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Regulation of human renal adenocarcinoma cell growth by retinoic acid and its interactions with epidermal growth factor

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Regulation of human renal adenocarcinoma cell growth by retinoic acid and its interactions with epidermal growth factor. Retinoic acid (RA) is a natural derivative of vitamin A which regulates the growth and differentiation of epithelia. We have previously proposed that RA participates in compensatory kidney growth and reported that RA inhibits rat mesangial cell growth. This paper describes the effects of RA on a human renal adenocarcinoma cell line (PAD) under different growth conditions, and its interactions with epidermal growth factor (EGF). PAD cells were shown to express RA receptors α and β by Northern blot analysis. In serum free cultures, addition of RA (10^{-7} M) markedly increased thymidine incorporation by PAD cells (155 ± 7% mean ± SE vs. control in 6 separate experiments; $P < 0.0001$). RA also caused a significant increase in thymidine incorporation by PAD cells under conditions of rapid growth in serum supplemented medium (115 ± 2% vs. control; $P < 0.001$). RA by itself was unable to reverse contact inhibition of PAD cell growth (NS vs. control), but it synergistically enhanced the mitogenic effect of EGF on confluent monolayers (110 ± 0.6% vs. EGF alone; $P < 0.05$). Northern blot analysis demonstrated that PAD cells express EGF receptor mRNA, and this was not significantly modified by the addition of RA. Growth arrested (serum starved) PAD cells expressed RAR-α mRNA which was up-regulated eightfold at three hours following the addition of 10% FCS. Thus, our data show that RA is directly mitogenic for serum starved human renal adenocarcinoma cells and that it exerts complex modulation of cell growth in the presence of EGF and serum components.

Cell replication and proliferation constitutes a complex process which is seen in normal and in pathological states. Remnant kidney hypertrophy following uninephrectomy is one of the few physiological occasions during which an organ grows in adulthood. Although it is known that in compensatory kidney growth increase in cell size is more important than cell division, the factors modulating this process are not completely understood [1].

Among the mechanisms of induction of kidney hypertrophy, the participation of renotropic factors has been widely studied [2]. However, no specific renal growth factor has been successfully characterized as the major determinant for compensatory kidney growth. The renal effects of polypeptide growth factors have been reviewed [3]. One of them, epidermal growth factor (EGF) has been related to compensatory kidney growth [4]. Glomerular mesangial cells respond to EGF [5] and receptors for this peptide have been identified in the basolateral side of tubular cells [6, 7]. The kidney is able to produce EGF and the main site of renal EGF production has been identified as the ascending limb of Henle [8, 9].

Proteins are, however, not the only factors involved in compensatory kidney growth. Previous studies with human kidney donors established a role for retinoids in the post-nephrectomy adaptation process [10]. A local modification of the cytosolic receptor for retinoic acid (CRABP, cellular retinoic acid binding protein) was observed as well as systemic modifications of retinol and retinol binding protein [10]. Since retinoids are essential for growth and maintenance of epithelia [11, 12], we postulated that retinoic acid (RA) may participate in human kidney hypertrophy [10]. However, apart from fibroblasts, few data are available describing the effects of RA on kidney cells. Recently, two reports have analyzed the effects of RA on animal-derived renal epithelial cell lines. The canine MDCK cells responded to RA addition with increased dome formation [13], and we observed changes in cell cycle and protein content of the rabbit RK13 cell line [14]. We have now extended these observations to a human renal adenocarcinoma cell line, including a study of the role of RA and its interactions with EGF in cell growth.

Methods

Cell culture

PAD cells were obtained from an axillary lymph node metastasis of an adenocarcinoma of renal origin. Although of tumoral origin, PAD cells conserve contact inhibition properties [15]. PAD cells were used between passages 20 and 40. For maintenance, cells were cultured in plastic flasks with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (GIBCO, Grand Island, New York, USA) and maintained at 37°C in 5% CO_2. PAD cell assays were performed in 96 well trays (Disposable Products). After trypsinization 5000 cells/well were randomly plated in 10% FCS supplemented RPMI 1640 and used for assay both before (day 1) and after confluent

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monolayers were obtained (day 4). PAD cells were also analyzed at days 1 and 4 in serum free medium in order to assess the effect of the retinoic acid and EGF in growth factor free medium as described by VanZoelen, vanOostwaard and de Laat [16].

Electron microscopy

PAD cell cultures were rinsed twice in 0.1 M phosphate buffered saline pH 7.4 and detached from plastic flasks with a cell scraper. The cell pellet was fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide. An albumin block of the cells was prepared by adding one drop of 20% FCS to one drop of 5% glutaraldehyde followed by centrifugation. The pellet was then fixed in 2.5% glutaraldehyde, processed through graded acetones and embedded in epon Araldite. Thin sections were examined in the electron microscope.

Immunohistochemistry

PAD cells were cultured on glass slides, fixed in 4% paraformaldehyde for 10 minutes, and permeabilized in 0.2% Triton X-100 for two minutes. The fixed cells were then incubated with specific primary antibodies followed by a biotinylated second antibody and horseradish peroxidase-conjugated streptavidin. Bound peroxidase enzyme was visualized using the diaminobenzidine substrate. Monoclonal antibodies used were: CAM 5.2, anticytokeratins 8, 18, 19 (Becton Dickinson, New Jersey, USA); 34 β E12, anticytokeratins 1, 5, 10, 14 (Dako, Copenhagen, Denmark); and anti-epithelial membrane antigen (EMA) (Dako). Rabbit polyclonal antibodies used were: anticarcinoembryonic antigen (CEA); antivimentin; and antiCD20 (pan B cells) (all from Dako).

Growth factors

RA, EGF and retinol (vitamin A) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). RA (10^{-7} M), EGF (4 ng/ml) and retinol (10^{-6} M) were added 24 hours before harvesting for thymidine incorporation assay. Other concentrations were used as specified.

DNA synthesis

DNA was monitored by thymidine incorporation as previously described [17]. A 16 hour pulse of 0.5 μCi/well ^{3}H radiolabeled thymidine (27 Ci/mmol; Amersham International, Amersham, UK) was used. One rinse (200 μl) with warm PBS and two rinses with 5% trichloroacetic acid were performed after removing the medium. Two successive 50 μl aliquots of 0.2 NaOH were added and left at 37°C for 10 minutes, then added to scintillation vials containing 100 μl of 0.2 N HCl and 3.5 ml of scintillation fluid. The counts per minute were determined with a LKB WALLACE 1214 (LKB, Upsala, Sweden) beta counter. Twelve replicates were performed in each experiment.

Cell cycle analysis

Cell cycle analysis of PAD cells was performed by flow cytometry as previously described [14] using propidium iodide dye [18]. Briefly, a single cell suspension in 2 ml PBS with 10% FCS was treated with 0.5 ml of propidium iodide (250 μg/ml in 1% NP-40) for 10 minutes in the dark on ice in the presence of 25 μg of RNase. Cells were passed through a 50 μm mesh to remove cell aggregates, and the fluorescence measured within two minutes on an EPICS 752 (Coulter Electronics, Miami, Florida, USA) flow cytometer. The EPICS argon laser was tuned to a wavelength of 488 nm, at a power of 600 mW, for excitation of the propidium iodide dye. Size measurements of the cells was based on light scatter collected in the forward angle (FALS) direction. DNA fluorescence emissions were measured through a 630 nm long pass filter. Fluorescence versus FALS scattergrams and single color histograms of the DNA staining pattern were analyzed. Cell cycle analysis based on DNA content distribution was performed using the PARA 1 software package. This program assumes Gaussian distributions for both the Go/G1 and G2+M cells and calculates the percentage of cells in these peaks. The percentage of cells in S phase is then measured by subtracting the other phases from the total.

RNA preparations and Northern blot hybridization

PAD cells grown in 175 cm^2 plastic flasks were harvested following trypsinization and RNA prepared by acid guanidinium thiocyanate-phenol-chloroform extraction [19]. Northern blotting of RNA samples used standard methods of denaturation with glyoxal and dimethylsulphoxide, size fractionation on 1.2% agarose gels and capillary blotting onto Hybond-N membranes (Amersham) [20]. Membranes were hybridized at 42°C overnight with ^{32}P-labeled cDNA probes in 5× SSPE/50% formamide/0.2 mg/ml herring sperm DNA/0.2 mg/ml yeast RNA/0.5% SDS/5× Denhardt's. Membranes were washed in 2× SSC/0.1% SDS at room temperature and in 0.2× SSC/0.1% SDS at 50°C for 30 minutes each. Hybridization with the 18S RNA oligonucleotide used the same buffer at 50°C overnight, followed by washing at room temperature as above and then in 0.1× SSC/0.1% SDS at 55°C. Autoradiographs were exposed for three to seven days with intensifying screens at −70°C. Densitometry measurements of autoradiographs used the Cue 2 Image Analyzer program (Olympus).

Probe preparation

cDNA probes used were as follows: human RAR-α was a 0.6 kb restriction fragment from the hT1R clone [21]; human RAR-β was a 1.4 kb cDNA clone containing the entire coding region [22]; human EGF-R probe was a Xba1/Kpnl 2.7 kb cDNA fragment [23]. Probes were labeled with [α-^{32}P]-dCTP (Amersham International) to a specific activity of $>10^{-9}$ cpm/mg DNA using a random priming kit (Boehringer, Mannheim, Germany). An 18 s rRNA 30 mer oligonucleotide probe (5′ GACACCATTAAGATCTCGATTATGTACGGC 3′) [24] was end labeled with [γ-^{32}P]-dATP (Amersham International) using a T4 polynucleotide kinase (Boehringer).

Statistical analysis

The Student's *t*-test was used to analyze differences between groups in each experiment. Student's *t*-test for paired data was used when several experiments were tested at the same time. Fisher's F analysis of the variance was used to assess differences between two groups with several data points. The results are expressed as mean ± standard error of the mean (SEM) and a P value lower than 0.05 is taken as significant.

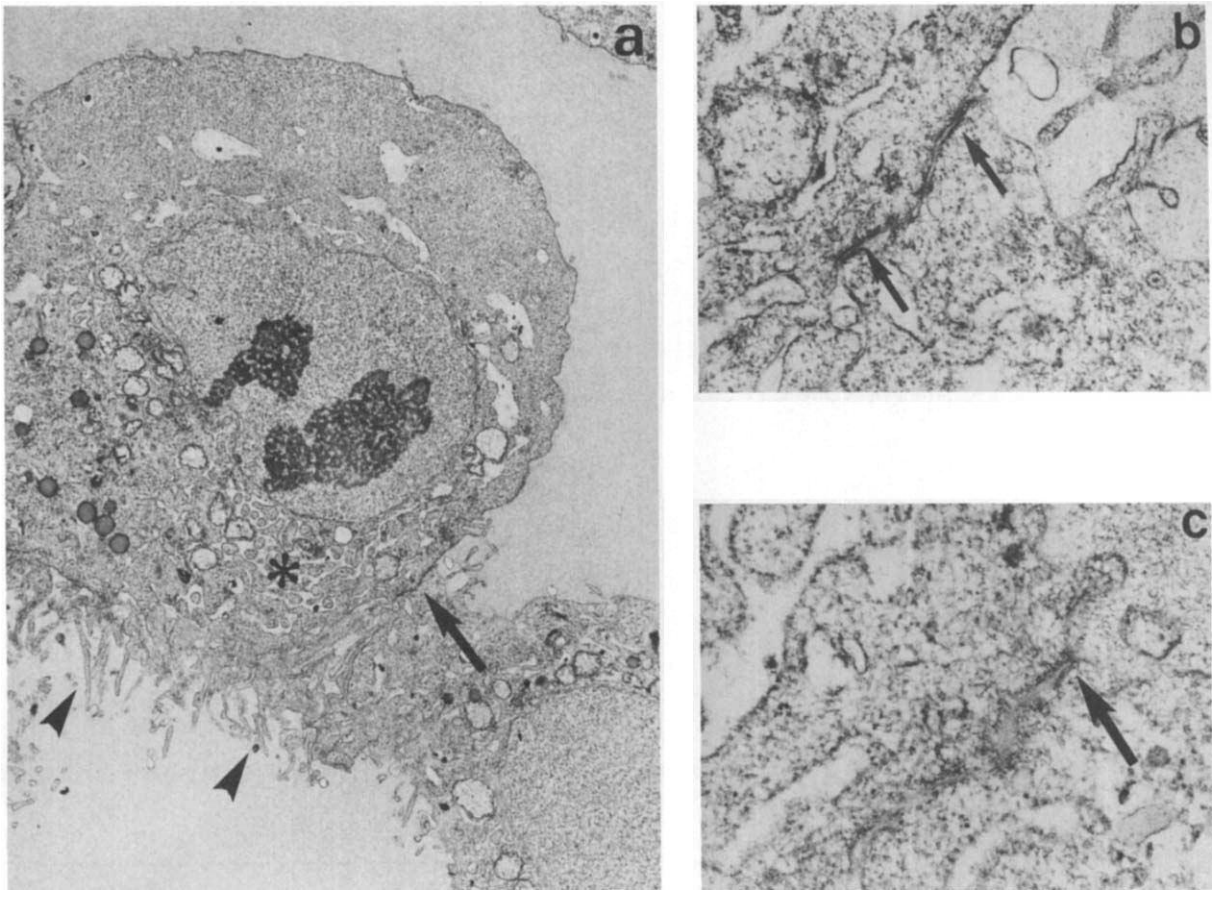

Fig. 1. *Transmission electron micrographs of cultured PAD cells.* **(a)** The cell surface shows short microvilli (arrowheads) and there is complex infolding of the cell membrane (*). The nucleus is irregular with two prominent nucleoli. An intercellular junction is present between the two cells (arrow) (×6100). **(b)** The intercellular junction is characterized by poorly differentiated desmosomes (arrows) (×30,000). **(c)** Further sections of the same intercellular junction reveal the presence of a poorly formed tight junction (arrow) (×30,000).

Results

Characterization of PAD cells

The epithelial nature of PAD cells was examined by electron microscopy and immunohistochemistry. On electron microscopy, PAD cells demonstrated marked pleomorphism, abundant often vacuolated cytoplasm, irregular surface microvilli and intracytoplasmic lumina. Occasional intercellular junctions were seen which were characterized by poorly differentiated desmosomes and poorly formed tight junctions (Fig. 1). PAD cells had an epithelial morphology under light microscopy and displayed an immunohistochemical phenotype consistent with their origin from a renal adenocarcinoma: strong staining with antivimentin (3+); moderate staining with CAM 5.2 anticytokeratin 8, 18, 19 antibody (2+); weak positive staining with anti-S100 (1+) (Fig. 2). This immunohistochemical phenotype was identical in both dispersed and confluent cell layers. No staining of PAD cells was apparent with antibodies to cytokeratins 1, 5, 10, 14(34βE12), EMA, CEA or CD20. Further characterization of PAD cells has been reported elsewhere [15].

Effects of RA and EGF in serum free medium

To determine the effects of RA and avoid interferences with other serum components we used growth factor free cultures as described by VanZoelen et al [16]. These cultures were maintained for four days without FCS, followed by the addition of either RA and/or EGF. RA alone significantly increased thymidine incorporation (155 ± 7% of the control; $P < 0.0001$) as did EGF (177 ± 10%; $P < 0.0001$; Fig. 3). The addition of both growth factors together had an additive effect (208 ± 25%; $P < 0.0001$; Fig. 3).

As an alternative means of assessing cell division, cell cycle analysis was used. Following exposure to 10^{-7} M RA, there was an increase in the number of cycling cells (G2+M phase cells) (Fig. 4). In three separate experiments, the mean increase of cells in G2+M phases was 18 ± 4% ($P < 0.001$ vs. control).

Effects of RA, EGF and retinol in serum supplemented medium

To assess whether RA could augment PAD cell proliferation under conditions of rapid cell growth, nonconfluent cultures supplemented with 10% FCS were examined. Addition of RA increased the rate of thymidine incorporation in rapidly growing

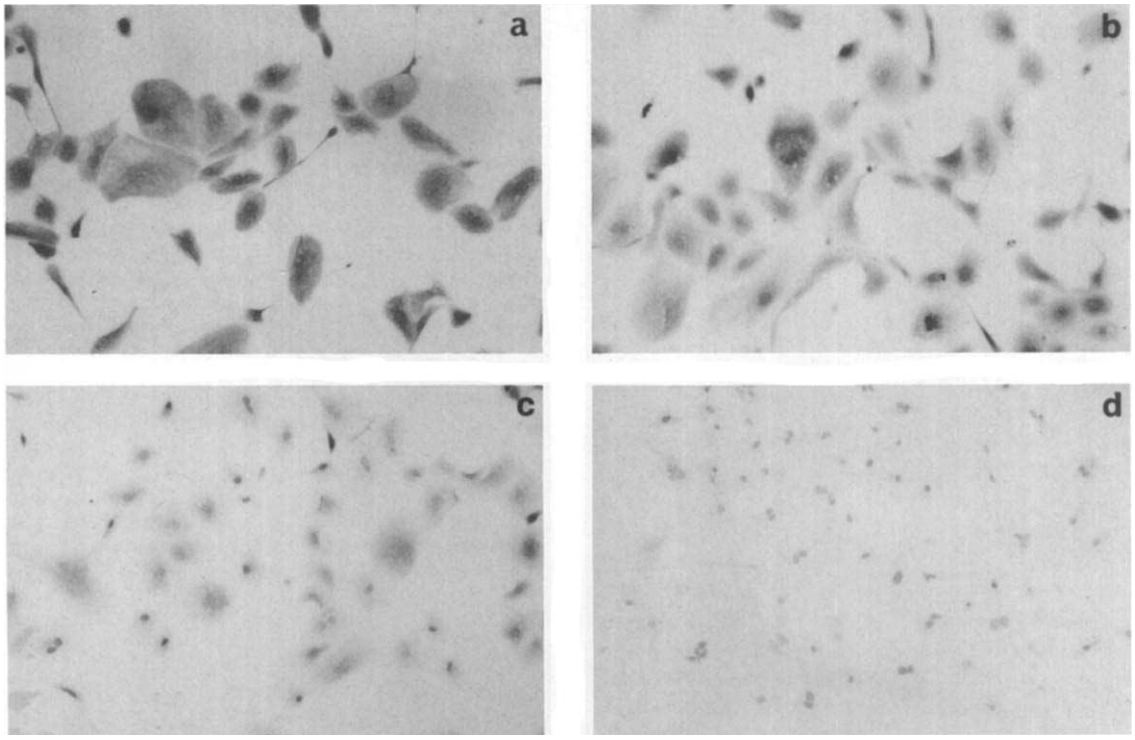

Fig. 2. *Antibody staining of cultured PAD cells by the immunoperoxidase method.* **(a)** Vimentin (3+), **(b)** cytokeratins 8, 18, 19 (2+), **(c)** S100 (1+), and **(d)** CD20 negative.

cells (Fig. 5a). To verify that increased thymidine incorporation was associated with an increase in cell proliferation, daily cell counts of PAD cultures in the presence and absence of RA were performed. The results in Figure 6 show a significant increase in cell number of rapidly growing cells in the presence of 10^{-7} M RA. The ability of RA to reverse contact inhibition was investigated. Addition of RA to PAD cells grown to confluence in FCS supplemented medium made no difference to thymidine uptake (101 ± 2% of control; NS). However, in these same cultures RA (10^{-7} M) enhanced the EGF stimulation of confluent cells, even though it had no direct effect by itself (Fig. 5b, c, d).

The effect of retinol on PAD cell growth was also assessed. Retinol (10^{-6}, 10^{-5}, and 10^{-4} M) was added to rapidly growing cells in serum supplemented medium and caused no significant change in thymidine incorporation (94%, 95% and 93% of control, respectively). A similar result was observed in confluent PAD cultures in the presence of serum: 10^{-5} M retinol did not modify thymidine incorporation (8902 ± 426 vs. control 8933 ± 447 cpm; NS).

Dose dependency of growth factor responses

The stimulation obtained with RA in serum deprived cultures (Fig. 7) and in rapidly growing cells (Fig. 8) was dose dependent. The magnitude of the RA effect on FCS stimulated cells was smaller as could be expected from cells already in rapid growth. In both cases, maximal stimulation was obtained at 10^{-7} M. Increasing the dose to 10^{-6} M resulted in a marginally lower response.

The stimulatory effect of EGF in confluent cultures was also dose dependent; optimum responses were obtained at 0.3 to 3 ng/ml. Addition of RA (10^{-7} M) increased the level of the EGF response by more than 30%, but did not change the half-maximal concentration stimulation value for EGF (Fig. 9). This

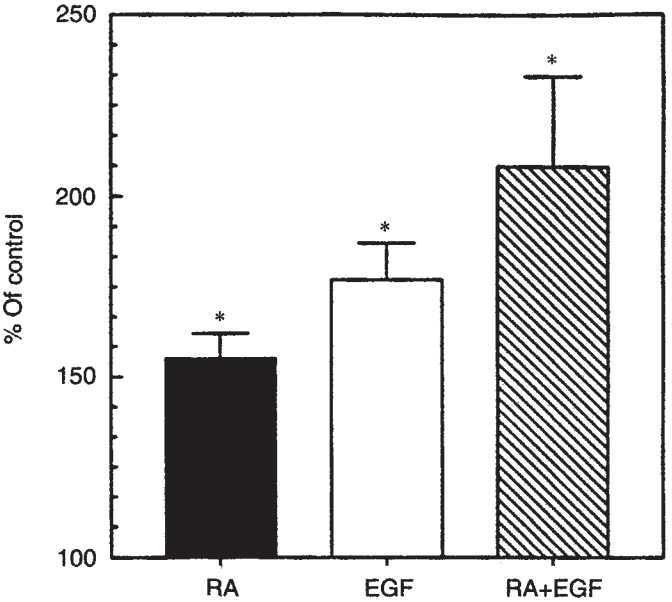

Fig. 3. *Effect of RA and EGF on serum-free cultures.* PAD cell proliferation was assessed by ^{3}H-thymidine incorporation. Each bar represents the percent variation from control values from 6 separate experiments, with 12 replicates in each experiment. Additive responses were observed in cultures containing both RA and EGF. (*$P < 0.001$ vs. control). Final concentrations of RA and EGF were 10^{-7} M and 4 ng/ml, respectively.

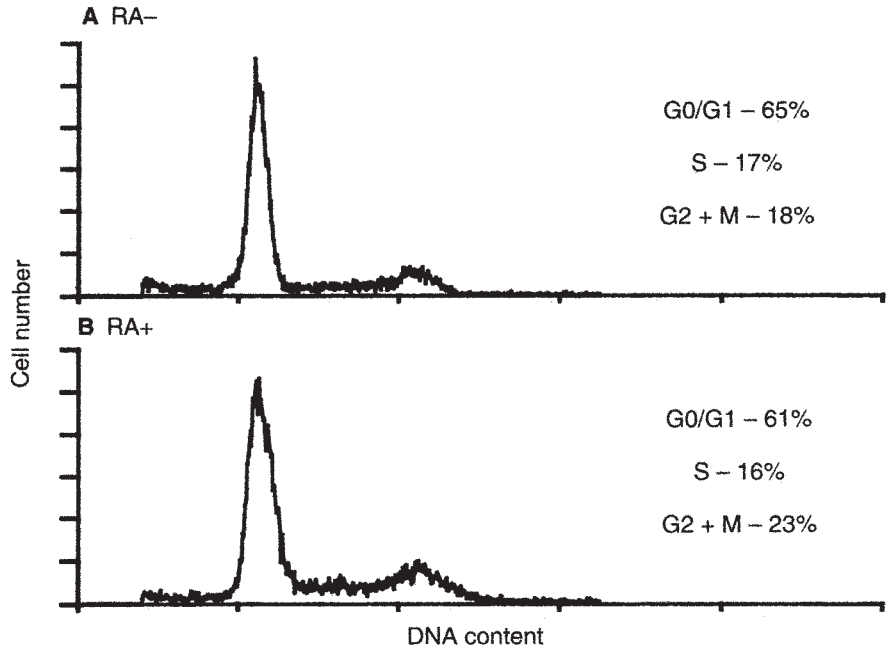

Fig. 4. *Cell cycle analysis in serum-free cultures.* Flow cytometric analysis showing that incubation of PAD cells with 10^{-7} M RA increased the number of cells entering into the cell cycle (S and G2+M phases).

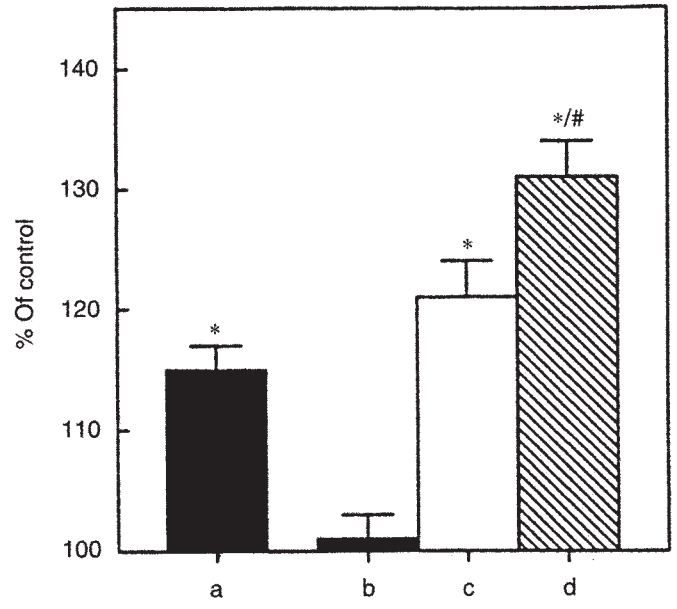

Fig. 5. *Effects of RA and its interactions with EGF in FCS supplemented cultures.* PAD cell proliferation was assessed by ^{3}H-thymidine uptake. Cells were maintained with 10% FCS supplemented RPMI 1640. Each bar represents the percent of the control values from 6 separate experiments, with 12 replicates in each experiment. (a) RA (10^{-7} M) effect on rapidly growing cultures, (b) RA (10^{-7} M) effect on contact inhibited cultures, (c) EGF (4 ng/ml) effect on contact inhibited cultures, and (d) EGF + RA effect on contact inhibited cultures. *$P <$ 0.05 vs. control, #$P < 0.05$ EGF vs. EGF + RA.

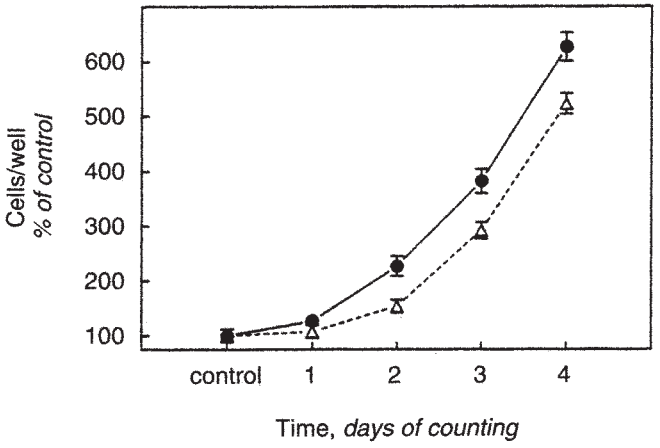

Fig. 6. *Effect of RA on daily cell counting of PAD cell cultures in rapid growth.* Four thousand cells/well were cultured in 96 well plates and counted daily after trypsinization. The results are expressed as percentage of the count on day 0, which is taken as control. Six wells contained 10^{-7} M RA (●) and 6 wells FCS alone (△). RA induced a significant increase in cell numbers on days 2, 3 and 4 ($P < 0.05$) (analysis of the variance of the two curves. $F = 7.97$; $P < 0.048$).

may indicate that RA does not alter the affinity of EGF for its receptor.

Expression of growth factor receptor mRNA species

PAD cells were analyzed for the expression of mRNA encoding receptors for EGF (EGF-R) and RA (RAR-α). Confluent PAD cells expressed both a major high molecular weight (approximately 10 kb) and a minor lower molecular weight (approximately 6 kb) mRNA species of the EGF-R (Fig. 10a).

Confluent PAD cells expressed two mRNA species for RAR-α (Fig. 10b). The size of the RAR-α mRNA bands was consistent with that found in studies of other cell types [21, 25]. In addition, confluent PAD cells expressed two mRNA species for the RAR-β (not shown). As a control, the human K562 erythroleukemic cell line was examined and shown to express to RAR-α (but not RAR-β mRNA) as previously described [25].

The effect of growth factor treatment on the expression of these receptor mRNA species was investigated. Confluent PAD

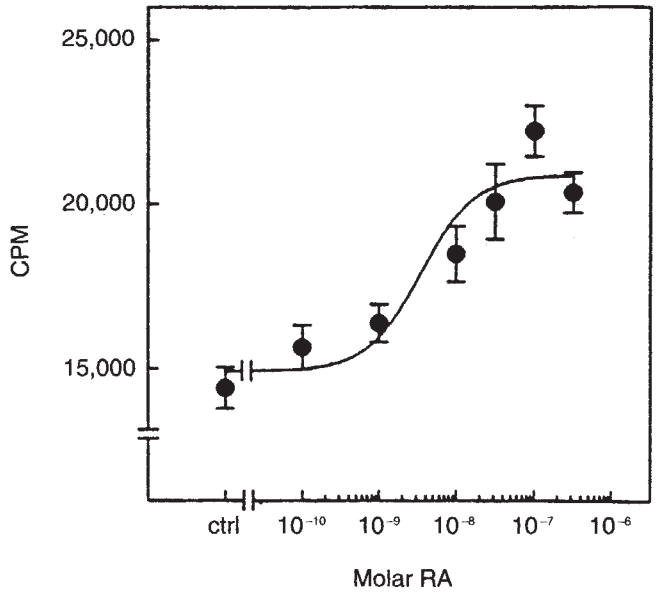

Fig. 7. *Dose-response study of RA effect on serum-free PAD cultures.* PAD cell proliferation was assessed by ^{3}H-thymidine uptake. Cells were maintained for 4 days in RPMI 1640 medium without FCS supplementation and then varying concentrations of RA added.

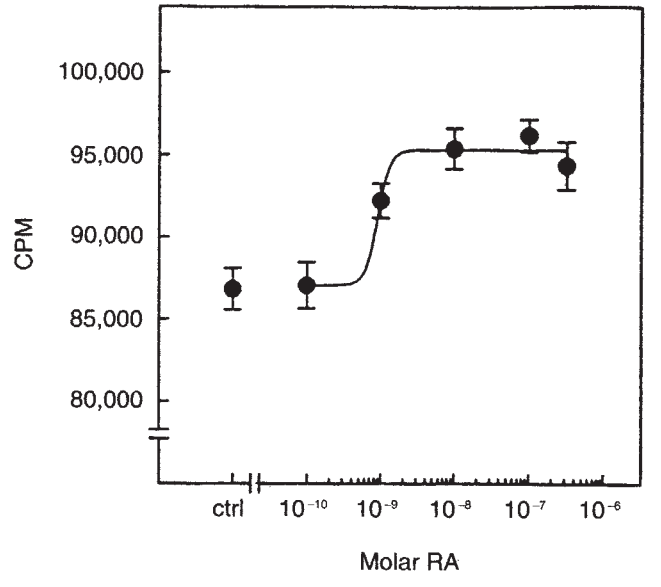

Fig. 8. *Dose-response study of RA effect on 10% FCS supplemented non-confluent PAD cultures.* PAD cell proliferation was assessed by ^{3}H-thymidine uptake. Cells were cultured with RPMI 1640 supplemented with 10% FCS, and the effect of varying concentrations of RA determined.

cells were incubated for three or six hours with 10^{-7}, 10^{-6}, 10^{-5} M RA and for 24 hours with 10^{-7} M RA. In addition, confluent PAD cells were incubated for three or six hours with 1 ng/ml of EGF. There was no consistent change in the level of expression of EGF-R, RAR-α, or RAR-β mRNA. Results from 24 hour incubation with 10^{-7} M RA treatment are shown in Figure 10.

The expression of RAR-α mRNA was investigated under different growth conditions. PAD cells in growth arrest ex-

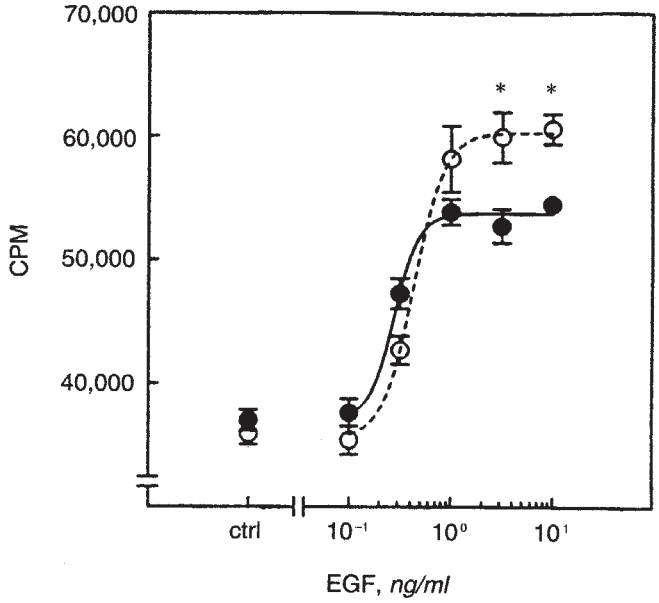

Fig. 9. *Dose-response curve of EGF effect in the absence (●) or presence of 10^{-7} M RA (○).* PAD cell proliferation was assessed by ^{3}H-thymidine uptake. Cells were grown to confluence in RPMI 1640 supplemented with 10% FCS and the effect of EGF and EGF + RA additions assessed.

pressed RAR-α mRNA, although this was eightfold lower than the level of expression in confluent cells (Fig. 11). The addition of FCS to growth arrested cells resulted in an eightfold up-regulation of RAR-α mRNA which was down-regulated by 24 hours (Fig. 11).

Discussion

Retinoids have been classically studied in dermatology and oncology [26], and the range of metabolic activities known to be induced by retinoids is very broad. RA has been shown to modify superoxide production in HL60 cells [27]; increase receptor numbers for vasoactive intestinal peptide in neuroblastoma cells [28]; up-regulate macrophage phagocytosis and lymphocyte response to interleukin-1 [29]; inhibit proliferation of monocyte-derived U937 cells [30]; or redifferentiate HL60 cell line, slowing down the cell cycle [31] and preventing c-*myc* overexpression [32]. RA and EGF are systemic growth factors [26, 33] that are locally synthesized in the kidney [8, 9, 34, 35] and the kidney expresses receptors for both of them [6, 7, 25, 36]. They are known to interact in several different metabolic systems [37–39], and both RA [10] and EGF [4] have been implicated in compensatory kidney growth. However, while EGF has been widely studied, fewer reports exist that assess RA effects on renal cells [13, 14, 40].

In the present study, the response of a human renal adenocarcinoma cell line to RA was determined. The malignant origin of PAD cells was confirmed by ultrastructural morphological examination. Evidence of secretory behavior and intracytoplasmic lumina and the identification of intercellular junctions strongly support the origin of the cells from an adenocarcinoma. The co-expression of low molecular weight cytokeratins [8, 18, 19] and vimentin by PAD cells is a characteristic pattern for

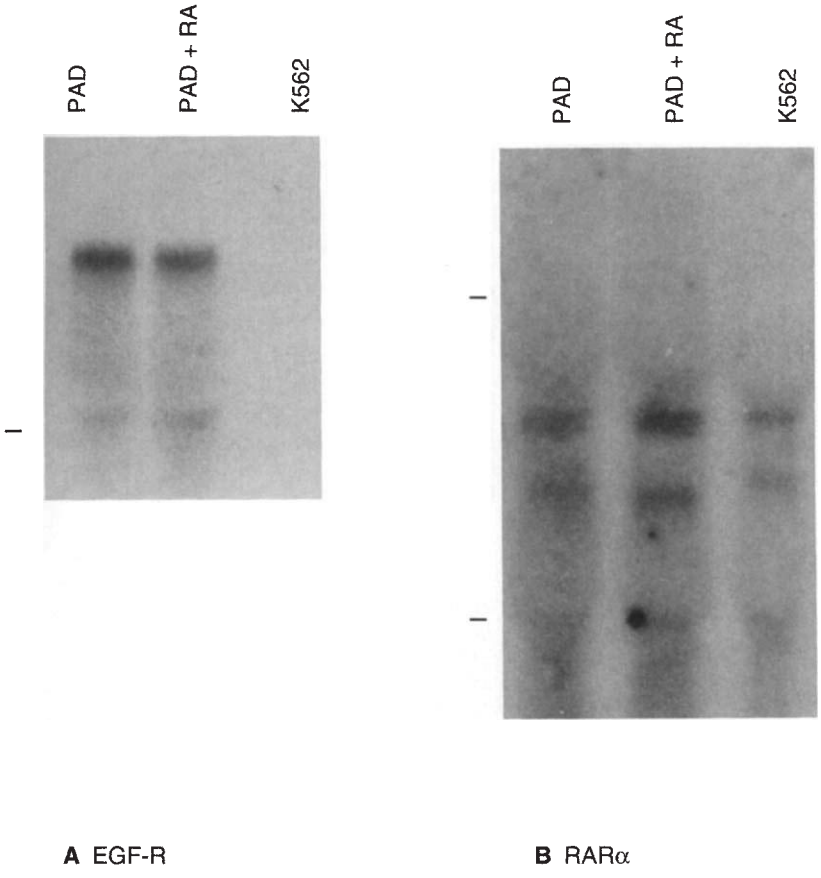

Fig. 10. *EGF-R and RAR-α gene expression by PAD cells.* Total cellular RNA was prepared from confluent PAD cells maintained for 24 hours with or without 10^{-7} M RA, and from K562 cell line (control). RNA (15 μg/lane) was analyzed by Northern blotting using the following cDNA probes: **(A)** EGF-R, and **(B)** RAR-α. The position of the 18s and 28s rRNA is shown.

renal cell carcinoma [41, 42]. In addition, S100 antigen expression by PAD cells is also consistent with a renal cell carcinoma [43].

It was demonstrated that RA alone was able to induce growth of serum starved PAD cells. Interestingly, RA was also able to further enhance DNA synthesis in already rapidly growing PAD cells and this mitogenic effect was observed at physiological concentrations (normal RA serum level is 3 to 4 ng/ml, or 10^{-8} M). However, no mitogenic response was observed when RA was added to confluent cultures. This finding contrasted with our previous observations in a rabbit renal epithelial cell line (RK13), where RA induced similar changes in thymidine incorporation in both confluent and rapidly growing cells [14].

It is known that RA can freely diffuse into the cell cytoplasm without the need for a specific carrier or membrane receptor [44]. The sequence and organ distribution for CRABP has been reported [36, 45] and four RA receptors with a nuclear binding portion (RAR alpha, beta, gamma and epsilon) have been identified [21, 22, 46, 47]. The RAR-β beta gene has a RA responsive element [48] which triggers RA up-regulation of RAR-β gene transcription [48]. However, RA has also been found to modify erythrocyte metabolism, therefore proving that not all RA actions require nuclear involvement [49].

While several tissues (including the kidney) have been found to express RA receptors and different cell lines have also been tested [21, 25, 47, 50], no published data are available regarding their expression on kidney cell lines or isolates. In the present study, we demonstrated that renal adenocarcinoma cells express both RAR-α and RAR-β mRNA. Further, we found RAR-α and RAR-β mRNA expression in confluent cultures, while in this situation RA did not elicit any change in thymidine incorporation. However, other factors related to contact inhibition may account for the lack of RA mitogenic activity. We cannot exclude though that RA induces metabolic changes other than thymidine incorporation, which may also be relevant for cellular growth [27, 39, 49, 51]. RAR-α mRNA was also expressed in starved PAD cells, and this expression was significantly increased at three and six hours following addition of FCS. These results suggest that RAR expression and RA activity are determined to some degree by the growth status of the cell.

EGF was mitogenic in confluent monolayers of PAD and this effect was also dose related. We observed that RA enhanced EGF mitogenic activity on confluent monolayers of PAD cells in serum supplemented medium. RA increased the maximal EGF response by an additional 30%. Mordan reported apparently contradictory results, observing that RA and retinyl acetate inhibited PDGF- and EGF-induced growth in rat fibroblasts [52]. Another report on rat mesangial cells showed that EGF can block RA-induced collagen synthesis [53]. Alternatively, RA has been found to increase EGF binding to its receptor in a fetal rat lung cell line [39] and in mouse mammary gland cells [54]. In mouse palatal mesenchymal cells, low concentrations of RA (10^{-9} M) enhanced EGF induced mitogenicity without modifying EGF receptor mRNA expression [55]. In our study RA enhanced EGF mitogenic effect on PAD cells

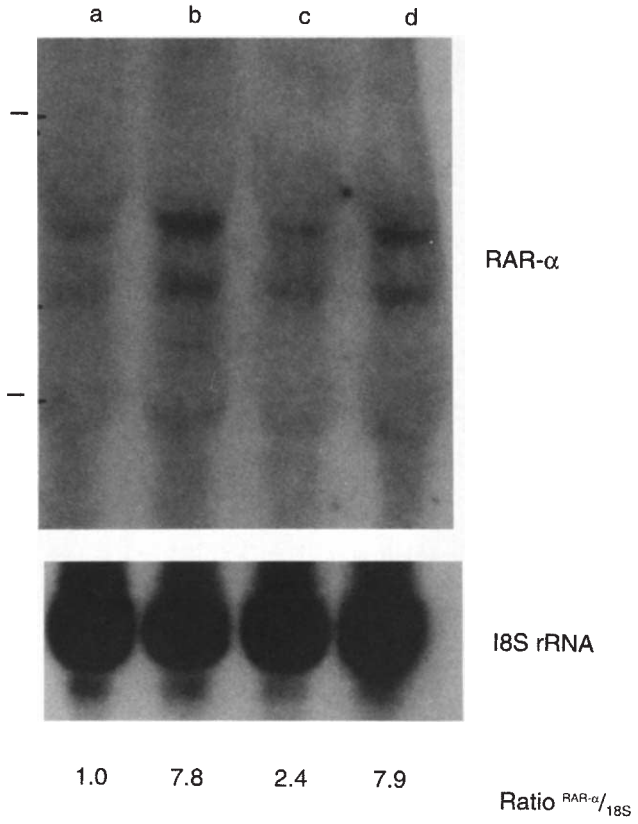

Fig. 11. *Effect of growth conditions upon PAD cell RAR-α gene expression.* Total cellular RNA was prepared from PAD cells under the following conditions: **(a)** growth arrested, **(b)** 3 hours and **(c)** 24 hours following the addition of 10% FCS to growth arrested cells, and **(d)** cells grown to confluence in 10% FCS. RNA (15 μg/lane) was analyzed by Northern blotting using probes for: RAR-α (upper panel) and 18S rRNA (lower panel). The position of the 18S and 28S rRNA is shown on the upper panel. The ratio of RAR-α mRNA/18s rRNA is shown, normalized to 1.0 for lane (a).

at 10^{-7} M without markedly increasing EGF receptor mRNA expression. Although post-translational changes are not ruled out by our experiments, it seems feasible that RA triggers other cellular modifications to increase EGF responsiveness in PAD rather than causing major increase in EGF receptor expression.

These findings indicate several potential modes of action by which RA could up-regulate kidney cell growth. The direct effect of RA in serum starved cultures suggests that it can act as a mitogen in its own right. The augmentation of fetal calf serum-mediated growth in rapidly growing cultures also argues that RA induces positive signals in the presence of other cytokines. In the context of compensatory kidney growth, however, it is likely that the confluent cultures most closely resemble the cell densities and proportion of dividing cells to be found in the intact organ. It is therefore of interest that in these conditions RA also acted synergistically with EGF. Given the putative role of the latter cytokine in compensatory kidney growth [4] we would suggest that RA may be similarly involved, particularly as increased cellular retinoic acid binding protein (CRABP) levels have been described in the remnant kidney [10].

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