

DEVELOPMENT OF NOVEL HUMAN HEPATOCYTE CELL LINES

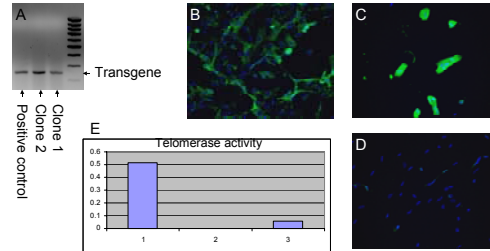
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Introduction

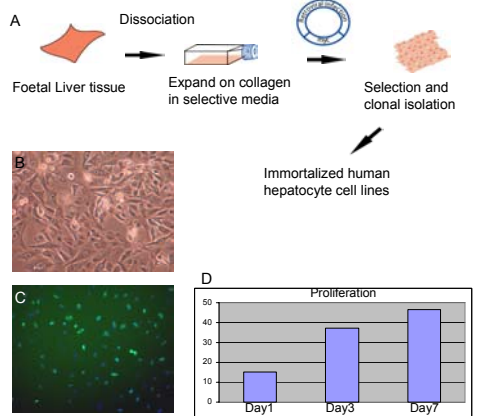
We have developed several conditionally immortalized human hepatocyte cell lines. Actively dividing primary hepatocytes from a 15 week fetus were immortalised by retroviral infection with a human c-myc gene fused to a mutated estradiol receptor, which can be activated by 4-hydroxytamoxifen (4-OHT). In this system, the presence of 4-OHT drives the hepatocytes to undergo proliferation. These cell lines express the immortalising transgene and hepatocyte markers including albumin, cytochrome-18 (ck-18), cyp3A7 and the main drug metabolising enzyme cyp3A4. The cell lines also lack expression of the immature hepatocyte marker α -fetoprotein (AFP).

Function of the immortalizing construct



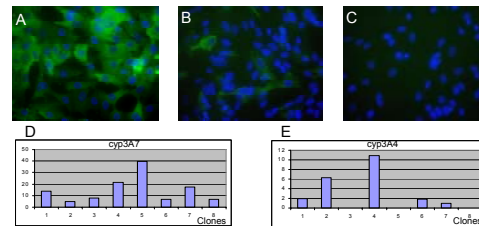
A) Confirmation of expression of the immortalizing transgene with RT-PCR. B) Immortalized hepatocytes stained for c-myc. C) Immortalized hepatocytes stained for c-myc after 1 week with no 4-OHT. D) Primary hepatocytes stained for c-myc. E) Telomerase expression for 1: Immortalised hepatocytes, 2: Immortalised hepatocytes with no 4-OHT for 1 week, 3: Primary hepatocytes.

Immortalized human hepatocyte cells



A) Primary fetal hepatocytes were dissociated, cultured and immortalized by c-myc retroviral infection. B) Image of proliferating hepatocytes in culture. C) Hepatocytes positive for the proliferation marker Ki-67. D) Percent of hepatocytes in culture after dissociation, which were positive for the Ki-67 proliferation marker.

Expression of hepatocyte markers



Immortalized hepatocytes stained for: A) Albumin, B) Ck-18, C) AFP. QRT-PCR results for several immortalized clones for: D) cyp 3A7, E) cyp3A4

Summary

Novel conditionally immortalized human hepatocyte cell lines expressing hepatocyte markers including the major drug metabolising enzyme cyp3A4, and lacking expression of the immature hepatocyte marker AFP have been established.