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Expression of cytochrome *P*-450 3A in HT29-MTX cells and Caco-2 clone TC7

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Abstract HT-29 sublines and Caco-2 clones were analyzed for the expression of cytochrome *P*-450 3A. The enzyme was found to be expressed in differentiated HT-29 cells selected by resistance to methotrexate and in one of seven Caco-2 clones, TC7. Its expression parallels the differentiation process, with highest levels being observed at late confluency. *P*-450 3A mRNA and protein patterns, as well as subcellular distribution, are intermediate between those observed in human adult intestine and fetal liver.

Key words: Cytochrome P450 3A; Caco-2 cell; HT-29 cell

1. Introduction

The cytochrome *P*-450 gene superfamily [1] encodes monooxygenases playing a major role in the metabolism of xenobiotics. Although the liver is generally the organ with the highest xenobiotic-metabolizing activity, *P*-450s are also present in other tissues, including the intestinal epithelium [2,12]. Most studies concerning intestinal *P*-450s have been performed in animal models [4-6]. In man, the major *P*-450s found in the small intestine belong to the CYP3A subfamily [7–12] and include CYP3A4 [11] and CYP3A5 [12]. Although considerable progress has been made in the knowledge of the transduction pathways involved in the transcriptional regulation of some *P*-450 genes like 1A and 2E [13–15], little is known of the regulation mechanisms which control intestinal CYP3A expression. One main reason to this situation is the lack of availability of reproducible in vitro models. The purpose of the present work was therefore to investigate whether differentiated populations isolated from the human colon carcinoma cell lines HT-29 and Caco-2 would express CYP3A. For this we used HT-29 sublines, either obtained by adaptation to hexose-free medium, which express an enterocytic phenotype [16] or selected by increasing concentrations of methotrexate (MTX) [17]. These latter cells, which form a mixed population of goblet cells and enterocytes when adapted to MTX 10^{-7} M, of goblet cells exclusively at 10^{-6} M, and of enterocyte-like cells at 10^{-3} M [17], maintain their differentiation characteristics when further subcultured in drug-free medium [17]. We also used 7 clones isolated from the Caco-2 cell line [18]. These clones all display an enterocytic differentiation but differ one from another as to the level of expression of membrane proteins involved in glucose utilization, namely sucrase-isomaltase and hexose transporters [18,19].

2. Materials and methods

HT-29 populations included parental cells, referred to as HT-29, cells resistant to MTX 10^{-7}, 10^{-6} and 10^{-3} M, referred to as HT29-MTX 10^{-n}, and cells adapted to glucose-free medium [16] and reversed to standard culture conditions, referred to as HT29-RevGlc$^-$. Clones PD7, PD10, PF11, TB10, TC7, TF3 and TG6, were isolated from early and late passages of Caco-2 cells as reported [18]. Culture conditions were the same as previously reported for HT-29 [17] and Caco-2 cells [18]. Normal adult and fetal tissues were obtained as reported [20]. Double labeling immunofluorescence was performed on cryostat sections of tissues and cell layer rolls as in [17,18], using mAb KO3 against human cytochrome *P*-450 3A [21] and a polyclonal rabbit antiserum against villin [22], obtained from D. Louvard (Institut Pasteur, Paris). Western blot detection of CYP3A was performed with mAb KO3 as in [21] on microsomal proteins from cells and tissues prepared as described [10]. CYP3A4 and CYP3A5 proteins were produced in yeast as reported in [23]. Extraction of RNA with guanidium isothiocyanate and centrifugation through a CsCl gradient [24], and Northern blot analysis were as described previously [18,25]. *P*-450 3A mRNA was detected using cDNA clone nf-25 [26], sucrase-isomaltase with cDNA probe SI2 [27], obtained from D. Swallow (MRC Human Biochemical Genetics Unit, University College, London), and dipeptidylpeptidase-IV with cDNA DPI-101 [28]. For quantitation of RNA, membranes were dehybridized and stained with Methylene blue. This was preferred to hybridization with actin or GAPDH as it was found that the level of these transcripts may vary with the phases of cell growth and from one cell line to another.

3. Results

Based on the growth-related variations of the differentiation process, screening of the different cell populations for the presence of CYP3A mRNA was performed on late post-confluent cells. As shown in Fig. 1, although trace amounts of CYP3A mRNA are detectable in most populations, it is present at a significant level only in HT29-MTX cells and in the Caco-2 clone TC7. Comparison with small intestine and fetal liver shows that, in HT29-MTX cells, only the 1.9 kb transcript is present, whereas TC7 express three transcripts, like in the small

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Abbreviations: MTX, methotrexate; CYP3A, cytochrome *P*-450 3A, named according to recent recommendations for standardization of *P*-450 gene nomenclature [1]; DPP-IV, dipeptidylpeptidase-IV; SI, sucrase-isomaltase.

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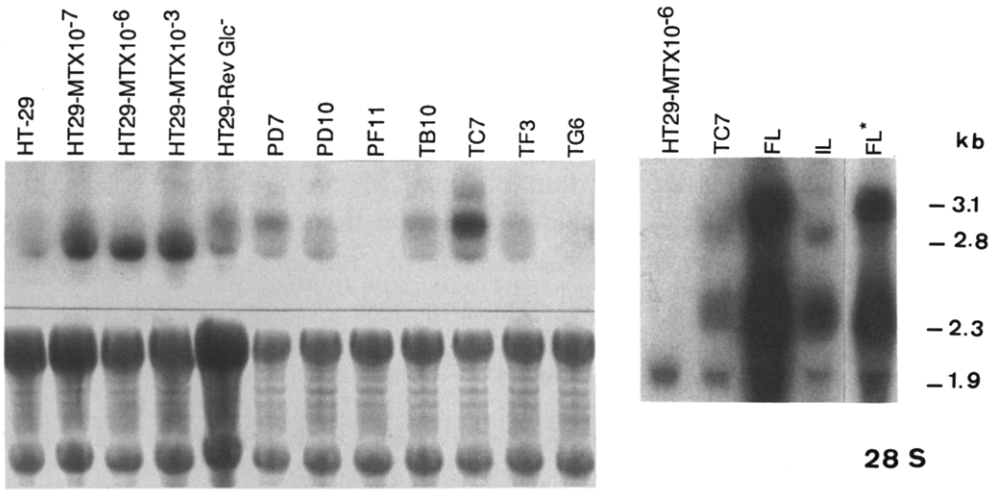

Fig. 1. Northern blot analysis of CYP3A in HT-29 subpopulations and Caco-2 clones. Left, upper panel, total RNA from post-confluent cultures (day 21) of the indicated cells (20 μg for HT-29 populations, 15 μg for Caco-2 clones) was hybridized with cDNA nf-25 (CYP3A); lower panel, Methylene blue staining of the same dehybridized membrane. Right, upper panel, the same quantity of total RNA (10 μg) from the indicated cells, fetal liver (FL) and ileum (IL) were allowed to migrate for a longer time than in left panel and hybridized with CYP3A probe; FL*, shorter exposure time in order to visualize the 1.9 kb transcript; lower panel, Methylene blue staining of 28 S from the same dehybridized membrane.

intestine and fetal liver. However, the pattern observed in TC7 cells differs from that observed in intestine and fetal liver with the upper band migrating between the 3.1 kb fetal liver and the 2.8 kb intestinal transcripts (Fig. 1).

In order to verify whether expression of CYP3A is associated with the differentiation process, CYP3A mRNA was analyzed at different stages of culture of HT29-MTX 10^{-6} and TC7 cells, in comparison with classical markers of differentiation, namely DPP-IV for HT29MTX [17] and sucrase-isomaltase for TC7 cells [18]. As shown in Fig. 2, there is a concomitant expression of CYP3A and differentiation markers, with highest levels being observed at late confluency.

For characterization of CYP3A expression at the protein level, HT29-MTX 10^{-6} and TC7 cells were therefore analyzed at late post-confluency. Western blot analysis of cellular microsomal fractions shows that CYP3A from the two populations migrates at a level which is intermediate between that observed with fetal liver and CYP3A5 protein on one hand and small intestine and CYP3A4 on the other hand (Fig. 3). As shown by immunofluorescence, CYP3A is diffusely expressed in the cytoplasm of TC7 cells, without apparent polarized compartimentalization, as shown by double labeling with villin (Fig. 4). In HT29-MTX cells it appears to be localized between the nucleus and the mucus compartment (Fig. 4). Although the intensity of staining of CYP3A is much lower in the cells than in fetal liver or small intestine, the diffuse cytoplasmic distribution observed in TC7 cells resembles that in the fetal liver (Fig. 4e). In both cell types it differs however from the apical compartmentalization which, as already reported [11], is observed in the small intestine (Fig. 4f).

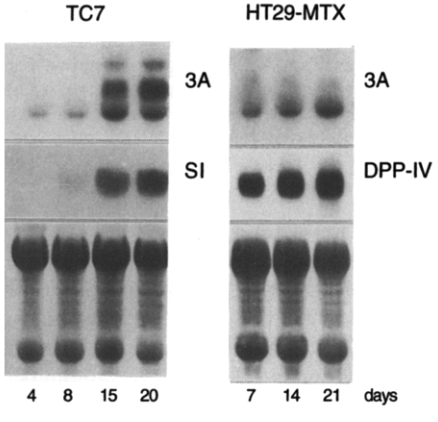

Fig. 2. Growth and differentiation-related expression of CYP3A mRNA in Caco-2-TC7 and HT29-MTX 10^{-6} cells. Total RNA (20 μg) from cells harvested at the indicated days of culture was successively hybridized with cDNA nf-25 (3A), SI2 (sucrase-isomaltase) or DPI-101 (DPP-IV), dehybridized and stained with Methylene blue.

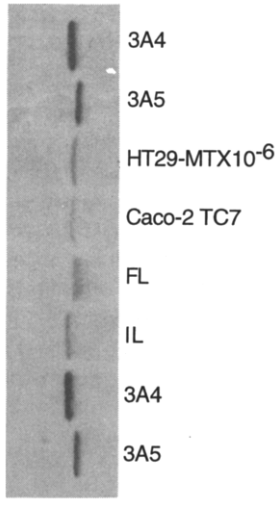

Fig. 3. Western blot analysis of HT29-MTX 10^{-6} and TC7 CYP3A isoforms. Microsomal proteins from post-confluent cultures (day 21) of the indicated cells (40 μg) were analyzed with mAb KO3 in comparison with microsomal extracts from IL, same normal adult ileum as in Fig. 1 (5 μg), FL, same fetal liver as in Fig. 1 (1 μg), CYP3A4 and CYP3A5 proteins expressed in yeast (0.1 pmol).

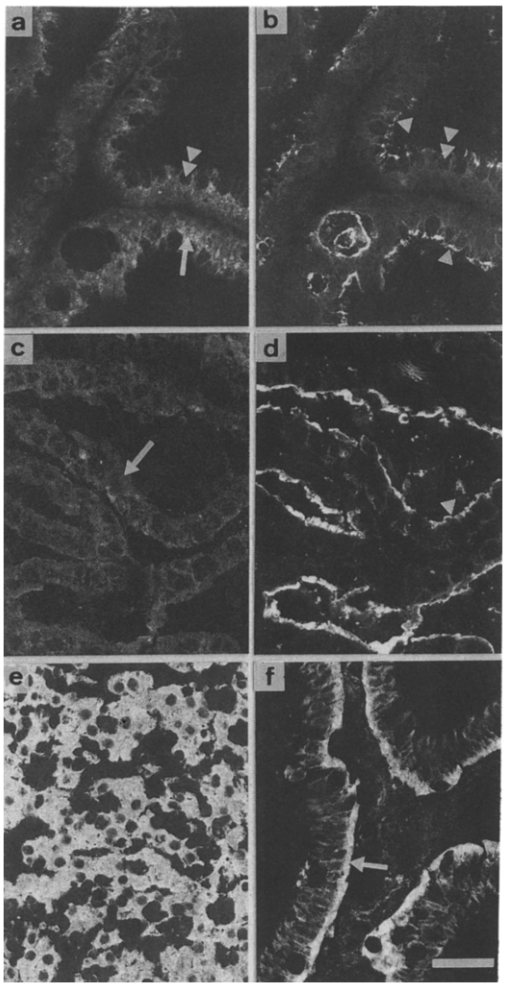

Fig. 4. Indirect immunofluorescent staining of CYP3A in HT29-MTX 10^{-6} and TC7 cells. Cryostat sections of postconfluent (day 21) HT29-MTX 10^{-6} (a,b) and TC7 cells (c,d) were double stained with (a,c) mAb KO3 against CYP3A and rhodamine-labeled anti-globulins and (b,d) a polyclonal rabbit antibody against villin, used as a marker of the brush border, and fluorescein isothiocyanate-labeled anti-globulins; (e) same fetal liver and (f) same adult ileum as in Fig. 1 stained with mAb KO3. Arrows point to CYP3A, arrow heads to villin, double arrow heads in a and b to mucus. Bar, 50 μm.

Finally, since the expression of CYP3A in the HT-29 populations analyzed is mainly associated with HT29-MTX cells, it was important to analyze whether this expression is a consequence of MTX treatment or a specific feature of the selected differentiated phenotypes. As shown in Fig. 5, reversion to drug-free medium for several passages of HT29-MTX cells has no effect on the level of expression of either CYP3A mRNA or protein.

4. Discussion

We show here that differentiated subpopulations isolated from the HT-29 cell line by resistance to methotrexate and Caco-2 cell clone TC7 do express CYP3A. Expression of CYP3A is closely associated with the differentiation process as substantiated by its growth-related appearance and concomitant expression of differentiation markers. This growth-related expression mimics that observed in the small intestine in which both mRNA and protein are absent from dividing crypt cells and appear when the cells migrate along the crypt-villus axis [5,11]. Although expression of CYP3A in HT-29 and Caco-2 cells is associated with the differentiation of the cells, it is independent from the phenotype of differentiation as it is present in mucus-secreting cells, like HT29-MTX 10^{-6}, and in enterocyte-like cells, like HT29MTX 10^{-3} and Caco-2 clone TC7. Conversely, cell differentiation is not sufficient for the expression of CYP3A, as it is significantly expressed neither in enterocytic HT29RevGlc$^-$ cells, nor in any of the other Caco-2 cell clones, although they express the same high degree of enterocytic differentiation as TC7 cells [18]. This would suggest that HT-29 and Caco-2 parental cells contain a minor population of cells able to express CYP3A which are selected during induction of MTX resistance of HT-29 cells or cloning of Caco-2 cells.

Both HT29-MTX and TC7 cells express a CYP3A mRNA transcript of 1.9 kb, a transcript shown to be mainly, but not exclusively, associated with fetal liver CYP3A [29]. This transcript is the only one expressed in HT29-MTX cells, thus differing from TC7 cells which also express two higher transcripts, like in the small intestine and fetal liver, with the upper one differing from that observed in these two conditions. At the protein level both types of cells express a protein which migrates at a level intermediate between CYP3A4 and CYP3A5, one of the isoforms also found in fetal liver [30]. This finding is consistent with the observation that most differentiation features of HT-29 and Caco-2 cells share fetal and adult characteristics [31].

These results are the first to demonstrate that cell lines of intestinal origin are able to express CYP3A. In view of the observation that the isoform expressed differs from both 3A4 and 3A5, further studies should determine whether it is CYP3A7 [32–34], a gene also expressed in fetal liver which

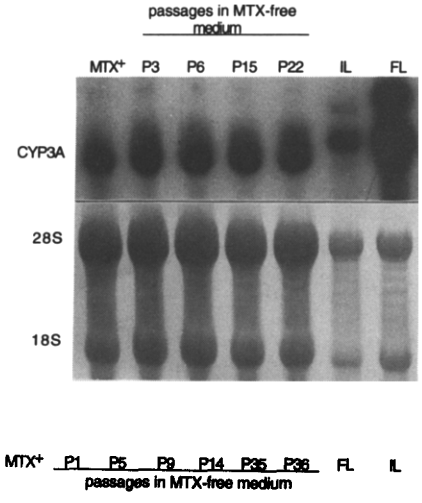

Fig. 5. Permanence of CYP3A expression in HT29-MTX cells reversed to drug-free medium. HT29-MTX 10^{-3} cells, passage 18 (MTX$^+$) were subcultured for the indicated number of passages (P) in MTX free medium and analyzed at late post-confluency (day 21). Upper panel, total RNA from cells (20 μg), same IL and FL (5 mg) as in Fig. 1, hybridized with cDNA nf-25; middle panel, same membrane dehybridized and stained with Methylene blue; lower panel, Western blot analysis of microsomal fractions of the cells (40 μg) and of same preparations of FL (1 μg) and IL (5 μg) as in Fig. 3.

shows 82% of amino acid homology with CYP3A5 [35], or a still uncharacterized 3A isoform. Whether these cells can be helpful models for investigating the regulation mechanisms which control CYP3A expression remains to be investigated.

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