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Transfection of adult primary rat hepatocytes in culture

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Abstract

The use of adult primary hepatocytes in culture is of importance for the understanding of hepatic processes at the cellular and molecular levels, and the possibility to employ transient transfection of reporter constructs is invaluable for mechanistic studies on hepatic gene regulation. Although frequently used, there is a lack of reports addressing optimization and characterization of transfection of primary rodent hepatocytes. Here, we have shown that the efficiency of biochemical transfection reagents varies significantly and that Lipofectamine2000 was a superior transfection reagent for adult primary rat hepatocytes when using luciferase reporter vectors. The efficiency increased when the cells were allowed ample time to adapt to the in vitro milieu. Cotransfection of a second reporter gene indicated a risk for promoter competition, and we found that relating reporter activity to total cellular protein content gave consistent and reliable results. Differentiation of the cells, achieved by including biomatrix from the Engelbreth–Holm–Swarm mouse sarcoma in the culture system, was to a larger extent required for hormonal/drug responses of transfected constructs than for responses of endogenous genes and assured responses of transfected constructs. Dexamethasone (Dex) is most often included in hepatocyte culture media, but we could not demonstrate a general beneficial effect of Dex on expression of luciferease reporter contructs. Using the established protocol, we have demonstrated responses of transfected constructs to growth hormone, glucocorticoid and LXR stimuli.

Keywords: Rat liver; Primary hepatocytes; Transfection; Lipofection; Luciferase reporter; Extracellular matrix

1. Introduction

Primary culture of liver cells is a valuable system for studying the biology and pathobiology of the liver. The system allows examination of direct effects of hormones and xenobiotics as well as studies on molecular mechanisms of hepatic gene regulation. For maintenance of the adult hepatocyte phenotype in vitro, the attachment support plays a dominant role, and numerous examples in the literature corroborate the beneficial effect of adhesion of the cells to an extracellular matrix, such as the biomatrix extracted from the Engelbreth–Holm–Swarm mouse sarcoma (EHS/Matrigel®) [1]. The effect is attributed to a morphology more closely resembling that of the hepatocyte in vivo, accompanied by maintained expression of liver enriched transcription factors [2,3]. Even transformed, poorly differentiated hepatoma cells have been shown to gain some differentiated functions when cultured in the presence of EHS [2].

A common way to identify gene regulatory sequences is by transient transfection of reporter gene constructs. Liposome mediated transfection (lipofection), probably the most versatile method for introducing exogenous DNA into cells, of primary hepatocytes is, however, severely reduced when the cells are cultured on EHS [4]. This has, as shown by Shih and Towle [5], been possible to overcome by transfecting hepatocytes shortly after plating, followed by an overlay of diluted EHS. A number of studies have emerged in which primary rat hepatocytes have been transfected using different transfection reagents, methods and reporter genes. It is generally accepted that primary hepatocytes are not easy to transfect and the sensitivity of the reporter is an issue. Luciferase is a favourable reporter due to its high sensitivity and the activity is easily measured. Moreover, for cotransfection of a reporter plasmid for normalization, the use of firefly and *Renilla* luciferase reporters makes it possible to measure the respective luciferase activity in a single sample with a dual assay. Despite this, no study has, to our knowledge, addressed optimization of lipofection of primary rat hepatocytes using luciferase as reporters. This is a pertinent task in

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that the kinetics of transient expression can vary with the reporter gene used and with the type of cell transfected [6]. Furthermore, cotransfection of a second plasmid to determine transfection efficiency can affect expression of the test plasmid or vice versa [7], and we have experienced squelching of hormonal response upon cotransfection of primary hepatocytes using electroporation [8]. In an attempt to apply the method of an overlay of diluted EHS for studies on growth hormone (GH)-regulation of gene expression in primary rat hepatocytes, preliminary results indicated that a GH-response was obtained also in the absence of EHS. This prompted us to reinvestigate the prerequisite of EHS for hormonal/drug inducibility of rat hepatic genes and, following optimization of a protocol for lipofection of primary rat hepatocytes, we have investigated hormonal/drug inducibility of luciferase reporter gene activity by different response elements with and without an overlay of EHS. We have also addressed the issue of normalization for transfection efficiency.

2. Materials and methods

2.1. Primary rat hepatocyte cultures

The Stockholm South Ethical Committee of the Swedish National Board for Laboratory Animals approved all animal procedures. Hepatocytes were isolated from 7 to 8-week-old female Sprague–Dawley rats purchased from B&K Universal (Stockholm, Sweden). Hepatocytes were isolated by non-recirculating collagenase perfusion through the portal vein of isoflurane anesthetized rats as previously described [9]. The cells were filtered through a double layer of gauze, pelleted and resuspended in fresh medium four times. Cell viability was assessed by trypan blue exclusion and averaged 90–95%. For studies on induction of endogenous genes, 10×10^6 cells were plated onto 100-mm dishes uncoated or coated with EHS. For transfection experiments, 2×10^6 cells, unless otherwise stated, were plated onto uncoated 60-mm dishes. Cells plated onto uncoated dishes were plated in medium containing 2% fetal calf serum, whereas cells plated onto EHS-coated dishes were in serum free medium. Culture dishes were obtained from Nunc A/S (Roskilde, Denmark). Williams' E medium with Glutamax (Invitrogen Life Technologies, Paisley, Scotland, UK) supplemented with 10 nM insulin (Sigma–Aldrich, Stockholm, Sweden) and 100 U/ml of penicillin and 100 μg of streptomycin/ml (Invitrogen, Life Technologies) was used in all experiments except in Fig. 1B where modified Waymouth's 752 medium [10] was used. The cells were maintained at 37 °C in a humidified 5% CO_2 incubator. Cells were allowed to attach for 15–18 h, thereafter all cultures received serum free medium. EHS-matrix was prepared from Engelbreth–Holm–Swarm sarcoma propagated in C57BL/6 female mice and stored at −20 °C as previously described [1]. The EHS (10 mg/ml) was thawed on ice, and 7 μl/cm^2 was evenly spread onto the dish using a rubber policeman and allowed to form a gel

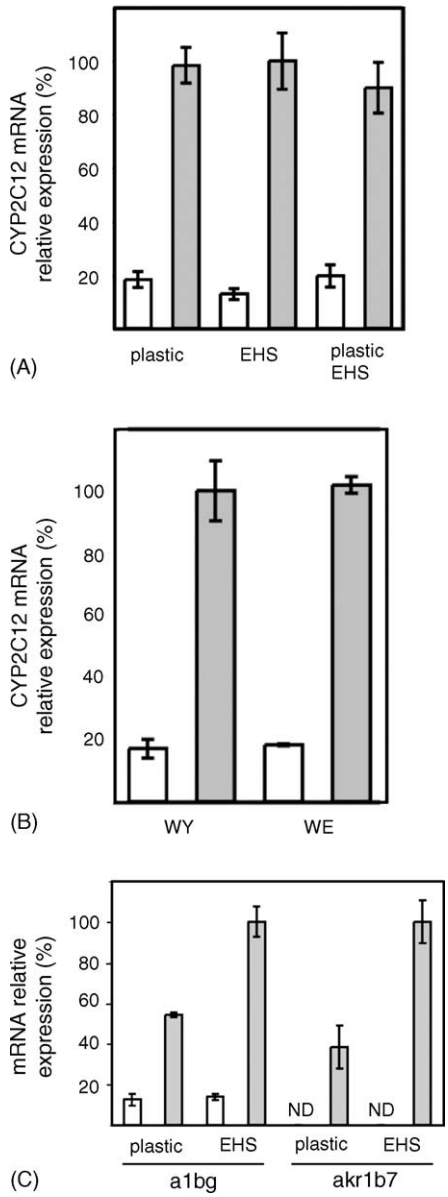

Fig. 1. Effect of different culture conditions on GH-induced gene expression in female derived primary rat hepatocytes. (A) Cells were cultured in modified Williams' E medium on uncoated dishes (plastic), on EHS-coated dishes (EHS) or on uncoated dishes with an overlay with EHS at the time of GH treatment (plastic/EHS). At 42 h of culture age, cells were treated (grey bars) or not (open bars) with 4.5 nM GH and harvested 20 h later. Prepared tNA samples were analyzed for CYP2C12 mRNA contents. The results are expressed in percent of the level in GH treated cells cultured on EHS-coated dishes. (B) Cells were cultured on uncoated dishes in modified Waymouth 752 (WY) or Williams' E (WE) medium, treated with GH and analyzed as in (A), the level of CYP2C12 mRNA in GH treated cells (grey bars) cultured in WY was set to 100%. (C) Expression of a1bg and akr1b7 mRNAs in response to GH treatment (grey bars) in cells cultured on uncoated (plastic) or EHS-coated (EHS) dishes. The levels of expression in cells cultured on EHS with GH treatment were set to 100%. Values shown are the mean ± S.D. from three independent dishes. ND, not detectable.