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Differential androgen sensitivity is associated with clonal heterogeneity in steroid metabolism, ornithine decarboxylase regulation and IL-1α action in mouse mammary tumor cells

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Abstract

Upon androgen deprivation, Shionogi (SC-115) mouse mammary tumors undergo phenotypic changes enabling their escape from growth dependence on androgens. Even within androgen-responsive cell populations, marked clonal heterogeneity is observed in the trophic effects of androgens. The present study compares several parameters of androgen action between three SC-115 cell clonal subpopulations exhibiting high (clone 107), low (clone S1A2) and no trophic response (clone 415) to androgens. These parameters pertain to (1) kinetics of androgen binding, (2) metabolism of 5α-dihydrotestosterone (DHT), 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol), (3) ornithine decarboxylase (ODC) activity and (4) interleukin-1α (IL-1α) action on cell proliferation. Only marginal differences in the affinity and abundance of androgen-specific binding sites were detected between the three clones. While clone S1A2 degraded DHT to 3α-diol at a much faster rate than the highly androgen-sensitive 107 cells and androgen-insensitive 415 cells, differences in the rates of intracrine conversion of 3α-diol and 3β-diol to DHT did not correlate with the ability of these steroids to stimulate cell proliferation. Induction of ODC activity at the onset of exponential growth was strongly DHT-dependent in 107 cells, whereas this dependence was markedly attenuated in androgen-hyposensitive cells. Unexpectedly, DHT strongly repressed the marked ODC induction resulting from fresh medium addition in 415 cells which show no growth response to androgens. Low IL-1α concentrations were mitogenic in all three SC-115 clones. Whereas the mitogenic action of IL-1α was completely androgen-dependent in 107 cells, this dependence was relieved in S1A2 cells, which responded to DHT and IL-1α in an additive fashion. Thus, clonal heterogeneity in the pattern of steroid metabolism within Shionogi tumors cannot solely account for loss of androgen dependence, which may rather correlate with the constitutive activation of transduction pathways controlling the expression of growth-associated genes (e.g. ODC) by serum growth factors, including IL-1α.

1. Introduction

The development of phenotypic heterogeneity within tumor cell populations is a well recognized phenomenon and is among the major problems facing cancer therapy [1–5]. The mechanisms responsible for tumor heterogeneity are multiple and include both genetic and epigenetic changes [2–5]. In the case of hormone-sensitive tumors such as prostate and breast carcinomas, progression to a hormone-independent state potentially results in some cases from selection among pre-existing, rare hormone-insensitive stem cells. However, multiple lines of evidence indicate that hormonal insensitivity arises from hormone-responsive cells through adaptive changes to a hormone-poor environment [6,7]. The nature of these changes is still poorly understood.

The Shionogi mouse mammary carcinoma (SC-115) has long been used as a model for the progression of androgen-sensitive cells to a hormone-independent state [1,8,9]. Androgen withdrawal from androgen-sensitive tumors initially causes cell proliferation arrest in

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vitro, or tumor regression in vivo. Restoration of SC-115 tumor cell proliferation by androgens can be observed only for a limited period after the onset of hormone deprivation [9,10]. Prolonged incubation under androgen-free conditions gives rise to an androgen-independent phenotype accompanied by characteristic morphological changes [8,9]. We have previously shown that a wide spectrum of growth responsiveness to 5α-dihydrotestosterone (DHT) exists even among androgen-sensitive SC-115 cell populations [1,10]. This clonal heterogeneity in trophic response affects not only the sensitivity, but also the amplitude of growth stimulation by DHT. Changes in androgen sensitivity may arise by adaptation to increases or decreases in androgen concentrations. For instance, a DHT-hypersensitive phenotype can be selected by chronic maintenance of SC-115 clonal cell populations in the presence of suboptimal androgen concentrations [10].

The mechanistic basis underlying differences in androgen responsiveness between clonal populations of SC-115 cells is unclear. No consistent changes have been observed in the kinetic and DNA transactivating properties of the androgen receptor in clones or sublines exhibiting differential hormone sensitivity [9,11] On the other hand, the possibility that changes in androgen responsiveness might arise from phenotypic differences in the rate of metabolic activation/inactivation [12] has not been investigated in SC-115 tumors. Furthermore, decreased growth sensitivity to androgens could coincide with altered sensitivity to exogenous growth factors [13] and/or to the spontaneous development of autonomy in the production of androgen-inducible peptide growth factors such as the fibroblast growth factor-like androgen-induced growth factor (FGF-8) [14,15].

To address these questions, we have compared the rate and pattern of metabolite formation from DHT, 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol) in three different clonal subpopulations of SC-115 cells respectively exhibiting high, low or no growth sensitivity to androgens. The kinetics of androgen binding, the induction of ornithine decarboxylase (ODC) activity and the trophic effect of the cytokine interleukin-1α (IL-1α), have also been compared between these clones. We are reporting marked differences in the pattern of metabolism of 5α-androstanes (DHT, 3α-diol and 3β-diol) between the three SC-115 clones, which correlate poorly, however, to their differential growth sensitivity to androgens. Moreover, differential patterns in the kinetics of ODC activity were associated with the attenuation of growth dependence on androgens. Finally, clonal heterogeneity in the mitogenic effect of DHT is associated with decreased androgen dependence for the stimulation of cell proliferation by IL-1α, which is shown for the first time to act as a mitogen in a mammary carcinoma cell line.

2. Materials and methods

2.1. Reagents

DHT, 3α-diol, 3β-diol, testosterone (T), androst-4-ene-3,17-dione (4-dione), androst-5-ene-3β-17β-diol (5-diol) and dehydroepiandrosterone (DHEA) were obtained from Steroids (Wilton, NH). L-[1-^{14}C]ornithine (58 mCi/mmol), [1,2,4,5,6,7,16,17-^{3}H(N)]-DHT (142.2 Ci/mmol), 5α-[9,11-^{3}H]-androstane-3α,17β-diol (56 Ci/mmol), 5α-[1,2,(N)-^{3}H]-androstane-3β,17β-diol (48.6 Ci/mmol) and [17α-methyl-^{3}H]-17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one ([^{3}H]R1881) (87 Ci/mmol) were obtained from Dupont/New England Nuclear (Lachine, Que., Canada). The radiochemical purity of these steroids was >98% and periodically checked by thin layer chromatography. The antiandrogen hydroxyflutamide (OH-FLU) was kindly provided by Dr. T.L. Nagabuschan and Dr. R. Neri (Schering Plough, Kenilworth, NJ). Collagenase (from *Clostridium histolyticum*) and β-glucuronidase were purchased from Boehringer Mannheim (Laval, Que. Canada). Fetal calf serum (FCS) was obtained from HyClone (Logan UT). IL-1α, hyaluronidase II, while all other reagents for cell culture and biochemicals were obtained from Sigma (St. Louis, MO).

2.2. Cell culture and cloning

Shionogi male mice bearing androgen-sensitive SC-115 tumors were provided by Dr. Y. Lefebvre (Department of Biochemistry, University of Alberta, Calgary, Canada.). Cells were dispersed from tumors by a 2 h treatment at 37°C in HEPES buffer (25 mM HEPES, pH 7.2; 137 mM NaCl; 5 mM KCl; 0.7 mM Na_2HPO_4; 10 mM D-glucose) containing 510 U/ml collagenase, 550 U/ml hyaluronidase II and 4% (w/v) bovine serum albumin (fraction V). Dispersed cells were collected by centrifugation (500 × *g* for 10 min), washed twice by suspension in Eagle's minimal essential medium (MEM) containing 5% (v/v) dextran-coated charcoal-treated FCS (DCC-FCS), MEM non-essential amino acids, 10 U/ml penicillin, 50 μg/ml streptomycin and 10 μM DHT. Stock DHT solutions were prepared in EtOH and added to media to yield a final EtOH concentration <0.01% (v/v). Cells were plated in the same medium in 75 cm^2 flasks at a density of 10^6 cells/flask under a 5% CO_2 humid atmosphere at 37°C. The medium was changed weekly. Cells were subcultured when nearly confluent by gentle digestion in a solution of 0.1% pancreatin (Flow,

Rockville, MD) in HEPES buffer containing 3 mM EDTA.

Soft agar cloning and selection of clonal SC-115 subpopulations with different androgen sensitivity were performed as described [1,10]. Three clones were selected for the present experiments, with high (clone 107), low (clone S1A2) and no growth sensitivity (clone 415) to androgens. Cell clones were maintained in MEM medium supplemented as above except for clone 415 which was routinely grown without DHT. The medium was changed every third day and the cells were subcultured as described above.

2.3. Effect of hormones on cell proliferation

Cell clones were seeded in 24-well culture plates at a density of 2×10^4 cells/well in MEM containing 2% (v/v) DCC-FCS medium and the above-described supplements except DHT. Test compounds were added 24 h after plating. Fresh experimental medium was replaced every other day. After a 12 day incubation, cell proliferation was measured by a colorimetric DNA assay [16] or by electronic sizing [17] using a Model ZM Coulter counter (Coulter Electronics, Hialeah, FL). The half-maximal effective concentration for growth stimulation (EC_{50}) was calculated using an iterative least-squares regression [18].

2.4. Determination of androgen-specific binding sites in intact cells

Cells were seeded in steroid-free MEM supplemented as described above in 24-well plates at a density of $1.0–1.5 \times 10^5$ cells/well. Medium was removed 24 h after plating and [^{3}H]R1881 (10^{-12}–10^{-8} M) was added to triplicate dishes in serum-free MEM containing 0.1% (w/v) of fatty acid-free bovine serum albumin and 1 μM triamcinolone acetonide. Nonspecific binding was determined in parallel dishes incubated in the same medium containing 1 μM of unlabeled R1881. After 1 h of incubation at 37°C, medium was carefully removed and cells were washed 3 times with 1 ml ice-cold buffer (5 mM sodium phosphate, pH 7.4, 120 mM sucrose, 10% (v/v) glycerol, 0.5% (w/v) fatty acid-free bovine serum albumin) as described [19]. EtOH (0.5 ml) was then added to the wells and after an overnight incubation at room temperature, radioactivity of the extracts was determined by liquid scintillation spectrometry. Kinetic analysis of binding parameters was performed as described [20].

2.5. Determination of steroid metabolism in intact cell cultures

Cells were plated in 24-well plates at a density of $1.0–1.5 \times 10^5$ cells/well in 1 ml of DHT-free MEM medium supplemented with 2% DCC-FCS as described above. Twenty-four hours after plating, 5 nM of either [^{3}H]DHT, [^{3}H]3α-diol or [^{3}H]3β-diol were added in triplicate at a final EtOH concentration of 0.1% (v/v). Incubation was stopped by the addition of 20 mM acetic acid. Medium was removed, transferred into 16×150 mm glass tubes and 5 ml of EtOH were then added.

Steroid extraction and analysis were then performed essentially as described [21]. Briefly, after centrifugation of the EtOH-containing medium sample, the pellet was washed twice with 2 ml EtOH and the combined EtOH extracts were then evaporated to dryness under a stream of N_2. Prior to use, reversed-phase C18 columns (Amprep C18, Amersham Canada, Oakville, Ont., Canada) were conditioned by consecutive applications of 10 ml MeOH, 10 ml H_2O and 10 ml of MeOH:H_2O (5:95, v/v; mixture A). The extracts were then solubilized in 2 ml of mixture A and applied onto the C18 columns. After washing the columns with 2 ml of mixture A, 5 ml of MeOH:H_2O (40:60, v/v) were added to elute the glucuronide derivatives. Addition of 5 ml of MeOH:H_2O (85:15, v/v) resulted in the elution of the unconjugated (free) steroids. Both fractions were dried in a Speed-Vac rotary evaporator (Savant Instruments, Farmingdale, NY, USA). The dried glucuronide fraction was solubilized with 1 ml of 0.1 M phosphate buffer (pH 6.5) and hydrolyzed with β-glucuronidase under constant agitation for 72 h at 37°C with two daily additions of 4 U of β-glucuronidase as described [21,22]. The steroids thus released were extracted twice with ethyl ether, the organic phase was evaporated and the extract then redissolved in 0.5 ml MeOH:H_2O (1:1, v/v). High performance liquid chromatography analysis was performed using a Waters model 510 chromatograph (Waters Instruments, Milford, MA) equipped with a Novapak C18 column (8×100 mm; Waters Instruments) with 4 μm particle size. The radioactive samples were eluted isocratically using a solvent system consisting of H_2O:MeOH:tetrahydrofuran-acetonitrile (50:35:10:5, v/v) at a flow rate of 1.5 ml/min. The scintillation cocktail was simultaneously pumped at a flow rate of 3 ml/min and radioactivity was determined with an on-line Berthold LB-506 radioactivity detector (Wildbad, Germany). The amount of radioactivity incorporated into each of the individual metabolites was calculated from the product of the total radioactivity recovered by the percentage of conversion to each radioactive metabolite, as described [23].

2.6. Determination of ODC activity

Cells were plated in 100 mm dishes at a density of 5×10^5 cells/dish in MEM supplemented as above with 5% DCC-FCS in the absence of DHT. After 24

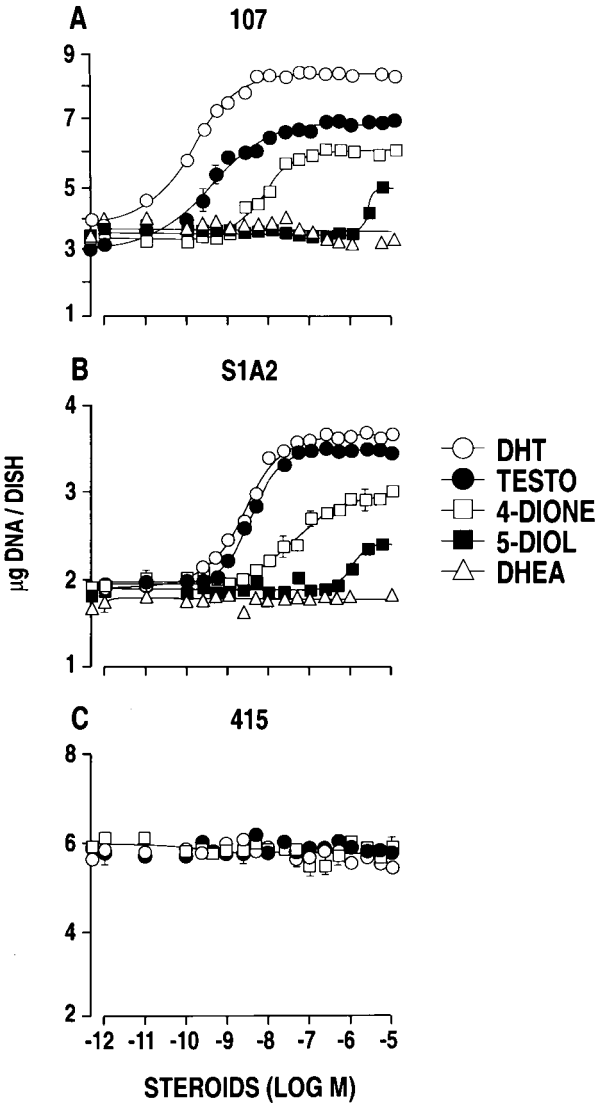

Fig. 1. Effect of various androgens or androgen precursors on growth in clonal cell populations with high (107), low (S1A2) and no androgen sensitivity. DNA content per culture was determined in clones 107 (A), S1A2 (B) and 415 (C) after a 12 day incubation with the indicated concentrations of DHT (○), T (●), 4-dione (□), 5-diol (■) and DHEA (△). Values are the mean ± SD of triplicate determinations.

h, 10 nM DHT or the vehicle (0.01% EtOH) was added to triplicate cultures. After incubation for the indicated time intervals, cells were harvested with 0.05% trypsin/0.02% EDTA (w/v), centrifuged at 2000 × *g* for 90 s at 4°C, washed with 10 ml of ice-cold phosphate-buffered saline (0.9 mM $CaCl_2$; 0.5 mM $MgSO_4$; 2.7 mM KCl; 140 mM NaCl; 1.5 mM KH_2PO_4; 8.1 mM Na_2HPO_4, pH 7.3) and resuspended in 200–300 µl of ice-cold buffer A (50 mM Tris–HCl, pH 7.5; 100 µM EDTA; 5 mM dithiothreitol). The homogenate was centrifuged at 12,000 × *g* for 20 min at 4°C and the supernatant was saved for the determination of ODC activity [24]. Protein content was determined according to the method of Bradford [25] using bovine gamma globulin as standard. Results are expressed as nmol $^{14}CO_2$ formed per h per mg of cytosolic protein.

Table 1
EC_{50} values of the growth response to various androgens and androgen precursors in Shionogi cell clones with high (107) and low (S1A2) androgen sensitivity; values are the mean ± SD for triplicate determinations

Steroid	EC_{50} (nM)	
	Clone 107	Clone S1A2
DHT	0.13 ± 0.02	4.0 ± 1.7[a]
T	0.40 ± 0.07[b]	4.5 ± 0.1[a]
4-dione	7.0 ± 0.9[b]	44 ± 9[a,b]
3α-diol	0.30 ± 0.01[c]	12.0 ± 1.5[a,b]
3β-diol	0.20 ± 0.02	7.0 ± 0.8[a,c]
5-diol	> 1000[b]	> 1000[b]

[a] Significantly different ($P < 0.01$) from value for clone 107.
[b] Significantly different ($P < 0.01$) from value for the corresponding DHT-treated group.
[c] Significantly different ($P < 0.05$) from value for the corresponding DHT-treated group.

2.7. Calculations and statistical analysis

Statistical significance was determined according to the multiple range test of Kramer [26]. All results are expressed as mean ± SD for determinations from triplicate cell cultures. When no error bars are shown, the symbol size exceeds the experimental deviation.

3. Results

3.1. Differential sensitivity of cell proliferation to androgens and androgen precursors

As shown in Fig. 1 and Table 1, the three selected clones showed a marked heterogeneity in their growth sensitivity to various androgens (DHT,T) or androgen precursors (4-dione, 5-diol and DHEA). Proliferation of clone 415 was completely insensitive to any of these steroids, as expected from the selection method used. Furthermore, the proliferation rate of 415 cells was 2- to 3-fold higher than in clones 107 and S1A2 under androgen-depleted conditions. On the other hand, all steroids tested except DHEA significantly stimulated cell proliferation in both clones 107 and S1A2, in the order of potency DHT > T > 4-dione ≫ 5-diol. The weak or absent effect of the adrenal C19 androgen precursors 5-diol and DHEA in these short-term (12 day) incubations is consistent with their previously reported slow kinetics of action in SC-115 cells [27]. The EC_{50}

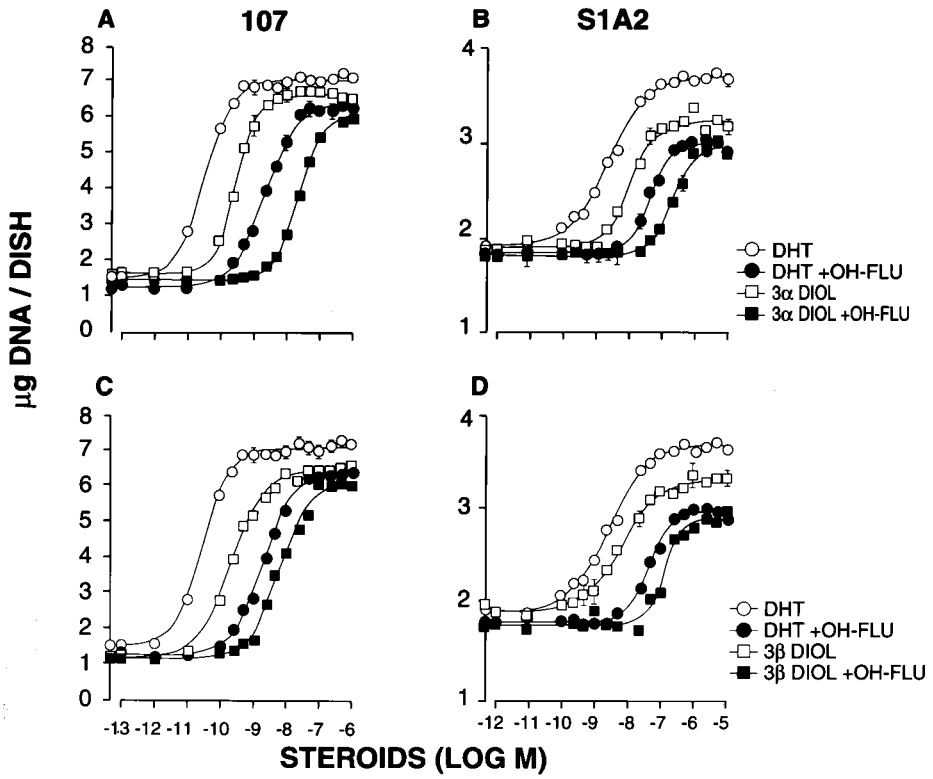

Fig. 2. Effect of DHT, 3α-diol and 3β-diol on cell proliferation in Shionogi clones 107 and S1A2. DNA content per culture was determined in 107 cells (A, C) and S1A2 (B, D) after a 12 day incubation with the indicated concentrations of DHT (○, ●), 3α-diol (panels A and B, □, ■) or 3β-diol (panels C and D, □, ■) in the presence (solid symbols) or absence (plain) of the antiandrogen hydroxyflutamide (OH-FLU, 1 μM). Values are the mean ± SD of triplicate determinations.

values observed for the mitogenic effect of DHT, T and 4-dione were respectively about 30-, 11- and 6-fold higher for S1A2 than 107 cells (Table 1). Moreover, the maximal fold stimulation of cell proliferation induced by DHT, T and 4-dione was about 2-fold higher in 107 than in S1A2 cells. Thus, clone S1A2 can be described as androgen-hyposensitive as compared with 107 cells both (i) in terms of the effective concentration of steroid required for half-maximal growth stimulation and (ii) in the maximal effect induced by androgen addition.

Interestingly, two metabolites of DHT, namely 3α-diol and 3β-diol, were also quite effective mitogens in clones 107 and S1A2 (Fig. 2 and Table 1). As shown by its competitive reversal with the pure antiandrogen hydroxyflutamide (OH-FLU) [28], the stimulatory effect of DHT as well as of 3α-diol and 3β-diol was mediated by the androgen receptor. As expected, cell proliferation in clone 415 was insensitive to 3α-diol and 3β-diol (data not shown).

3.2. Androgen-specific binding sites in Shionogi clones 107, S1A2 and 415

We next assessed whether the differential growth sensitivity of the three clones studied arose from heterogeneity in the binding parameters of the androgen receptor expressed in these cells [11]. Using the nonmetabolizable androgen [^{3}H]R1881 as ligand, the number of androgen-specific binding sites was shown to be similar in both androgen-sensitive clones (2.8 and 2.5 fmol/μg DNA for clones 107 and S1A2, respectively) and only 30–35% lower (1.8 fmol/μg DNA) in clone 415. Likewise, only minor differences could be found in the K_D values of [^{3}H]R1881 binding between these three clones (data not shown).

3.3. Metabolism of DHT, 3α-diol and 3β-diol in clones 107, S1A2 and 415

One possible cause for decreased growth sensitivity to androgens might be an increased rate of steroid catabolism. We thus determined the kinetics and pattern of metabolism of [^{3}H]DHT, [^{3}H]3α-diol and [^{3}H]3β-diol (each at 5 nM) in the three SC-115 clones. These studies were also designed to evaluate whether 3α-diol and 3β-diol could exert their mitogenic stimulation directly or following metabolic transformation to DHT through the action of the appropriate dehydrogenase(s) [29].

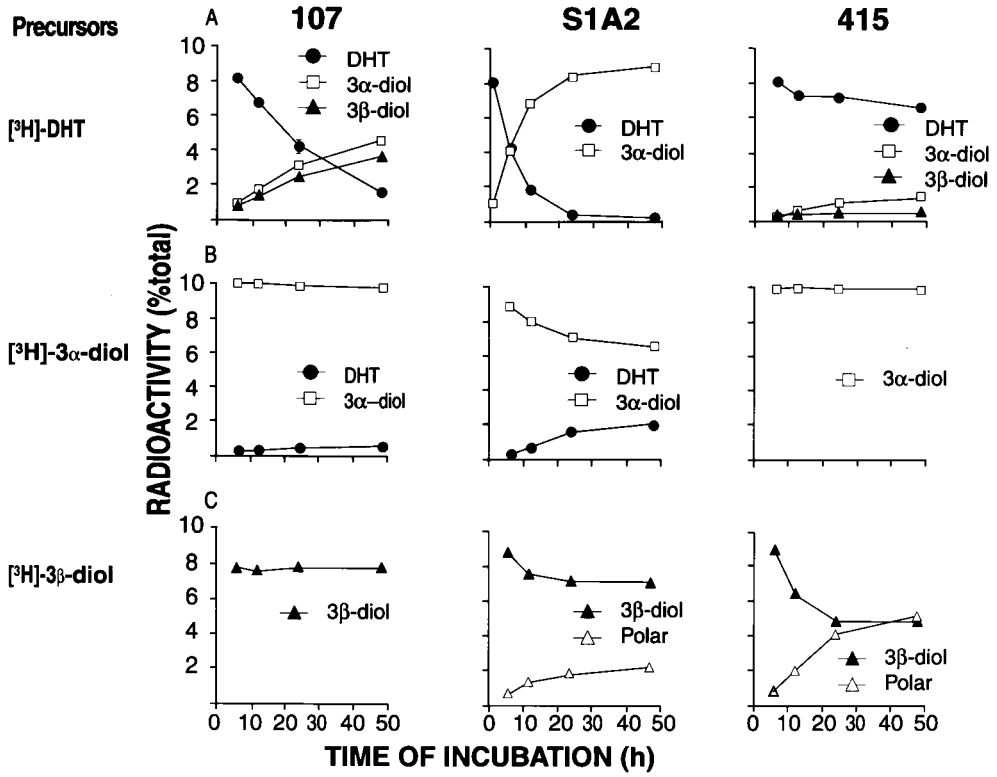

Fig. 3. Time course of metabolism of [^{3}H]DHT, [^{3}H]3α-diol and [^{3}H]3β-diol in Shionogi clones 107, S1A2 and 415. Clones 107 (left panels), S1A2 (center panels) and 415 (right panels) were incubated for the time indicated with (A) 5 nM [^{3}H]DHT, (B) 5 nM [^{3}H]3α-diol or (C) [^{3}H]3β-diol and medium was analyzed for radioactively labelled steroids as described in Materials and methods. Metabolites found are identified as DHT (●), 3α-diol (□), 3β-diol (▲) or polar metabolites, including glucuronides (△). Values are the mean ± SD of triplicate determinations.

As shown in Fig. 3A, [^{3}H]DHT was differentially metabolized through 3α- and/or 3β-reduction in the three clones examined. The most striking difference was in the extent of DHT degradation found in the androgen-sensitive clones 107 and S1A2. In fact, less than 3% of the DHT initially added remained intact in S1A2 cells at the end of a 24 h incubation, whereas 53% of the precursor could be recovered in 107 cells after the same period. While clone 107 degraded DHT to both 3α-diol and 3β-diol (32 and 25% of total radioactivity, respectively, after 24 h), 80% of total radioactivity was recovered as 3α-diol after the same period in S1A2 cells, with no evidence of 3β-diol formation in the latter clone. Thus, 3α-hydroxysteroid dehydrogenase activity was considerably higher in S1A2 than 107 cells, while 3β-hydroxysteroid dehydrogenase activity was detected only in clone 107. Clearly, steroid degradation could not explain the growth independence from DHT found in clone 415 since these cells exhibited an even lower rate of DHT catabolism than clone 107 (72, 10 and 5.5% of total radioactivity being recovered as DHT, 3α-diol and 3β-diol, respectively, after 24 h). Interestingly, after a 3 month period of androgen deprivation, a 2-fold decrease in the rate of 3α-diol formation and a loss of 3β-diol formation from DHT were observed in 107 cells, together with a complete loss of growth responsiveness to androgens (results not shown). Thus, although phenotypic differences in DHT metabolism were stable and reproducible under steady-state conditions, chronic androgen deprivation could lead to significant changes in the pattern of DHT metabolism in a given clonal cell subpopulation.

Catabolism of [^{3}H]3α-diol was extremely limited (< 5%) or undetectable in clones 107 and 415, respectively, while S1A2 cells converted about 20% of the precursor to DHT after 48 h (Fig. 3B). On the other hand, an important fraction of [^{3}H]3β-diol was metabolized to glucuronide derivatives (up to 22 and 51% after 48 h in S1A2 and 415 cells, respectively) (Fig. 3C). In marked contrast, metabolism of [^{3}H]3β-diol was virtually absent in the most androgen-sensitive clone (107 cells).

3.4. Clonal heterogeneity of ODC regulation

An often dramatic induction of ODC activity is associated with the onset of macromolecular synthesis in most mammalian cells stimulated to progress into the cell cycle, especially at the G_1/S transition [30,31].

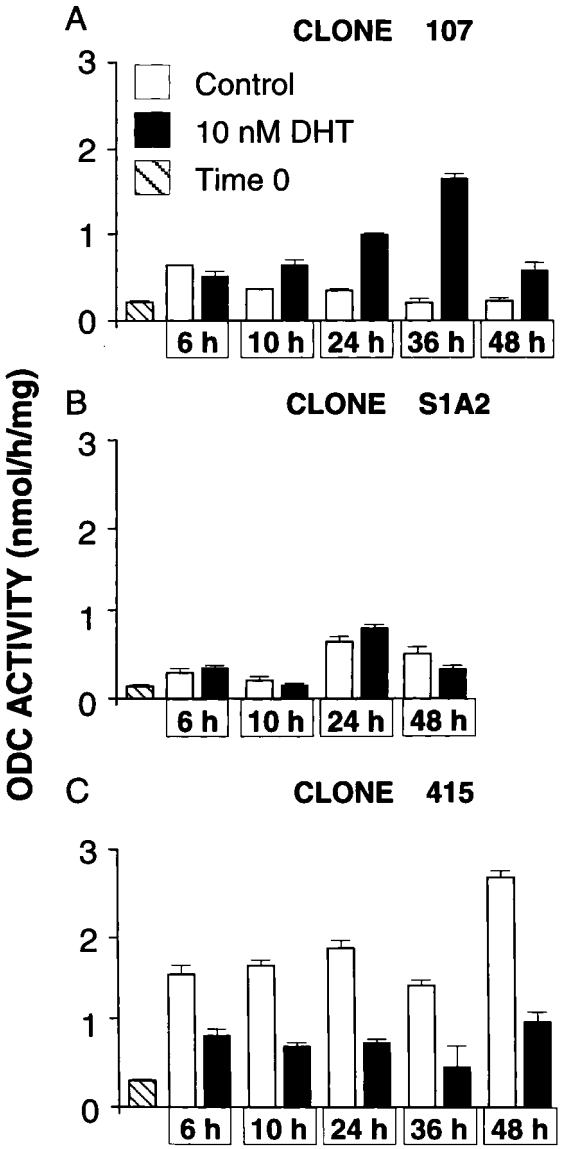

Fig. 4. Time course of induction of ODC activity in Shionogi clones 107, S1A2 and 415. At time zero, 10 nM DHT (solid bars) or the EtOH vehicle (0.1%, v/v) (plain bars) were added to (A) 107, (B) S1A2, or (C) 415 Shionogi cell clones and cells were harvested at the indicated time for the determination of ODC activity (cf. Materials and methods). Values are the mean ± SD of triplicate determinations.

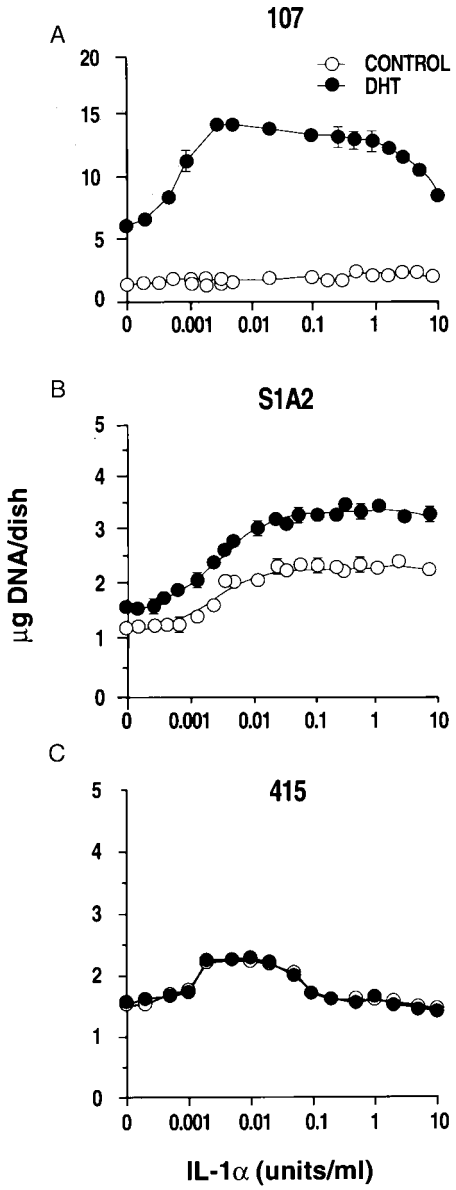

Fig. 5. Effect of IL-1α on cell growth in 107 and S1A2 Shionogi clones 107, S1A2 and 415. DNA content per culture was determined in (A) 107, (B) S1A2 and (C) 415 cells after a 12 day incubation with the indicated concentration of IL-1α in the presence (•) or absence (○) of 0.3 nM DHT. Values are the mean ± SD of triplicate determinations.

Moreover, ODC expression is stimulated by androgens in tissues where these hormones exert trophic effects such as the rat ventral prostate and mouse kidney [32,33]. Because an early increase in ODC expression often precedes the hormonal stimulation of DNA synthesis, we compared the effect of DHT on ODC activity levels between the three clones as a potential parameter of phenotypic heterogeneity in the mechanism of androgen action. As shown in Fig. 4, the sole addition of fresh, DHT-free medium rapidly (≤6 h) increased ODC activity in all three clones. However, this DHT-independent ODC induction quickly subsided in the most androgen-sensitive clone (107 cells) and the enzyme activity went back to its original value within the next 30 h (Fig. 4A). On the other hand, the basal induction of ODC activity had a biphasic pattern in S1A2 cells, with maxima detected at approximately 6 and 24 h (Fig. 4B). In contrast, the early induction of ODC by DHT-free medium in the androgen-insensitive 415 cells was quite stable through time, with evidence of a secondary increase up to 2.5 nmol/h/mg at 48 h (Fig. 4C).

As predicted from its strong mitogenic effect in clone 107, DHT progressively increased ODC activity in these cells up to a maximal 8-fold increase observed 36 h after androgen addition, followed by a rapid decrease between 36 and 48 h. On the other hand, DHT had little effect on the biphasic profile of ODC induction observed in S1A2 cells after fresh medium addition. Surprisingly, DHT markedly repressed the basal induction of ODC activity in 415 cells, which are growth-insensitive to androgens, as early as 6 h after steroid addition. Thus, DHT differentially modulates the early induction of ODC activity which precedes the onset of exponential cell proliferation in three SC-115 clones displaying high, low and no growth sensitivity to androgens.

3.5. Clonal heterogeneity of proliferative response to IL-1α

IL-1α is known to inhibit cell proliferation in several tumor types, including human mammary carcinomas [34,35]. In pilot experiments, however, we unexpectedly found that IL-1α potently stimulated cell proliferation in some SC-115 cell sublines. It was thus of interest to examine whether the mitogenic response to a peptide growth factor such as IL-1α displayed clonal heterogeneity with respect to differential growth sensitivity to androgens.

As shown in Fig. 5, IL-1α significantly influenced cell proliferation in the two androgen-dependent clones in a concentration-dependent fashion, but with markedly different characteristics between these sublines. The marked mitogenic response to IL-1α observed in clone 107 was strictly androgen-dependent, unlike in S1A2 cells, in which DHT (at the suboptimal concentration of 0.3 nM) and IL-1α both independently increased cell proliferation in an additive fashion (Fig. 5A,B). Increasing concentrations of IL-1α (up to 1 U/ml) had a mitogenic effect in clone 107, whereas concentrations >1 U/ml were less effective. IL-1α exerted its mitogenic effect over a much wider range of concentrations in S1A2 cells, with an EC_{50} of about 0.02 U/ml. In androgen-independent 415 cells, IL-1α had a weak, biphasic mitogenic effect, with a maximal 40% increase in cell mass after 12 days (Fig. 5C). These data show for the first time that IL-1α is mitogenic in a murine mammary carcinoma and that this effect is strictly androgen-dependent in highly androgen-sensitive cells, whereas decreased androgen sensitivity is associated with a loss in the DHT requirement for growth enhancement by the cytokine.

4. Discussion

We have compared several parameters related to the androgenic control of cell proliferation between cell clones derived from a single mouse mammary tumor to obtain further insight into the mechanism underlying escape from androgen dependence. For this purpose, we have selected SC-115 clones exhibiting high (or normal), low and no growth response to androgens. The lack of well-marked differences in the characteristics of androgen-specific binding between these three clones agrees with previous studies suggesting that the degree of growth responsiveness to androgens is unrelated to androgen receptor levels [11]. On the other hand, our data suggest that decreased mitogenic action of androgens could be correlated with other clonal characteristics which might underlie escape from androgenic control of cell proliferation.

The ≧20-fold higher EC_{50} value found for the mitogenic action of DHT in androgen-hyposensitive S1A2 cells as compared with 107 cells could partly result from their markedly higher reductive 3α-hydroxysteroid dehydrogenase activity and thus from a more rapid decrease in actual DHT concentration. However, differences in pattern of 3α- and 3β-diol metabolism between the two androgen-responsive clones did not correlate with the nearly 40- and 30-fold differences, respectively, in the EC_{50} value for the mitogenic effect of these steroids. Thus, considerable heterogeneity in the pattern of steroid metabolism may exist within a tumor cell population, but other factors must be involved in the differential androgen sensitivity of SC-115 clones.

Competitive reversal of the mitogenic effect of 3α- and 3β-diol by OH-FLU unequivocally demonstrates that 5α-androstane-3,17-diols, generally considered as inactivation products of DHT, can indeed provide a strong androgenic stimulation in SC-115 tumor cells. The mitogenic effect of 3α- and 3β-diol might be due to their prior conversion to DHT. In the most androgen-sensitive clone, however, very little DHT was synthesized from 3-α-diol, whereas DHT formation from 3β-diol, if any, was below detection limits. On the other hand, 3α- and 3β-diol can bind to the androgen receptor with a relative affinity of about 8 and 3.5%, respectively, as compared with DHT [36], which would be consistent with their relative effectiveness as mitogens through a direct, androgen receptor-mediated action (cf. Table 1). The androgenic action of 3α-diol has been thought to result from its reversible conversion to DHT [29], while 3β-diol has been described as an estrogen in some systems [36]. The present results lead to the important suggestion that these steroids might activate a potent androgenic trophic response without prior conversion to DHT. The generality of the androgenic potential of 3α- and 3β-diol will await the assessment of their biological activity in other androgen-responsive systems.

Androgens strongly induced ODC activity in andro-

gen-sensitive cells, in agreement with in vivo observations in castrated male mice bearing androgen-dependent SC-115 tumors [37]. This progressive induction of ODC by DHT with a maximum observed at 36 h is not unlike that described in vivo in the androgen-treated mouse kidney [32], which occurs primarily at the transcriptional level [38]. On the other hand, the rapid increase in ODC activity observed in all clones 6 h after fresh medium addition is typical of that induced by peptide growth factors [39] and likely results from posttranscriptional mechanism(s) such as a decrease in ODC mRNA turnover, translational activation and/or decreased enzyme turnover [39–41]. However, while this early ODC induction subsequently vanished in 107 cells, a secondary increase in ODC activity was clearly observed in androgen-hyposensitive cells, which roughly paralleled the DHT-dependent increase in ODC observed in 107 cells. The fact that this second phase of ODC induction was virtually DHT-independent suggests that in the androgen-hyposensitive phenotype, serum mitogens might activate ODC gene expression in the absence of androgens. Thus, androgens might play a permissive role in ODC induction by medium growth factors and a lesser dependence on androgens might more or less completely relieve cells from this 'priming' effect. A similar role for estrogens has been noted in their interplay with growth factors for synergistic activation of gene expression [43,44].

An unexpected finding was the marked down-regulation of ODC activity by DHT observed in the androgen-insensitive clone. Androgens can still regulate the expression of a transfected gene under control by an androgen-inducible promoter in SC-115 cell variants having lost a trophic response to androgens [9]. The present observation further supports the notion that the pathway of androgenic stimulation is still functional in 'androgen-insensitive' SC-115 cells. How DHT might negatively regulate ODC activity with rapid kinetics in androgen-independent cells remains to be determined. In rat Sertoli cells, androgens repress constitutive ODC gene transcription and thus rapidly decrease enzyme activity due to the high rate of ODC mRNA turnover [45]. Such a mechanism could also apply here if ODC mRNA expression was constitutively elevated in 415 cells. Indeed, the nearly maximal increase in ODC activity observed in 415 cells within 6 h after medium addition is consistent with the rapid mobilization of a translationally inactive ODC mRNA pool [46,47].

Finally, we are reporting for the first time a mitogenic action for IL-1α in a mammary tumor. This finding is in striking contrast with the potent growth inhibitory effect of this cytokine in human breast cancer cells [34,35]. Moreover, our data clearly show that androgens are absolutely required for this response in a highly androgen-responsive SC-115 clone. Whether this requirement reflects an androgen dependence for IL-1 receptor expression or for downstream intracellular signalling by the cytokine is unknown. Nevertheless, our observation that the strict androgen dependence for both the mitogenic action of IL-1α and ODC induction is lost in androgen-hyposensitive cells suggests that transition to androgen dependence may coincide with the basal activation of genes involved in proliferation control. The androgen-dependent stimulation of the autocrine production of FGF-8 has been described in SC-115 cells [15,42] and the constitutive secretion of polypeptide growth factors appears to be involved in the transition to androgen insensitivity [42]. In this context, the sustained, androgen-independent increase in ODC activity observed in androgen-insensitive cells (cf. Fig. 4C) could reflect the build-up of such peptide mitogen(s) in the extracellular medium. In fact, the present data agree with recent evidence indicating that progression to androgen independence might be accompanied by a differential responsiveness to peptide growth factors [13]. Thus, an increased rate of steroid degradation, such as in the case of DHT metabolism in S1A2 cells, might be only an accessory factor leading to androgen hyposensitivity.

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