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Optimization of the fractionation of HepG2 cell line by differential centrifugation

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Background and Aims: The expression and study of cellular kinetic in mammalian culture systems can be achieved during cell lysis, cell fractionation and organelle isolation. No standard and simple protocol exists for the subcellular fractionation of HepG2 cell line. Currently published protocols involve time consuming centrifugation steps requiring expensive equipments and commercially available kits can be prohibitively expensive when handling ultiple samples. The isolation of plasma membrane fraction is difficult and needs ultracentrifuges or gradient centrifugation. The aim of this study was establishing a subcellular fractionation method for HepG2 and subsequently defining the intracellular kinetic of medicines and poisons.
Methods: Several techniques for cell homogenization and fractionation were compared and their suitability, advantages, and limitations for HepG2 were discused, resultig to the the most effective method as explained below. The cells were scraped into PBS, treated with lysis buffer and subjected to liquid shear and homogenized in a Dounce homogenizer with 20 strokes. Then the organelles were separated by differential centrifugation. The Nuclear pellet was centrifuged out at 1000 g for 10 minutes. Then the supernatant was centrifuged at 15000 g to pellet the mitochondrial fraction. Plasma membranes were isolated by a cationic colloidal silica isolation technique and electron microscopy was used to confirm the fractionantion.
Results: This technique may easily be used in cell biology to purify a specific organelle from a homogenate of HepG2 cells. All used buffers can be prepared inexpensively and easily and the protocol requires no costly equipment.
Conclusions: We have established a protocol to sequentially extract organells from cultured HepG2 cells in fractions enriched for membrane, nuclear mitochondiria and cytosolic organelles. We describe a protocol for the crude subcellular fractionation of cultured mammalian cells that is both straightforward and cost effective and may facilitate the more accurate study of cellular kinetic.

Keywords: HepG2; Homogenization; Differential centrifugation; Colloidal silica