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DIFFERENTIAL EXPRESSION OF CYTOCHROME P-450 IN PROLIFERATING AND QUIESCENT CULTURES OF MURINE LUNG EPITHELIAL CELLS

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SUMMARY: Expression of the cytochrome P-450 monooxygenase activity 7-ethoxyresorufin O-deethylase (7-ERD) was surveyed in proliferating and quiescent cultures of murine cell line C-10, a non-tumorigenic line of presumed alveolar type II origin. 7-ERD activities were undetectable in subconfluent/proliferating cultures but became detectable once the cultures had become confluent and their growth had arrested due to contact inhibition. Serum deprivation of subconfluent cultures resulted in a rapid inhibition of cell proliferation and the subsequent expression of 7-ERD. These results suggest that 7-ERD expression is regulated as a function of the proliferative status of C-10 cells.

The cytochrome P-450 system represents a supergene family of proteins that catalyze the metabolism of several classes of chemicals. Expression of individual isozymes in the same organism varies markedly form tissue to tissue, and from cell type to cell type within the same tissue (1,2). Among the many different pulmonary cell types, immunocytochemical analyses have localized most of the P450IA1 cytochrome to alveolar type II cells and bronchial Clara cells (3).

Recent studies have demonstrated that rodent epidermal cells express some P-450 dependent monooxygenase activities following the cessation of proliferation and during early stages of terminal differentiation (4,5). A relationship between cell proliferation and P-450 expression is also suggested by the observations that

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regenerating liver (6,7) and neoplastic hepatic tissues (8-12) generally have lower P-450 contents than normal liver. In the current study we investigated the expression of 7-ERD activity, a measure of P450IA1 (13), in proliferating and quiescent cultures of the murine lung epithelial cell line, C-10. This cell line expressed biochemical features of Type II cells at early passage (14). C-10 cells ceases to proliferate upon reaching confluence (contact inhibition) or after removal of serum from culture media. By both procedures we demonstrate that 7-ERD expression is suppressed in proliferating but not quiescent C-10 cultures.

MATERIALS AND METHODS

Chemicals. Ethynylpyrene was the gift of Dr. W.L. Alworth, Tulane University, New Orleans. 7-Ethoxyresorufin was obtained from the Pierce Chemical Co. 7-Hydroxyresorufin was purchased from the Aldrich Chemical Co.

Cell culture. C-10 type II granular pneumocytes were derived from adult BALB/cJ mouse lung epithelium (14) and grown in 60 mm culture dishes in CMRL 1066 medium supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, 100 IU/ml penicillin, 3.5 g/L glucose and 0.1 g/L L-glutamine. Cells were grown in a humidified 5% CO_2 incubator at 37°C and passaged by treating cultures with a 0.25% solution of trypsin diluted in phosphate buffered saline (PBS). C-10 cells are contact inhibited in culture and non tumorigenic when injected into nude mice (14,15).

7-ERD measurement. At various times after plating some cultures were washed twice with PBS and stored at -70°C for 7-ERD analyses after the addition of 0.75 ml of 100 mM Hepes, pH 7.8, 1.6 mg/ml BSA and 5 mM $MgSO_4$. Other plates were used for estimation of cell numbers. 7-ERD was assayed by measuring the production of the fluorescent metabolite 7-hydroxyresorufin. Analyses were performed directly in culture dishes that had been stored at -70°C. The assay we employed has been described in detail (16). The only modifications to the published assay were the inclusion of 50 μM dicumarol in the assay cocktail, and the lysing of the cultures by the addition of SDS to terminate the reaction. Specific activities are expressed as pmol product per hr per 10^6 cells.

RESULTS

7-ERD activities as a function of culture confluence. Cultures of C-10 cells were harvested at various times after plating in order to assess the effects of culture confluence on 7-ERD activities (Fig. 1). Cultures were visually confluent at a density of ~1.4 x 10^6 cells/plate, but continued to proliferate and pack until the cells reached a density of ~1.8 to 2.1 x 10^6 cells/plate. 7-ERD activities were detectable in confluent cultures but not subconfluent cultures (Fig. 1), and similar to 7-ERD activities measured in cultured murine keratinocytes following the induction of terminal differentiation (5).

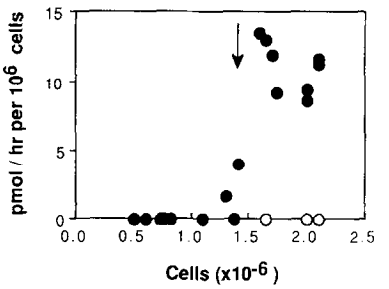

Fig. 1. Expression of 7-ERD activity as a function of cell density. C-10 cultures were harvested for 7-ERD assays and determination of cell number at various times after plating. Cultures were refed with fresh medium every day. 7-ERD analyses were performed in the presence (•) or absence of 50 μM dicumarol (o). The arrow represents the density at which cultures were visually confluent, but sparsely packed.

7-ERD could only be detected in C-10 cultures if the assay mixtures were supplemented and preincubated with dicumarol. Dicumarol is an inhibitor of the enzyme NAD(P)H: quinone oxidoreductase (17), which is found in relatively high levels in C-10 cells (18). Since the product of the 7-ERD reaction is a substrate for quinone reductase (19), it appears that C-10 cells have concentrations of this enzyme sufficient to mask 7-ERD activity.

Ethynylpyrene is a suicide substrate specific for P450IA1 (20). Incubation of confluent cultures with 10 μM ethynylpyrene for 2 hr prior to harvest completely eliminated 7-ERD activity (data not shown). Consequently, the 7-ERD activities measured in C-10 cells reflect the presence of active P450IA1.

The cell densities designated in Fig.1 were achieved by harvesting the cultures at various times after plating. Consequently there existed the possibility that 7-ERD expression was regulated as a function of time in culture as opposed to degree of confluence. Experiment 1 in Table I demonstrates that 7-ERD expression was clearly associated with cell density. However, experiment 2 in Table I suggests that once cells arrest a period of time must elapse before optimal 7-ERD expression occurs. We have noted a similar result in two additional studies.

Effects of serum deprivation on cell proliferation and 7-ERD expression. Subconfluent C-10 cells stopped proliferating upon removal of serum from the culture media (Fig 2A). Cell numbers remained reasonably constant following

Table 1. Effects of time in culture on 7-ERD expression. C-10 cells were seeded at various densities and later harvested at the same time (Exp. 1), or seeded at the same density and subsequently harvested at different times (Exp. 2).

Exp.	Time in Culture (hr)	Cells/plate ($x10^{-6}$)	7-ERD Activity (pmol/hr per 10^6 cells)
1	72	0.6	nd
	72	1.3	1.7
	72	2.0	8.6
2	24	0.7	nd
	48	2.0	2.7
	72	2.0	11.3
	96	2.0	11.7

nd, activity was not detected.

serum deprivation and represented culture densities at which 7-ERD activity was normally not expressed. However, 7-ERD activities were expressed in subconfluent cultures within 24 hr of serum deprivation (Fig. 2B). In contrast, in the same experiment cultures maintained in serum containing medium only expressed 7-ERD once they reached confluence.

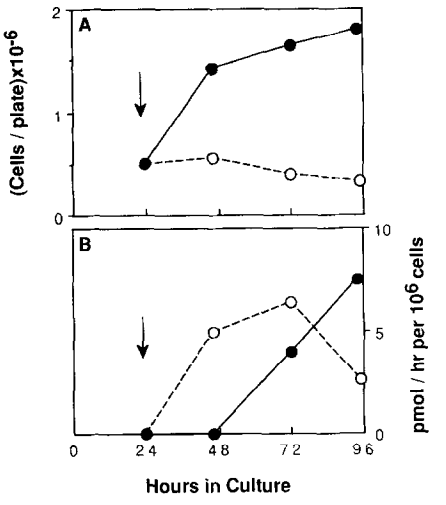

Fig. 2. Inhibition of cell proliferation (panel A) and induction of 7-ERD activity (panel B) by serum deprivation. C-10 cells were plated in medium containing 10% FBS. The following day half of the cultures were washed twice with PBS and refed with medium lacking FBS. Cultures were refed every day thereafter. Cells cultured in serum containing (•) or serum deficient (o) medium were harvested at various times for cell counts or 7-ERD analyses. The arrow indicates the time of serum removal.

DISCUSSION

In the current study we used contact inhibition and serum deprivation as methods to arrest the proliferation of cultured C-10 cells. Manipulation of C-10 proliferation by both of these techniques suggests that 7-ERD expression occurs in quiescent cultures but is suppressed in proliferating cultures. To date, this is the first use of either of these approaches to demonstrate a relationship between cell proliferation and P-450 expression in epithelial cells.

The relationship between cellular 7-ERD activities and the proliferation status of C-10 cultures is not unique to this lung cell line. Keratinocytes cease to proliferate once they become committed to terminal differentiation. Two groups (4,5) recently reported that several monooxygenase activities markedly differ in proliferating and differentiating epidermal cells. Specifically, monooxygenase activities were consistently greater in differentiating keratinocytes. Fetal liver, regenerating liver, neoplastic hepatic nodules and hepatocellular carcinomas are all rapidly proliferating tissues, relative to normal liver. P-450s are virtually absent in fetal liver until just before birth (21,22). Overall P-450 contents and some monooxygenase activities are dramatically decreased on a per mg protein basis following partial hepatectomy (6,7). P-450 content and some P-450 isozymes and activities are also low in rapidly growing spontaneous and chemically induced hepatic nodules and hepatomas (8-12). Collectively these studies suggest that the expressions of some constitutive (basal) P-450 activities in epithelial cells are regulated as a function of cellular proliferative status. Whether a comparable relationship holds for inducible P-450 activities and other enzymes involved in xenobiotic metabolism is not known. However, the recent demonstration of differential expression of glutathione S-transferase subunits in subconfluent and confluent rat liver epithelial cells (23) suggests that phase II drug metabolism enzymes may also be regulated as a function of the proliferation status of the cell.

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