen resistance, we investigated the growth arrest states induced by SERMs and pure antiestrogens and the mechanisms for their differential sensitivity to growth factor-mediated cell cycle reentry.

Materials and Methods

Antioestrogens. Stock solutions of antiestrogens were prepared as follows: ICI 182780 (a kind gift from Dr. Alan Wakeling, AstraZeneca Pharmaceuticals, Alderley Park, Cheshire, United Kingdom), hydroxytamoxifen (from Dr. A. Wakeling), CI 628 (from Dr. E. Elslager, Warner-Lambert Parke Davis, MI, ICI 164384 (from Dr. A. Wakeling), and RU 58668 (a kind gift from Dr. P. Van de Velde, Hoechst Marion Roussel, 93235 Romainville, France) were dissolved in ethanol to 0.01 M and a working dilution in RPMI 1640 medium

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[4] The abbreviations used are: SERM, selective estrogen receptor modulator; ER, estrogen receptor; CDK, cyclin-dependent kinase; N-CoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoid and thyroid hormone receptor; MEK, mitogen-activated protein/extracellular signal-regulated kinase.

was prepared from this stock immediately before each experiment. Hydroxyclomiphene (from Merrell-Dow Pharmaceuticals, OH) stock solutions were prepared as 0.01 M stocks in DMSO and working dilutions were prepared in RPMI 1640 on the day of use.

TAT-p27 Protein. The TAT-p27 construct was a kind gift from Dr. Steven Dowdy (Washington University School of Medicine, St. Louis, MO). Tat-p27^{Kip1} protein was produced as described (17), except protein was dialyzed exhaustively and concentrated by acetone precipitation.

Cell Culture. The MCF-7 and MDA-MB-231 cell lines were obtained from EG & G Mason Research Institute (Worcester, MA). These cell lines were cultured and DNA flow cytometry was performed as described previously (10). Antiestrogens were added to cells in exponential growth phase and the cells harvested after 48 h of treatment for flow cytometric determination of cell cycle phase distribution or for Western/immunoblot analysis. Except as shown in Fig. 1*A*, the following maximally effective concentrations of antiestrogen were used: ICI 182780, 10 nM; ICI 164384, 100 nM; RU 58668, 10 nM; and hydroxytamoxifen, hydroxyclomiphene, and CI628, 1 μM. For experiments involving serum starvation, after culture in RPMI containing 10% FCS overnight, MCF-7 cells were grown in a defined serum-free, phenol red-free RPMI 1640 medium supplemented with transferrin (24 μg/ml) and gentamicin (10 μg/ml). This medium was changed daily for the length of the experiment. Hydroxytamoxifen or hydroxyclomiphene was added for a further 24 h. In some experiments, MCF-7 cells were serum-starved in the presence of hydroxytamoxifen (or the appropriate controls) for 24 h, after which TAT-p27 was added directly to the growth medium at a final concentration of 500 nM for a further 16 h. Insulin stimulation involved the addition of insulin to a final concentration of 10 μg/ml directly to the media, 6 h before harvest. Nocodazole (Sigma, Castle Hill, NSW, Australia) was added to cells at a final concentration of 50 ng/ml to prevent cell division of insulin-stimulated cells. Wortmannin (Sigma) was used at a final concentration of 100 nM and U0126 (Promega, Annandale, NSW, Australia) was used at 10 μM.

Immunoblot Analysis. Cells were lysed and proteins separated by SDS-PAGE as described previously (10). The following antibodies were used: E2F4 (Santa Cruz Biotechnology Inc, Santa Cruz CA, C-20); p27^{Kip1} (Transduction Laboratories, Lexington KY); ERα (Dako A/S, Glostrup, Denmark).

Immunoprecipitation. Antibodies were chemically cross-linked to the beads to reduce background and immunoprecipitation performed as described previously (10). Polyclonal antibodies raised against human p130 (C-20), SMRT (C-19), N-CoR (C-19), SRC-1 (M-341), and p/CIP/SRC-3 (M-397) were obtained from Santa Cruz Biotechnology Inc.

Chromatin Immunoprecipitation. Cells were fixed with formaldehyde (1% final concentration) and treated as described previously (16). Briefly, the soluble chromatin was extracted, precleared with salmon sperm DNA and protein A-Sepharose beads, then immunoprecipitated overnight at 4°C using the SRC-1 and p/CIP antibodies described above. DNA was extracted from the immunoprecipitates and purified before PCR. The following primers were used for PCR of the pS2 promoter region (16): forward, 5′-CTATGAATC-ACTTCTGCAGTGAGT-3′; and reverse, 5′-CCGGATTTTATAGGGCAG-GCTCTG-3′.

Indirect Immunofluorescence. MCF-7 cells were initially grown on acid-washed coverslips in RPMI/10% FCS, then serum-starved in the presence of hydroxytamoxifen as described above. After insulin stimulation (as described) cells were washed twice in PBS and fixed in 3.7% paraformaldehyde, permeabilized in 0.2% Triton X-100 and blocked in 2% BSA, 0.05% Tween 20 in PBS. Cells were subsequently incubated with antibodies against N-CoR and/or SMRT. After extensive washes in PBS containing 0.05% Tween 20, cells were incubated with fluorescein-conjugated secondary antibodies for 1 h (FITC donkey antigoat). Cells were washed extensively and mounted in 90% v/v glycerol in PBS. Cells were viewed using a Leica DMR confocal microscope with a TCS software package. Images were recorded digitally and processed using Adobe PhotoShop software.

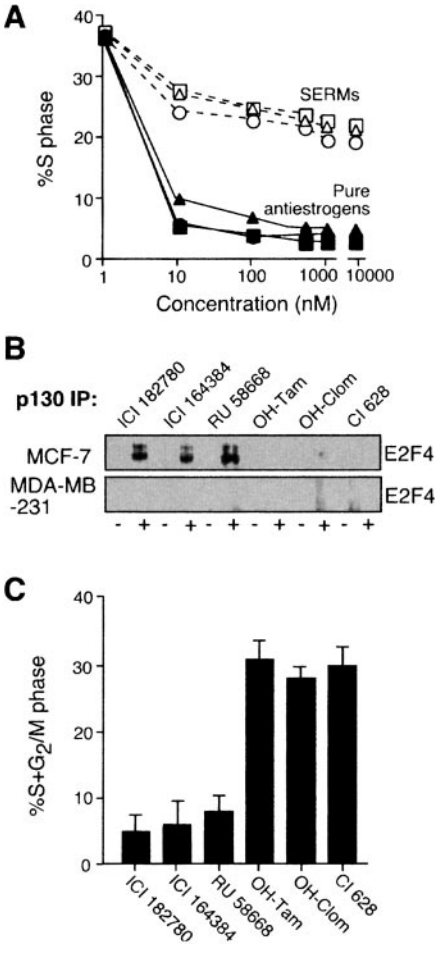

Fig. 1. SERMs and pure antiestrogens induce different states of growth arrest and response to mitogen stimulation. *A*, proliferating MCF-7 cells were treated with increasing concentrations of SERMs (○, hydroxytamoxifen; △, hydroxyclomiphene; □, CI 628) or pure antiestrogens (■, ICI 182780; ▲, ICI 164384; ●, RU 58668). *B*, lysates of MCF-7 cells (and ER-negative MDA-MB-231 cells as controls) were immunoprecipitated using p130 antibody and Western blotted with an antibody to E2F4. *OH-Tam*, hydroxytamoxifen; *OH-Clom*, hydroxyclomiphene. *C*, MCF-7 cells were serum-starved in the presence of a maximally effective concentration of the indicated antiestrogens and subsequently treated with insulin or vehicle in the presence of nocodazole to block G_2/M progression. The data are presented as the difference in the S + G_2/M phases in the presence and absence of insulin and are the mean of three experiments ±SD. *OH-Tam*, hydroxytamoxifen; *OH-Clom*, hydroxyclomiphene.

Results

Pure Antiestrogens and SERMs Induce Different Growth Arrest States. Our previous observation that ICI 182780 arrests MCF-7 human breast cancer cells in quiescence (10) raised the question of whether this response was also induced by other classes of antiestrogens. We compared the responses of MCF-7 cells treated with three SERMs [hydroxytamoxifen (the active metabolite of tamoxifen), hydroxyclomiphene, and CI 628] and three pure steroidal antiestrogens (ICI 182780, ICI 164384, and RU 58668). The pure steroidal antiestrogens decreased the S-phase fraction from 37% to 6–8%, but the SERMs were significantly less effective even at high concentrations, decreasing the S-phase fraction to 22–24% (Fig. 1*A*). p130-E2F4 association, a marker of quiescence, was apparent in cells treated with pure antiestrogens, but not SERMs (Fig 1*B*), indicating that the SERMs did not arrest breast cancer cells in quiescence. No changes were observed in the ER-negative cell line, MDA-MB-231, after treatment with either SERMs or pure antiestrogens (Fig. 1*B* and data not shown), confirming that these responses are ER-mediated.

We reasoned that if distinct growth arrest states were induced by the different classes of antiestrogens, the ability of cells to reenter the cell cycle after mitogen exposure might be differentially altered. The proportion of cells that responded to mitogenic stimulation from an antiestrogen-arrested state in the first 24 h after insulin stimulation was only 5–8% after pretreatment with pure antiestrogens, but ~30% after treatment with the SERMs (Fig. 1*C*). Cells stimulated with IGF-I, EGF, or TGFα behaved in a similar manner, demonstrating

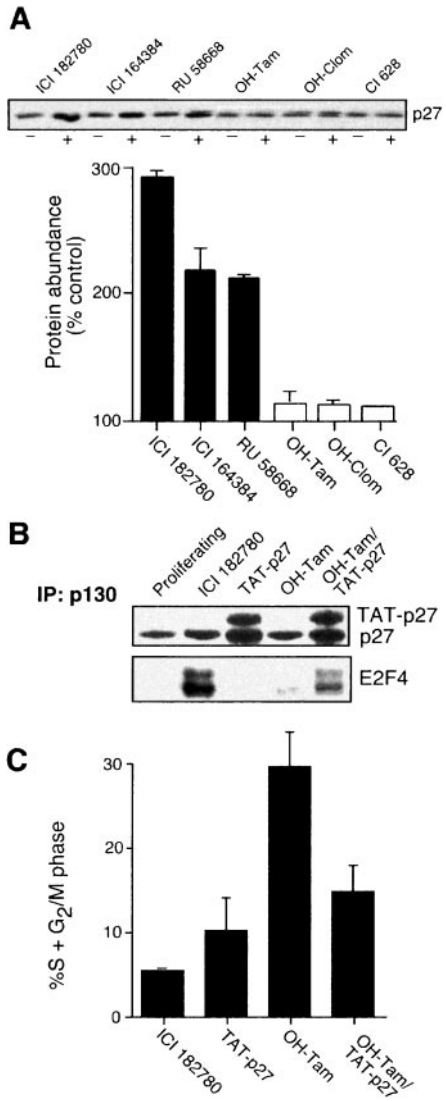

Fig. 2. p27Kip1 induces quiescence in hydroxytamoxifen-treated MCF-7 cells. *A*, proliferating MCF-7 cells were treated with antiestrogen or vehicle and lysates Western blotted. The relative increase in p27Kip1 compared with vehicle control in three separate experiments (mean ± SD) is plotted. *B*, TAT-p27 protein was introduced into cells treated with hydroxytamoxifen or vehicle control, in parallel with proliferating and ICI 182780-treated cells. Total cell lysates were collected and Western blotted with an antibody to p27 or used for p130 immunoprecipitations followed by Western blotting with an antibody to E2F4. *OH-Tam*, hydroxytamoxifen; *OH-Clom*, hydroxyclomiphene. *C*, MCF-7 cells were serum-starved in the presence of ICI 182780, hydroxytamoxifen, TAT-p27, or both hydroxytamoxifen, and TAT-p27, then stimulated with insulin in the presence of nocodazole to block G_2/M progression. The differences in the percentage of cells in S and G_2/M between insulin treated and control cells are plotted as the mean ± range of two separate experiments. *OH-Tam*, hydroxytamoxifen; *OH-Clom*, hydroxyclomiphene.

clear differences in the ability of cells to reenter the cell cycle after arrest with the two classes of antiestrogen and consistent with the conclusion that they induce different growth arrest states.

Transduction of Hydroxytamoxifen-treated Cells with TAT-p27 Mimics ICI 182780-mediated Growth Arrest. An increase in p27Kip1 plays an essential role in maintaining growth arrest of MCF-7 cells after ICI 182780 treatment (10, 11). Because p27Kip1 has been implicated in the maintenance of quiescence (18, 19), differential regulation of p27Kip1 is a potential mechanism through which different antiestrogens might induce different states of growth arrest. Consistent with this hypothesis, 48-h treatment with the pure antiestrogens increased the level of p27Kip1 2–3-fold, whereas SERM treatment had little effect (Fig. 2*A*).

To determine whether the increased p27Kip1 abundance observed after treatment with pure antiestrogens was a pivotal event mediating entry into quiescence and consequent mitogenic insensitivity, we assessed the effects of introduction of exogenous p27Kip1 via TAT-mediated protein transduction (17) on the potency of hydroxytamoxifen. In serum-starved cells treated with hydroxytamoxifen or TAT-p27 alone, little or no p130-E2F4 association was seen (Fig. 2*B*). However, the combination led to significant p130-E2F4 association, as seen after ICI 182780 treatment (Fig. 2*B*). Furthermore, exogenous p27Kip1 also decreased sensitivity to mitogenic stimulation, because the addition of TAT-p27 decreased the percentage of insulin-stimulated cells that escaped hydroxytamoxifen-mediated growth arrest from 30% to 15% (Fig. 2*C*). Thus, the transduction of TAT-p27 into hydroxytamoxifen-treated cells elicited a response similar to that induced by ICI 182780, *i.e.*, growth arrest in quiescence and decreased sensitivity to mitogens.

Pure Antiestrogen and SERM Effects on ERα Abundance and Association with the Corepressors N-CoR and SMRT. Previous work identified the significant down-regulation of ERα by ICI 182780 (20) and the recruitment of the corepressors N-CoR and SMRT by tamoxifen (16) as potential mechanisms by which different antiestrogens can mediate growth arrest. ERα decreased after treatment with pure antiestrogens, but not after treatment with any of the SERMs, which, instead, increased ERα levels (Fig. 3*A*). In addition, various mitogenic signaling pathways can negatively regulate the ability of N-CoR and SMRT to associate with ERα (21). Therefore, we investigated the role of corepressor association with ERα in the mitogen sensitivity of cells treated with different SERMs. A significant increase in SMRT and N-CoR association with ERα was apparent after

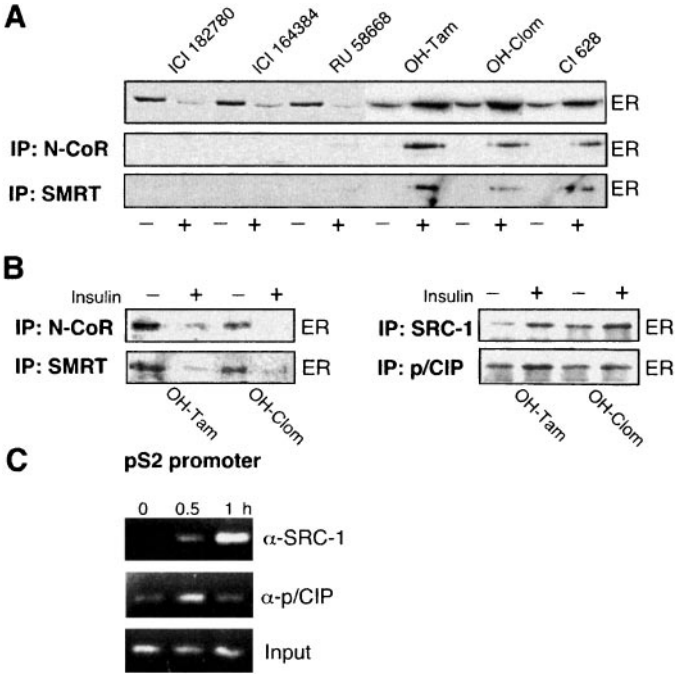

Fig. 3. Changes in ER levels and association with coregulatory proteins after antiestrogen treatment and mitogenic stimulation. *A*, ERα levels were determined after treatment with the indicated antiestrogens. N-CoR or SMRT immunoprecipitates were Western blotted with an antibody to ERα. *OH-Tam*, hydroxytamoxifen; *OH-Clom*, hydroxyclomiphene. *B*, MCF-7 cells were serum-starved in the presence of hydroxytamoxifen or hydroxyclomiphene then stimulated with insulin. Two corepressors (N-CoR and SMRT) and two coactivator proteins (SRC-1 and p/CIP) were immunoprecipitated and Western blotted with an antibody to ERα. *OH-Tam*, hydroxytamoxifen; *OH-Clom*, hydroxyclomiphene. *C*, the experimental design was the same as in *B*. SRC-1 and p/CIP were immunoprecipitated from fragmented chromatin and the associated DNA purified. The promoter region of the estrogen responsive gene *pS2* was amplified as described in the "Materials and Methods." *Input* shows the result of PCR from total formaldehyde-fixed cell lysate.

SERM treatment but the pure antiestrogens did not induce detectable association of ERα with either of the corepressors (Fig. 3*A*). Thus, the previously described mechanisms are characteristic of these classes of antiestrogens.

We next investigated whether corepressor association with ERα could be regulated by growth factors that reinitiate cell cycle progression in SERM-treated cells. MCF-7 cells were serum-starved in the presence of hydroxytamoxifen or hydroxyclomiphene and then stimulated with insulin. Corepressor-ERα association was markedly decreased after insulin stimulation, and, conversely, the association of two coactivators, SRC-1 and p/CIP, was increased (Fig. 3*B*). To determine whether this exchange between corepressors and coactivators resulted in changes in ERα-mediated transcription, we measured coactivator occupancy of a known estrogen-regulated gene promoter, pS2, using chromatin immunoprecipitation. In cells serum-starved in the presence of hydroxytamoxifen and then restimulated with insulin, SRC-1 associated with the pS2 promoter within 30 min of insulin stimulation and this increased after 1 h (Fig. 3*C*). Interestingly, p/CIP association with the same site in the pS2 promoter increased after 30 min, but returned to baseline by 1 h (Fig. 3*C*). These data indicate that mitogen stimulation alters coactivator recruitment and hence ERα activity at the promoters of estrogen-regulated genes.

We next investigated whether subcellular relocalization might be a cause of modulation of ER binding and transcriptional inhibition by the corepressors N-CoR and SMRT. Indirect immunofluorescence of cells serum-starved in the presence of hydroxytamoxifen revealed nuclear localization of both N-CoR and SMRT (Fig. 4*A*). However, after insulin stimulation, cytoplasmic distribution of both N-CoR and SMRT increased substantially (Fig. 4*A*). To further define the signaling pathways used by insulin to regulate the corepressor-ER complex, we used specific chemical inhibitors of either the mitogen-activated protein kinase or phosphatidylinositol 3′-kinase pathways: U0126 and Wortmannin, respectively. MEK inhibition before insulin stimulation inhibited the cytoplasmic relocalization of N-CoR (Fig. 4*B*). Similar results were obtained for SMRT (data not shown). In marked contrast, inhibition of phosphatidylinositol 3′-kinase with Wortmannin did not influence the insulin-mediated redistribution of N-CoR or SMRT to the cytoplasm (Fig. 4*B* and data not shown). Thus, the cytoplasmic redistribution of N-CoR and SMRT appears to be mediated by signaling through MEK.

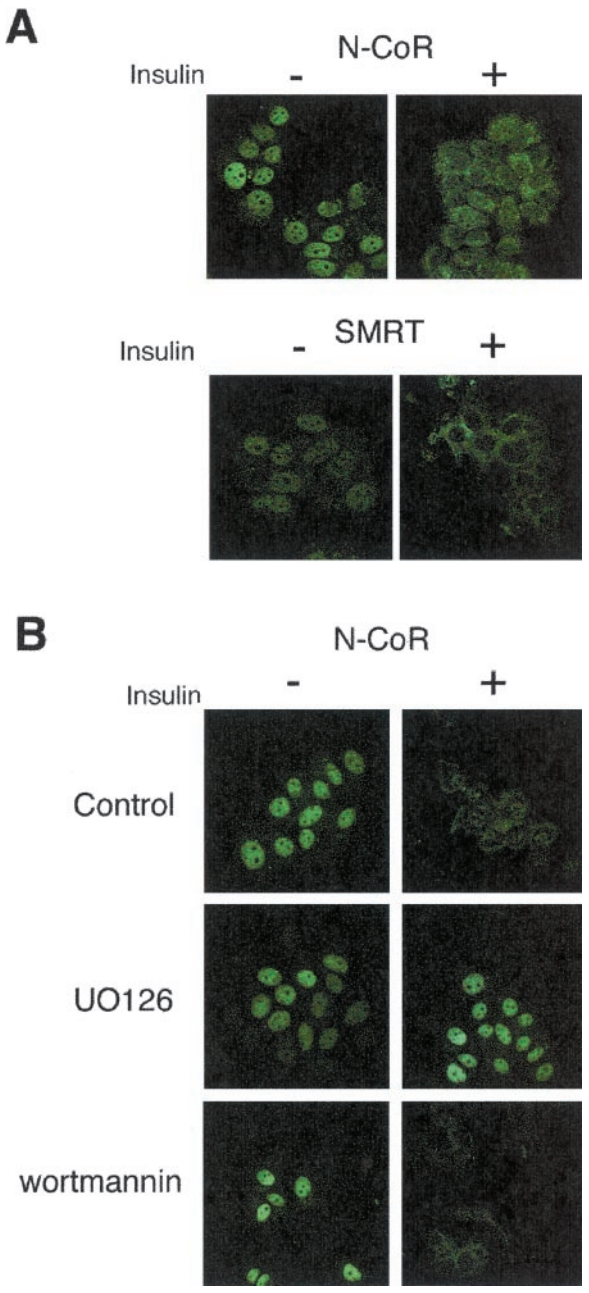

Fig. 4. Mitogenic stimulation of MCF-7 cells treated with SERMs induces cytoplasmic localization of N-CoR and SMRT that is mediated by the ERK pathway. *A*, MCF-7 cells were serum-starved in the presence of hydroxytamoxifen then stimulated with insulin. Localization of N-CoR and SMRT was visualized by confocal microscopy. *B*, MCF-7 cells were serum-starved in the presence of hydroxytamoxifen, pretreated with either the MEK inhibitor U0126 or the PI3 kinase inhibitor Wortmannin and then stimulated with insulin. Localization of N-CoR was visualized by confocal microscopy.

Discussion

In these experiments, we have shown that not all antiestrogens induce the same growth arrest state: all three pure antiestrogens arrested MCF-7 cells in quiescence, but the SERMs did not. In addition, whereas SERM-treated cells responded to mitogenic stimulation, cells treated with pure antiestrogens were essentially insensitive. These data raised the question of the molecular mechanisms of growth arrest. One candidate mechanism that could potentially account for this disparity was modulation of the CDK inhibitor $p27^{Kip1}$, which is essential for long-term growth arrest by ICI 182780 (10, 11), and is induced in MCF-7 xenografts after long-term administration of RU 58668 concomitant with a significant decrease in tumor volume (22). Despite this, accumulation of $p27^{Kip1}$ did not appear to be a major mechanism of growth regulation by the SERMs. The presence of hydroxytamoxifen alone or $p27^{Kip1}$ alone did not cause quiescence, suggesting that although either can inhibit cell cycle progression, neither is sufficient to induce quiescence. Significantly, however, the addition of exogenous $p27^{Kip1}$ to hydroxytamoxifen-treated cells resulted in growth arrest in quiescence as indicated by p130-E2F4 association, and conferred mitogen insensitivity.

The absence of p130-E2F4 complexes in SERM-treated cells indicated that they were arrested in G_1 rather than in G_0, consistent with the observation that after treatment with any of the three SERMs, a third of the cell population reentered the cell cycle from an arrested state in the first 24 h of mitogen stimulation. These restimulated cells apparently maintained key priming events required for DNA synthesis and cell division that were not present in cells treated with pure antiestrogens, because in contrast with the response of SERM-treated cells, very few of the cells that were treated with the pure antiestrogens could reenter the cell cycle after insulin stimulation. This lack of response did not simply reflect an elongated period of time required for exit from G_0 into the cell cycle, because insulin stimulation for >48 h did not increase the percentage of cells synthesizing DNA (data

not shown). These data support the hypothesis that quiescence requires initial cell cycle arrest, followed by a secondary event (likely p27^{Kip1} induction) that causes the transition from G_1 into G_0. The effects of hydroxytamoxifen treatment and subsequent p27^{Kip1} addition are reminiscent of ICI 182780-mediated growth arrest, which initially involves inactivation of cyclin E-Cdk2, followed by accumulation of p27^{Kip1} and subsequent growth arrest in quiescence (10). These data emphasize the importance of the accumulation of p27^{Kip1} in induction of quiescence after treatment with the pure antiestrogens, consistent with previous observations in other cell types that implicate p27^{Kip1} in the induction and maintenance of a quiescent state (18, 19).

A second mechanism of growth regulation investigated was modulation of ERα, which was strongly down-regulated by pure antiestrogens but not by the SERMs. The decline in ERα protein levels after pure antiestrogen treatment likely inhibits both classic ER-mediated transcription and growth factor-mediated activation of the ER complex and may be the fundamental reason why the pure antiestrogens are solely estrogen antagonists. Although the SERMs did not decrease ERα protein levels, they did recruit the corepressors N-CoR and SMRT to the ER. Evidence including the correlation between tamoxifen resistance and decreased levels of N-CoR and SMRT (23) or increased levels of the coactivator AIB1 (24) indicates that this is likely to be an important *in vivo* growth inhibitory mechanism for SERMs.

The ability of mitogens to attenuate the effects of tamoxifen and structurally similar compounds was identified more than a decade ago (14, 15). We now provide a mechanistic explanation for these observations, whereby recruitment of corepressors to the ER is impaired by the presence of growth factors that induce redistribution of the corepressors to the cytoplasm. The MEK signaling pathway increases SMRT phosphorylation, which induces subcellular relocalization of the corepressor and, therefore, decreases transcriptional inhibitory activity (25). The dissociation of the corepressors from the ER may destabilize the scaffolding of the multisubunit transcriptional repressor complex containing SMRT, Sin3A, and histone deacetylases (26), thereby impairing ER-mediated repression. We show that in the presence of mitogenic growth factors the association of the corepressors N-CoR and SMRT with tamoxifen-bound ER is greatly diminished and replaced by association with the transcriptionally active coregulators SRC-1 and p/CIP. Furthermore, the presence of SRC-1 and p/CIP-associated ER correlated with the localization of the coactivators to the promoter of the ER-regulated gene *pS2*. Thus coactivator association with tamoxifen-bound ER may be regulated by mitogen-mediated subcellular redistribution of corepressors and consequently the ability of tamoxifen to growth-arrest MCF-7 cells may be greatly influenced by the presence of mitogens that activate signaling pathways capable of regulating corepressor association with ER-containing complexes.

In summary, we have shown transduction of p27^{Kip1} into hydroxytamoxifen-treated cells mimics the effects of pure antiestrogens. We also show that growth factor-induced reversal of tamoxifen arrest is accompanied by modulation of ERα-cofactor interactions. Overall, these data suggest that therapeutic strategies aimed at elevating p27^{Kip1} or inhibiting MEK should be tested for their ability to reverse antiestrogen resistance in preclinical models and in the clinic.

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