Gene Expression in Clonally Derived Cell Lines Produced by in Vitro Transformation of Rat Fetal Hepatocytes: Isolation of Cell Lines Which Retain Liver-specific Markers

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ABSTRACT

The pattern of gene expression in fetal hepatocytes transformed in culture with a hepatocarcinogen (FRL cells) is studied with respect to a range of markers which are either developmentally regulated and/or shown to be expressed at high levels in hepatoma cells. The relative abundance of the respective mRNAs is determined and immunocytochemistry is used to detect the respective proteins in cultured cells. When compared with its normal counterpart, FRL cells retain the expression of transferrin, α,2-acid glycoprotein, γ-glutamyltranspeptidase, and tyrosine aminotransferase at near normal levels, while expression of the liver-specific isoenzymes of pyruvate kinase (L form) and aldolase (B form) is reduced. The cell lines are different in that they fail to express albumin, α-fetoprotein, thiostatin and α,2-macroglobulin, and they express high levels of MFG-pyruvate kinase and aldolase A, markers often found in abundance in hepatoma cells. Therefore transformation has resulted in different effects on different genes. Furthermore, it is of interest to find that the cells coexpress both forms of the pyruvate kinase isoenzymes which does not occur in the normal developing hepatocyte.

These results indicate that it is possible to use this model to study changes which accompany transformation of fetal rat hepatocytes. The resulting cell lines have a stable phenotype and retain the changes which result from transformation even after extended passaging. This facilitates comparisons between the precursor cell and the tumor cell, both of which can be maintained under controlled conditions which exist in culture.

INTRODUCTION

The pattern of gene expression in cancer cells often bears a closer resemblance to that observed in normal immature cells than fully differentiated cells for a given tissue. This is true for cells in the liver lineage (1, 2). It is also established that different hepatoma lines display different subsets of liver markers. Weinhouse (1) concluded that some hepatoma cells possessed patterns of gene expression which resembled more immature liver, whereas others were akin to the pattern which existed in less immature cells. In particular, many hepatoma cells display isoenzyme patterns which are found in early liver during development. It has been reported that the isoenzymic pattern with respect to alcohol dehydrogenase, pyruvate kinase, aldolase, and hexokinase in liver tumors show a fetal pattern (2). In established cell lines, for instance, the McA-RH7777 cell, expression of α-fetoprotein, which is characteristic of fetal liver is observed (3), whereas the H4-II-E-C3 line produces tyrosine aminotransferase (4) which is found only after birth. Often, the process of transformation leads to a loss in expression of liver-specific genes (2). Therefore it is possible to classify genes into three groups when comparing the pattern of expression observed in vivo for normal liver and that of hepatoma cell lines in vitro. The first group of genes are those which remain expressed; and in contrast the second group is suppressed. The third group comprises those genes which were expressed early in development, become suppressed during the course of normal development, and are reactivated as a consequence of transformation. The latter have been termed “oncodevelopmental” markers by Fishman (5). To better understand why particular genes are influenced one way or another during oncogenesis, it is necessary to compare their expression in the normal cell with its transformed counterpart under similar conditions.

We have previously shown that it is possible to transform fetal hepatocytes in culture by exposing cells to 3′-methyl-4-dimethyl-aminobenzene and phenobarbital (6). Cells lines derived by inoculating such cultures into immunodeficient nude mice were shown to possess hepatocytic properties in some instances, whereas others were completely devoid of any hepatocytic phenotype. Occasionally, cell lines possessing an intermediate phenotype were obtained. It was concluded that properties of the cell lines reflected the cellular origin of the transformed cell (6). Thus only those which were derived from parenchymal cells exhibited liver properties. Cultures which possessed intermediate characteristics were shown to comprise two cell types; one with a parenchymal morphology, the other resembling clear epithelial cells. In order to study changes in gene expression accompanying hepatocyte transformation, it is desirable to have a model whereby one can reproducibly isolate transformed cells which belong in the hepatocytic lineage. Then it is possible to compare the pattern of gene expression in the cell from which the transformed progeny was derived and the resulting tumor cell. This communication describes a procedure by which colonies of putative transformed parenchymal cells in fetal hepatocyte cultures exposed to MDAB2 can be selected for study. Five such cell lines are surveyed for a range of phenotypic markers which are characteristic of liver. In particular those markers such as α-fetoprotein (3), the acute phase proteins (7), aldolase (8), pyruvate kinase isoenzymes (9), and tyrosine aminotransferase (10) which are developmentally regulated are examined. Their expression is assessed with respect to the abundance of mRNA and the presence of proteins by immunocytochemistry.

MATERIALS AND METHODS

Animals. Fetal hepatocyte cultures were established from 19-day gestation Wistar albino rats of the species Rattus norvegicus. Putative transformed cells were inoculated in an athymic immunodeficient Swiss mouse designated Swiss ARC-nu/+ obtained from the Animal Resources Centre, Murdoch, Western Australia.

Chemicals. All chemicals were of analytical grade. Eagle’s MEM, Williams’ medium and fetal calf serum were obtained from Flow

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2 The abbreviations used are: MDAB, 3′-methyl-4-dimethyl-aminobenzene; MEM, minimal essential medium; GGT, γ-glutamyl transpeptidase; TAT, tyrosine aminotransferase; PK, pyruvate kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; cDNA, complementary DNA; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline.

7593
Gene Expression in Transformed Fetal Hepatocytes

Fig. 1. Photomicrograph of FRL 1 at passage 10 (a) and passage 21 (b) and FRL 1.1 (after passage through a nude mouse) at passage 3 (c) and 21 (d). Giant cells are arrowed in (c) and (d). × 100.

Laboratories, Annandale, New South Wales, Australia. Fungizone, penicillin, streptomycin, trypsin-EDTA, and glutamine were purchased from Grand Island Biological Co., Grand Island, NY. Sodium phenobarbitone was purchased from BDH Chemicals, Ltd., Poole, England, and MDAB was supplied by Tokyo Kasei Kogyo, Japan. 32P-dCTP was obtained from Amersham Australia Pty Ltd., Sydney, Australia. SDS and protein dye reagent concentrate were purchased from Bio-Rad Laboratories, Richmond, CA. EDTA, Tris, phenyl methylsulfonyl fluoride, diaminobenzoic acid, and dexamethasone were obtained from Sigma Chemical Co., St. Louis, MO. Collagenase used in this study is a product of Boehringer Mannheim, West Germany. GeneScreen was obtained from New England Nuclear, Sydney, Australia. Antibody to albumin, α-fetoprotein, transferrin, aldolase B, and TAT were prepared in our laboratory by immunization of rabbits with the respective purified proteins. Antibodies to L-PK and M2-PK were provided by Dr. T. Noguchi, Osaka University, Japan.

Culture Conditions. The basic culture medium was modified MEM supplemented with 10% fetal calf serum, glutamine (2.4 mM final concentration), Fungizone (28 μg/ml), and penicillin/streptomycin (57 units/ml and 28 μg/ml, respectively). This medium, referred to as MEM hereafter was used to maintain all the fetal liver cell lines. Cultures were maintained in a 5% CO2:95% air water-saturated atmosphere at 37°C on plastic culture dishes or in culture flasks gassed with 5% CO2:95% air mixture. Fresh medium was added the day after subculture and every second day thereafter. For subculturing, cells were overlayed with 0.25% trypsin-EDTA in a Ca2+, Mg2+-free balanced salt solution and then incubated for 10 min at 37°C. Detached cells were removed, centrifuged (200 × g) for 2 min, and resuspended in MEM by aspiration. For total RNA extraction approximately 20% of the cells from a confluent 25-cm2 flask were plated into 90-mm-diameter collagen-coated plastic culture dishes and supplemented with medium to a final volume of 10 ml. For propagation, cells were subcultured when the culture was confluent by placing 10% of the cells in a fresh culture flask. Cells were taken for assay when they reached confluency, usually after 3–4 days in culture.

Transformation of Fetal Hepatocytes. Fetal hepatocytes derived from 19-day-gestation rats were isolated and cultured as described by Yeoh et al. (10). Livers were aseptically removed and diced by using a Mickle
chopper (Mickle Laboratories Engineering Co., Surrey, United Kingdom) fitted with a razor blade. The preparation was incubated at 37°C with collagenase (0.5 mg/ml in Hanks’ balanced salt solution). After 15 min the mixture was filtered through cheesecloth and the suspension of cells was washed twice with balanced salt solution by centrifugation at 50 × g for 1 min and finally suspended in culture medium. The cells were exposed for 3 days to MDAB which was added to the medium dissolved in propylene glycol to give a final concentration of 5 μM. Following this, they were maintained in MEM supplemented with 10^-6 M dexamethasone and 1.5 × 10^-6 M phenobarbital. Such cultures can be maintained for 4–6 weeks and then undergo a crisis stage between 6 and 8 weeks when many cells die. After about 10 weeks, colonies consisting of clusters of rounded cells appear. By 12 weeks many of these are visible macroscopically. Under phase-contrast illumination, colonies comprising of cells with a hepatocyte morphology are marked and subsequently removed from the flask, using a cloning ring by trypsinization. These are then maintained and frozen at low passage (less than 10), while others are deliberately passaged frequently to obtain high passage cells (greater than 20). These cells are termed FRL 1, 2, 4, 5, 6 (FRL 3 was lost due to contamination). The low passage cells were also injected s.c. into immunodeficient nude mice, producing tumors from which cell lines were obtained. These are termed FRL 1.1, 2.1, etc.

γ-Glutamyl Transpeptidase Assay. The culture medium was dis-
The fixed cells were washed 3 times in PBS, incubated for 15 min with 0.1 M lysine in PBS-saponin (0.2%), followed by a 1-h incubation in 10% fetal bovine serum in PBS-saponin. Then circular areas of cells (about 5-mm diameter) were made by removal of surrounding cells using cotton swabs. This method enabled a single culture to be tested with a variety of antibodies. Each circle of cells was then reacted with a 1:200 and 1:400 dilution of first antibody, or non-immune rabbit IgG for 1 h. Subsequently, the cells were washed with PBS-saponin 3 times and a 1:200 dilution of the second antibody (peroxidase-coupled goat IgG directed against rabbit IgG) was added and incubated for 1 h. The washing procedure was repeated, followed by a final wash in 0.05 M Tris, pH 7.5. A solution of 0.05% diaminobenzidine and 0.01% H2O2 in 0.05 M Tris, pH 7.5, was added and allowed to react for 20 min. The cells were then washed with PBS. In all experiments, cells exposed to only the second antibody or to the IgG fraction of nonimmune serum were incorporated as negative controls.

The intensity of staining in selected samples was amplified by using a dimethylaminobenzene enhancement kit (Amersham International, United Kingdom) according to the protocol supplied with the kit.

Nucelic Acid Hybridization to Liver-specific mRNAs. Total RNA was isolated from FRL cells according to the method of LeMeur et al. (13) as soon as possible after the cultures had attained confluency. The samples were dispersed in 5 ml of buffer containing 3 M LiCl, 6 M urea, 10 mM sodium acetate (pH 5.0), 200 µg/ml heparin, and 0.1% SDS, using an Ultra-Turrax, and then left overnight at 4°C. The precipitated RNA was collected by centrifugation at 15,000 x g for 20 min, washed 4 times with 4 M LiCl, 8 M urea in water, dissolved in 50 mM sodium acetate (pH 5.0) in 1% SDS, and extracted with phenol-chloroform, then ether, prior to precipitation of RNA with ice-cold ethanol. The RNA was recovered by freeze drying the sample, dissolved in water, and its concentration was estimated by measuring its absorbance at 260 nm. For Northern analysis, 20 µg of RNA were electrophoresed according to the method of Lehrach et al. (14), and then electrorephoretically transferred to GeneScreen by using a Bio-Rad Transblot apparatus.

TAT mRNA was detected by hybridization to a 32P-labeled probe pCTAT3 (15), and L-PK and M2-PK mRNA were detected by using plPK1 (16) and pM2PK33 (17), respectively. Aldolase B was detected by using a 770-base pair cDNA which was isolated from a rat liver cDNA library and was isolated from a rat liver cDNA library using a 17-mer oligonucleotide based on the published sequence of the gene. The aldolase A (18), transferrin (19), albumin (20), α-fetoprotein (21), and glyceraldehyde-3-phosphate dehydrogenase (22) cDNA probes were generously supplied by the laboratories which cloned the respective sequences. Radioactivity was visualized by autoradiography. The cDNA probes were labeled by nick translation, using reagents obtained from International Biotechnologies, Inc. Hybridization in 50% deionized formamide, 5× standard saline citrate (1× is standard strength), 0.1% SDS, 5× Denhardt’s solution, and 250 µg/ml sonicated salmon sperm DNA was performed at 46, 55, and 42°C for TAT, L-PK, and M2-PK, respectively, for 3 h after 5 h of incubation at 37°C and the absorbance change at 405 nm was determined.

Cell line: Passage

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**Table 1** Relative levels of mRNA in FRL cells

The results are presented as mRNA for the respective genes relative to the level observed for GAPDH. Densitometric scans of Northern gels were performed for TAT, transferrin (TN), liver isoenzyme form (L-PK), and the embryonic form (M2-PK) of pyruvate kinase, and GAPDH. The intensity of the autoradiographs for TAT, transferrin (TN), liver isoenzyme form (L-PK), and the embryonic form (M2-PK) of GAPDH, and reprobed for thiostatin was determined for two sets of RNA by scanning on a densitometer. The mean values are presented.

**Fig. 4.** Northern analysis of mRNA derived from FRL cells probed for α2-macroglobulin (Mac) and α2-acid glycoprotein (App) on one membrane (a) and reprobed for thiostatin (b). L, low passage (<10); H, high passage (>20). M is RNA isolated from muscle, and L is RNA isolated from an adult rat undergoing an acute phase response.
Fig. 5. Photomicrographs showing immunocytochemical localization of tyrosine aminotransferase under standard conditions (a and b), after silver amplification of the dimethylaminoazobenzene stain (c and d), Mr-pyruvate kinase (e and f), transferrin (g and h), and L-pyruvate kinase (i and j). In each case the pairs of photomicrographs depict the cells viewed after phase contrast (a, c, and d) and bright field (b, d, and f) illumination. × 200.
GENE EXPRESSION IN TRANSFORMED FETAL HEPATOCYTES

Table 2 Relative intensity of staining for liver antigens in FRL cells compared with primary cultures of 19-day-gestation liver cells

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*ALB, albumin; AFP, α-fetoprotein; TN, transferrin; Ald-B, aldolase B.

RESULTS

Morphology. The cell lines FRL 1, 2, 4, 5, and 6 used in this study were morphologically similar and each did not change appreciably prior to, and after passage through a nude mouse. There was no change in morphology observable after extended passaging of the cells, although the tendency for cells to round up increased as a result of extended passaging. Fig. 1 shows the morphology of FRL 1 prior to passage through a nude mouse at passage 10 and 21 (Fig. 1, a and b, respectively), and after passage through a nude mouse at passage 3 and 21 (Fig. 1, c and d, respectively). The cells are epithelial-like with clearly defined nuclei and they invariably contain multiple nucleoli. There are always giant cells with multiple nuclei present (arrows in Fig. 1, c and d). When plated out initially, they form colonies of adherent cells which even at low density show evidence of some cells rounding up and piling up (Fig. 1, a and b). Rounded cells are observed to a greater extent when the culture attains a high density.

mRNA for Liver-specific and Development-specific Markers. Expression of liver-specific genes, in particular those which have been shown to be expressed at specific stages in liver development, was assessed in the cell lines designated FRL 1, 2, 4, 5, and 6. Total RNA from the cell lines before and after inoculation in nude mice at high and low passage number was isolated and Northern analyses of the respective mRNAs were carried out with the following cDNAs: albumin, α-fetoprotein, transferrin, the liver-specific isoform of pyruvate kinase (L-PK), the “embryonic” form of pyruvate kinase (M1-PK), aldolase A, aldolase B, and TAT. The level of GAPDH mRNA was assessed in total RNA extracts in order to control for the mRNA input on Northern gels as it is constitutively expressed in liver cells. Control experiments have shown that its level of expression correlates well with the intensity of staining of the ribosomal RNA bands with ethidium bromide.

GAPDH levels shown in Fig. 2a and Fig. 3a indicate that the amount of mRNA applied to the Northern gels is reasonably constant with the exception of FRL 2 (L), FRL 2 (H) and FRL 4 (L). The levels of albumin and α-fetoprotein mRNA was barely detectable; only faint signals were obtained for RNA extracted from all cell lines at low and high passage number (data not shown). Aldolase B mRNA was more abundant, but nevertheless was present at low levels (data not shown). Intermediate levels of mRNA for L-PK (Fig. 2b) and transferrin (Fig. 3c) were observed for all extracts. In contrast, high levels of TAT (Fig. 3b), M2-PK (Fig. 3c) and aldolase A (Fig. 2d) expression were seen with the FRL cell extracts. The results of densitometric analysis of the autoradiograms is presented in Table 1. No significant changes in expression were observed with extended passaging of the cells, or as a result of passaging the cells through nude mice with all the cell lines except FRL 4 and possibly FRL 6. In the FRL 4 line, a diminution in TAT and transferrin expression was observed after the cells were passaged through a nude mouse, resulting in a drop in index of mRNA level from 0.95 to 0.34 and from 1.61 to 0.73, respectively. In addition, there was a decline in the index for TAT, transferrin, and both L-PK and M2-PK as a consequence of passaging the cell line FRL 4.1 derived from the nude mouse. FRL 6 showed a decline in TAT expression between passage 7 (1.56) and passage 20 (0.44) which is possibly significant.

Hybridization analysis of the mRNA for the acute phase proteins shows that the FRL cells do not express detectable levels of α2-macroglobulin or thiostatin (Fig. 4, a and b). In contrast, α1-acid glycoprotein mRNA is present at levels which approach those observed in an adult rat undergoing acute inflammation in all the cell lines with the exception of FRL 6 and FRL 6.1 (Fig. 4a). Specificity of the hybridization conditions is demonstrated by the positive signal obtained for RNA isolated from the liver of a rat undergoing an acute phase response, and negative signals for RNA derived from muscle. High levels are observed in FRL 2, 4, and 5 with no appreciable alteration as a result of passaging or reintroduction into nude mice. In contrast, a reduction in expression in FRL 5.1 after increased passaging was observed. In FRL 1, reintroduction into nude mice results in a diminution of expression. Levels of α1-acid glycoprotein expression in FRL 6 and 6.1 are lower than that observed for the other cell lines.

7598

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Fig. 6. Photomicrographs showing immunocytochemical localization of liver proteins in 3-day-old cultures established from 19-day-gestation hepatocytes. Tyrosine aminotransferase (a and b) shows a heterogeneous pattern of staining. In contrast, staining for transferrin is homogeneous (c and d). In cocultures of primary 19-day-gestation hepatocytes and FRL cells, all the FRL cells are positive (arrows), but not the primary fetal hepatocytes (e and f). Transferrin stains both populations evenly (g and h), while the M2 form of pyruvate kinase only stains the FRL cells, and the primary fetal hepatocytes remain negative (i and j). × 200.

Immunocytochemical Studies. Cultures of the FRL cell lines were processed for immunocytochemistry to identify cells containing albumin, transferrin, aldolase B, L-PK and M2-PK, and TAT. Fig. 5 shows the pattern of staining obtained for TAT, L-PK, M2-PK, and transferrin, respectively. The cells are strongly positive for TAT (Fig. 5, a–d) and M2-PK (Fig. 5, e...
and f). However, staining for both TAT and M_2-PK is heterogeneous, with some cells very much more positive than others (Fig. 5, b, d, and e). Transferrin also stains positively (Fig. 5, g and h); staining is less intense with antibodies directed against L-PK (Fig. 5, i and j). In the case of transferrin and L-PK, all the cells are positive and a more even pattern of staining is observed (Fig. 5, h and j, respectively). Albumin and α-fetoprotein antibodies stained the FRL cells, but only marginally. The signal was significant above background, but it was extremely weak and could not be recorded in photomicrographs. The overall results from the immunocytochemical analyses are summarized in Table 2. These studies reveal that a good correlation exists between the mRNA levels and the intensity of staining with the respective antibodies. However, the pattern and intensity of staining with the different markers between 19-day-gestation hepatocytes and FRL cells differ.

In agreement with the mRNA data, FRL 1 cells show very intense staining for tyrosine aminotransferase. The staining pattern in these cells is different from that of primary cultures established from 19-day fetal hepatocytes because all the FRL cells are positive, although the level of staining varies (Fig. 5b). When the dimethylaminoazobenzene deposit is visually enhanced by silver staining, a gradation of intensity can be demonstrated for reaction against the TAT antibody in FRL cells (Fig. 5d). In the primary cultures of 19-day-gestation liver some fetal hepatocytes are positive, whereas a significant number are completely negative (Fig. 6a and b). This contrasts with the even pattern obtained with antibody directed against transferrin (Fig. 6, c and d). In order to compare both the pattern and the intensity of staining of TAT, transferrin, and M_2-PK in primary fetal hepatocytes and FRL cells, immunocytochemistry was performed on cocultures of the two cell types. These show the very similar morphology of the fetal hepatocytes and the tumor cells which are derived from them (Fig. 6, e, g, and i). Since the primary culture is established first, and FRL cells are added 2 days later, and the cultures are fixed for immunocytochemistry the following day, the FRL cells are present in these cultures more as individual cells in contrast to the primary cells which are in large groups, which helps to distinguish the two populations. Fig. 6f shows that positive and negative cells for TAT are only observed in the primary cells and that all of the FRL 1 cells (arrows) are strongly positive in the coculture. The intensity of staining for the two cell types is about the same when the strongly positive cells are compared. In contrast, transferrin is present in all FRL 1 cells as well as all of the primary cell culture derived from fetal rat liver (Fig. 6a). The pattern of staining for M_2-PK is quite different as only the FRL 1 cell line is positive, whereas the primary hepatocytes are negative (Fig. 6j).

Inducibility of Tyrosine Aminotransferase. The inducibility of tyrosine aminotransferase by dexamethasone was assessed in FRL 1 and FRL 2 cells in terms of enzyme activity, mRNA abundance, as well as by immunocytochemistry. The results summarized in Fig. 7 show that tyrosine aminotransferase activity is induced by dexamethasone in primary cultures established from 19-day-gestation liver and FRL 1 and FRL 2 by 6.6-, 6.3-, and 9.8-fold, respectively. The induction in tyrosine aminotransferase is reflected in alterations in the level of mRNA in the FRL 1 and FRL 2 cells as a consequence of exposure to dexamethasone relative to levels of GAPDH as shown by the Northern gel probes for TAT (Fig. 8a) and GAPDH (data not shown), respectively. Fig. 8b compares the counts obtained by cutting out the radioactive bands from membranes probed for TAT and GAPDH which show an induction of 5.7- and 4.8-fold for FRL 1 and FRL 2, respectively. Cultures of FRL 1 and FRL 2 maintained in medium supplemented with dexamethasone stained much more intensely for TAT when compared with cultures which did not receive the steroid analogue (data not shown).

γ-Glutamyl Transpeptidase. All the FRL cell lines showed significant levels of GGT activity. This was maintained in spite of repeated passaging of cells as well as reintroduction into nude mice. Levels of GGT observed in the FRL cell lines is summarized in Table 3. The GGT level observed for FRL cells is similar to that which was reported for cultured fetal hepatocytes derived from 19-day-gestation rats (6).

**DISCUSSION**

Different hepatoma cells express different liver-specific genes. Some display a “dedifferentiated” pattern of gene expression (1, 2). The Reuber hepatoma cell retains TAT expression (26), whereas in other cell lines it is suppressed. It is generally true that the isoenzymic forms of lactate dehydrogenase, pyruvate kinase, and aldolase in hepatomas are those which are found in fetal liver, and are not forms normally expressed in adult liver. These differences between hepatoma cells and normal liver may be the result of transformation or they may reflect differences between the cells from which the cancer cell originated. Alternatively, they may be attributable to varying environmental influences such as the hormonal status of the animal versus the culture conditions for maintaining the cell lines.

This study compares the pattern of gene expression in primary cultures of fetal hepatocytes established from 19-day-gestation rats and transformed cells derived from these cultures. These cells are morphologically similar to other established hepatoma cell lines such as McA-RH7777 (3) or H4-II-E-C3 (4), and they resembled hepatocytes which are derived from 19-day-gestation rat liver (see Fig. 6, c, d, and e). All the cell lines thus obtained also displayed some hepatocytic markers.

The initial cell lines FRL 1, FRL 2, etc., possess a phenotype which closely resembles that of the original primary culture of fetal parenchymal cells in certain respects. However, in other respects they are very different, for they contain very low or undetectable levels of mRNA for albumin, α-fetoprotein, α-2-macroglobulin, and thiostatin, and a low level of L-PK and aldolase B mRNA. Aldolase A and M_2 isoenzyme of pyruvate kinase mRNAs are expressed at high levels. Since hepatocytes derived from 19-day-gestation fetal liver express albumin (27),
aldolase B (18), L-PK (28), and high levels of thiostatin and \( \alpha_2 \)-macroglobulin (29), the FRL cells are different from normal fetal hepatocytes. They display intermediate levels of mRNA coding for transferrin and \( \alpha_1 \)-acid glycoprotein as well as the liver-specific isoenzyme of pyruvate kinase. We have shown that primary fetal hepatocytes express transferrin (30), the L-PK (31) and \( \alpha_1 \)-acid glycoprotein (32); therefore the FRL cells resemble their normal counterparts with respect to expression of these markers. \( \alpha_1 \)-Acid glycoprotein has been shown to be dexamethasone inducible. In this respect they are also similar to 19-day-gestation fetal hepatocytes. Furthermore, like the primary cultures, TAT expression is dexamethasone inducible. In this respect they are also similar to fetal hepatocytes derived from 19-day-gestation rats. Further studies involving transformation of fetal hepatocytes derived from primary cultures which are established from rats of different developmental age will show whether alterations in gene expression will be similar for hepatocytes of a particular developmental stage, and if the pattern between hepatocytes of different developmental age will display different patterns of gene expression. Thus it will be possible to determine if the pattern of expression of the transformed cell is at least in part determined by the pattern of the precursor cell, and what changes are obligatory to the transformed state.

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REFERENCES


Unpublished data.


Gene Expression in Clonally Derived Cell Lines Produced by \textit{in Vitro} Transformation of Rat Fetal Hepatocytes: Isolation of Cell Lines Which Retain Liver-specific Markers

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