

# New immortalised human fetal liver cell line, cBAL111, differentiates into hepatocytes *in vivo* and *in vitro*

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## Background

The success of Bio-Artificial Liver (BAL) systems relies on the development of a human hepatocyte cell line that combines both *in vitro* functionality and proliferation capacity. The aim of this study was therefore to develop a human hepatocyte cell line with sufficient hepatic function for *in vitro* applications.

## Generation of cBAL111

Human fetal liver cells (HFLC) were isolated from fetal livers at 16 weeks gestational age. A clonal derivative of HFLCs was immortalised by restoration of telomerase activity by lentiviral introduction of the hTERT cDNA into a monoclonal HFLC cell line. This cell line was named cBAL111.

## *In vitro* functionality cBAL111

Hepatic function of cBAL111 was tested 2 days after seeding and compared with that of primary mature and fetal human liver cells, 2 days after seeding and expanded HFLCs 2 days after the fourth passage (Table 1). Although many hepatic functions of HFLCs in primary culture are comparable to primary mature human hepatocytes, their functionality rapidly decreased after *in vitro* expansion. Under standard culture conditions, this cell line showed low hepatic functionality.

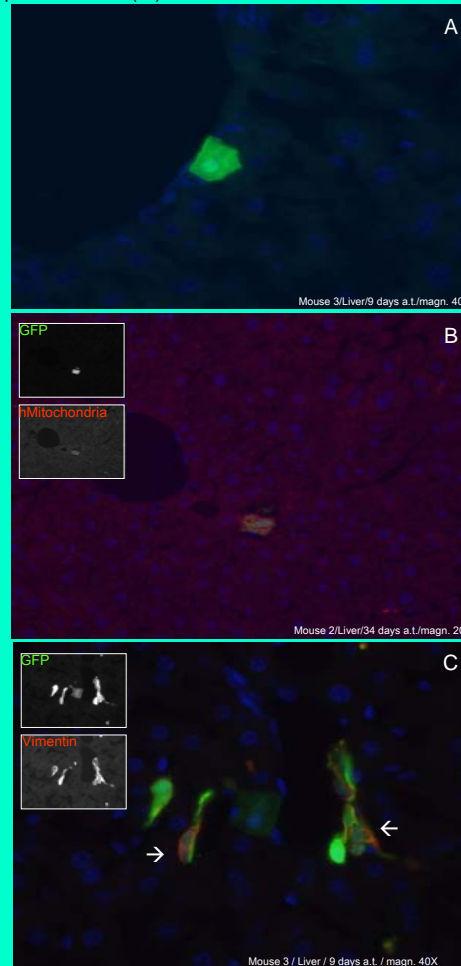
**Table 1:** Functionality of cBAL111 under standard culture conditions, compared to primary mature human hepatocytes, primary and expanded human fetal liver cells. Functions are expressed as nmol/h/mg protein, unless stated otherwise. Mean  $\pm$  SD, ND=not determined, -=undetectable.

Function		Cells			
		Primary mature liver cells	Primary fetal liver cells	Expanded fetal liver cells	cBAL111
Albumin production (ng/h/mg protein)		38 $\pm$ 8	47 $\pm$ 16	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0
Urea production		92 $\pm$ 34	21 $\pm$ 7	0.6 $\pm$ 0.5	15 $\pm$ 7
Ammonia elimination		95 $\pm$ 61	-	-	-
Lidocaine elimination		8 $\pm$ 10	-	ND	-
Galactose elimination		0.3 $\pm$ 0.4	0.1 $\pm$ 0.1	ND	-
mRNA levels (% of mature liver <i>in vivo</i> )	Albumin	100% Set as a reference	29	1	<1
	Transferrin		43	<1	<1
	AAT		180	<1	<1
	HNF 1 $\alpha$		179	24	1
	HNF 4		196	51	35
	Cyp 3A4		<1	<1	ND
	Cyp 3A7		1924	296	1944
	GST $\pi$		6818	21	89
	AFP		578	20	ND

## *In vivo* differentiation cBAL111

One million cBAL111 cells overexpressing GFP were transplanted in the tip of the spleen of Rag2<sup>-/-</sup> $\gamma$ C<sup>-/-</sup> mice. Livers were examined nine or 34 days after transplantation (Fig. 1). The cells had engrafted in the liver, adapted hepatocyte morphology and lost vimentin expression, indicating complete differentiation potential.

**Figure 1:** Transplanted GFP+ cBAL111 cells are present in the liver of Rag2<sup>-/-</sup> $\gamma$ C<sup>-/-</sup> mice (A). These cells contain human mitochondria (B) and do not express vimentin (C), where the undifferentiated cBAL111 cells do express vimentin ( $\rightarrow$ ).

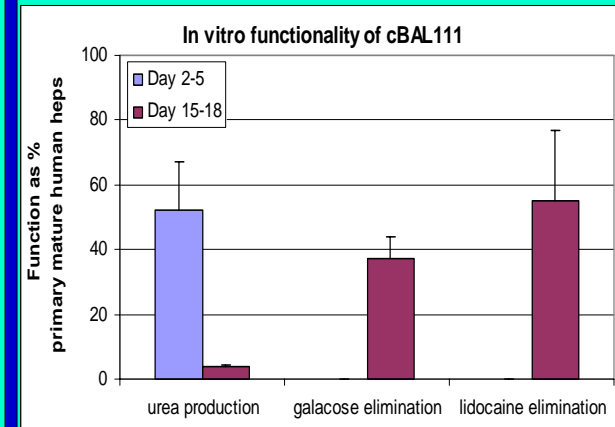


## Stimulation of *in vitro* differentiation

To induce hepatic differentiation *in vitro*, we studied the effect of cell density. Lidocaine and galactose elimination increased with cell density to levels comparable with mature human hepatocytes; surprisingly, urea production was inversely correlated with cell density (Fig. 2).

When cBAL111 was cultured in a bioartificial liver system, ammonia elimination was detected, up to 5% compared to mature human hepatocytes, whereas HFLC produce ammonia.

**Figure 2:** Hepatic functionality of cBAL111 in relation to cell density. The functionality of cBAL111 was tested at 2-5 and 15-18 days after seeding.



## Conclusions

We developed a new human hepatic cell line, cBAL111, with full differentiation potential *in vivo*. *In vitro* functionality of cBAL111 is strongly dependent on cell density but is higher as compared to its parental HFLCs in e.g. ammonia eliminating capacity.

Currently, cBAL111 is further characterised using microarray analysis to identify genes differentially expressed between cBAL111 and mature human hepatocytes. This information will be used to further enhance hepatocyte function of cBAL111 prior to testing in a BAL system in an animal model of acute liver failure.