Induction of Cytochrome P-450s and Expression of Liver-specific Genes in Rat Primary Hepatocytes Cultured on Different Extracellular Matrices

Natsuki Matsushita,†† Hiroaki Oda, Kazuikiyo Kobayashi,* Toshihiro Akaike,** and Akire Yoshida††

Laboratory of Nutritional Biochemistry, School of Agricultural Sciences, Nagoya University, Nagoya 464-01, Japan
* Laboratory of Polymer Chemistry, School of Agricultural Sciences, Nagoya, Nagoya University, Nagoya 464-01, Japan
** Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 227, Japan

Received January 11, 1994

Freshly isolated hepatocytes were cultured on an EHS-gel prepared from EHS-tumor, poly-N-p-vinylbenzyl-d-lactonamide (PVLA), and type I collagen (TIC). Hepatocytes on EHS-gel showed a spherical shape and much more strongly maintained the inducible expression of cytochrome P-450 genes which were lost on PVLA and TIC. Further, the expression of liver-specific genes were maintained on EHS gel at the highest level, and then higher on PVLA than TIC.

Adult rat hepatocytes in primary culture have been used for studying the mechanisms by which the growth and functions of differentiated cells are regulated. But monolayer hepatocytes rapidly lose some liver functions (e.g., induction of some cytochrome P-450s) after isolation on type I collagen (TIC) under the usual conditions. Therefore, a variety of attempts have been made to model cell culture systems involving culture media, extracellular matrices, and so on. Recent studies have demonstrated that a mixture of components (EHS gel) consisting of laminin, type IV collagen, and heparan sulfate proteoglycan extracted from a basement membrane of the EHS-tumor is found to be more effective for liver-specific gene expressions in hepatocytes. On the other hand, Kobayashi et al. synthesized a lactose-carrying styrene homopolymer, poly-N-p-vinylbenzyl-d-lactonamide (PVLA) to enhance the adhesion of hepatocytes to culture dishes and reported that PVLA enhanced the survival efficiency of hepatocytes. Hepatocytes on PVLA interestingly form spherical shapes similar to those of cells on EHS-gel, while liver-specific gene expression in cells on PVLA has not been examined. This study deals with the induction of cytochrome P-450s by xenobiotics and the mRNA levels of other liver-specific genes in hepatocytes cultured on these materials (i.e., TIC, EHS gel, and PVLA). The research reported here used of a primary hepatocyte culture system in which cytochrome P-450 can be expressed in response to xenobiotics. This system promises to be of substantial use in the future investigation of the regulation of the cytochrome P-450 genes and other liver-specific genes.

Parenchymal hepatocytes were isolated from rat liver by the method of Seglen and cultured in serum-free Waymouth 752/1 medium (Flow Lab., Scotland) containing 10⁻⁸ m insulin (Sigma Chemical Co., U.S.A.) as the sole hormone, together with amphotericin (0.25 µg/ml), penicillin (5 I.U./ml), and streptomycin (5 µg/ml). Hepatocytes were plated at 10⁵ cells in 3.5 ml of culture medium onto 60-mm plastic dishes coated with the following materials, (a) dry TIC (Corning Co., Ltd.), (b) the EHS gel (1 mg of protein per dish) prepared from the EHS-tumor as described previously, or (c) PVLA as described previously, and then incubated in a humidified atmosphere of 5% CO₂ and 95% air at 37°C. Four h after plating, the medium was replaced to remove dead cells.

Twenty-four h after plating, hepatocytes were morphologically examined with an Olympus inverted phase-contrast microscope. As shown in Fig. 1, hepatocytes cultured on TIC were flat and extended (Fig. 1A), but they remained round on EHS gel and PVLA 24 h after isolation (Figs. 1B and 1C). Their spherical shapes were continually observed without changes for at least 7 days (data not shown). Cell survival on PVLA was the same as on TIC, and improved on EHS gel as reported previously. It is known that the addition of epithelial growth factor (EGF) and insulin to hepatocytes on PVLA makes them form into spheroids, three-dimensional multicellular aggregations, which maintain their functions at a higher level, but our hepatocytes did not form spheroids without EGF in this study.

Twenty-four h after plating, 3-methylcholanthrene (3-MC) (1 µg/ml) or sodium phenobarbital (PB) (2 µM), was added and then hepatocytes were cultured for 48 h more. Isolation of total RNA from these hepatocytes was done as described by Chomczynski and Sacchi. Northern blot analysis was done as described by Thomas. RNA samples (10 µg) were electrophoresed on denaturing formaldehyde/agarose gels, transferred to Zeta-Probe nylon membranes (Bio-Rad), and then hybridized to 32P-labelled cDNA probes. The cDNA clones of rat CYPIA1, CY2B1, albumin, and apolipoprotein A-I (apo A-I) were obtained from Dr. K. Kawajiri (Saitama Cancer Research Institute, Japan), Dr. Y. Fujii-Kuriyama (Tohoku University, Japan), Dr. K. Nakamura (Nagoya University, Japan), and Dr. J. I. Gordon (Washington University, U.S.A.), respectively. As shown in the Fig. 2A, the induction of CY2B1/2B2 gene expression by PB was observed only in cells on EHS gel, but not on TIC and PVLA. The induction of CYPIA1 by 3-MC, which was not observed by Schuetz et al., was also observed only in hepatocytes on EHS gel as shown in Fig. 2B. On the other hand, the induction of CYPIA1 gene expression by 3-MC was observed in all the three cultures. Hepatocytes on EHS gel had the highest mRNA levels of albumin and apo A-I, and these two liver-specific gene expressions were higher on PVLA than on TIC (Fig. 2C and 2D).

†† This work was supported in part by a grant from the Elizabeth Arnold Fuji Foundation, Japan.
* To whom correspondence should be addressed. Present address: Bio-Mimetic Control Research Center, The Institute of Physical and Chemical Research (RIKEN), 3-8-31, Rokuban-cho, Atsuta-ku, Nagoya 456, Japan.
** Present address: Nagoya Bunri College, Nagoya 451, Japan.

Abbreviations: apo, apolipoprotein; EHS, Engelbreth-Holm-Swarm tumor; 3-MC, 3-methylcholanthrene; PB, phenobarbital; PVLA, poly-N-p-vinylbenzyl-d-lactonamide; TIC, type I collagen.
A recent study\textsuperscript{7} showed that the extracellular matrix may activate liver-enriched transcriptional regulatory factors, HNF3\textalpha{} and eH-TF, which regulate the transcription of the albumin gene. Thus, the EHS gel may affect the gene expression via the transcription machinery.

It is reported that under the culture conditions tried so far, hepatocytes have lost the ability to have cytochrome P-450s induced by xenobiotics and to express albumin and apo A-I genes.\textsuperscript{2} However, our findings demonstrated that the EHS gel provided the best conditions for long-term maintenance of differentiated functions, especially, the expression of cytochrome P-450 and other liver-specific genes. Longevity of cells on PVLA was similar to that on TIC, but PVLA expressed higher levels of liver-specific genes. PVLA as well as EHS gel permitted cells to remain round. Ben-Ze'ev \textit{et al.}\textsuperscript{5} have reported that hepatocytes cultured on hydrated type I collagen gel, when cultured at high density, are spherical and maintain a pattern of gene expression resembling that of hepatocytes on EHS gel. It seems that there may be some relationship between round shape and cell functions. However, as the induction of CYP2B1/2B2 was observed only in EHS gel but not in PVLA, this induction may not be due to the round shape of hepatocytes. Caron\textsuperscript{6} have demonstrated that addition of dilute EHS gel to hepatocytes cultured on TIC increased albumin production without morphological changes. Therefore, our observation suggests that cell shape is not a primary regulator for phenotypic expression, but the extracellular matrix might transmit signals through the membrane to let the cells express liver-specific genes. On the other hand, it has been demonstrated that DNA synthesis was similar in hepatocytes inoculated at high density (3.5 \times 10^6 cells per 60-mm dish) on either TIC or EHS gel and proliferative response to growth factors was low throughout on both substrates.\textsuperscript{4,5} This information points to the importance of cell-matrix interactions in determining the differentiated phenotype of hepatocytes. The molecular mechanisms by which these extracellular matrices affect the organization of hepatocytes and the differentiated functions, remain to be identified.

Acknowledgments. We thank Dr. K. Kimata (Aichi Medical University, Japan) for his kind gift of mouse EHS-tumor and technical advice for its passage. We are grateful to Dr. K. Kawajiri (Saitama Cancer Research Institute, Japan), Dr. Y. Fuji-Kuriyama (Tohoku University, Japan), and Dr. K. Nakamura (Nagoya University, Japan), Dr. J. I. Gordon (Washington University, U.S.A.), for cDNA probes for rat CYP1A2, rat CYP2B1, rat albumin, and rat apo-A-I, respectively.

References