# HUMAN MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE: DIFFERENTIATION INTO HEPATIC LINEAGE

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# BACKGROUND

- ***Liver transplant is the only way to treat patients with heavily damaged livers.***

- **Present limitations**: **Not enough livers available**

  **Rejection is not resolved**

- **Future prospects**: **To find an alternative therapy to LT**

  **Temporally bridging to LT**

- **Alternatives proposed:** **Systems for hepatic artificial support**

  **Xenotransplantation**

  **Gen therapy *ex-vivo* or *in-vivo***

  **Tissue engineering**

# BACKGROUND

- **There is growing evidence of stem cells reservoirs in several types of adult tissues.**

- **These cells may retain the potential to transdifferentiate from one phenotype to another.**

- A **future goal** of **liver-directed cell therapy**:

  - **Replacement of diseased hepatocytes by stem cells**

  - **Stimulation of endogenous or exogenous regeneration by stem cellls**

# BACKGROUND

- Adipose tissue is a rich source of mesenchymal stem cells (MSC), providing an abundant and accessible source of adult stem cells.

- A putative stem cell population within the adipose stromal compartment has been identified (ADSCs).

- ADSC compared with MSC from other sources, show better adaptability to culture conditions and higher proliferation capacity.

- Hence, adipose tissue might be an ideal source of large amounts of autologous stem cells attainable by a less

# AIM OF OUR RESEARH:

**To INVESTIGATE the differentiation of hMSCs from ADIPOSE TISSUE towards HEPATOCYTES**

# METHODS

- **Cell cultures**
- **Hepatic differentiation protocol**
- **Transduction of ADSCs with adenoviral vector cEBPβ**

# METHODS: Cell culture

**Human adipose tissue was obtained by lipectomy from six healthy patients (between 38 and 49 years).**

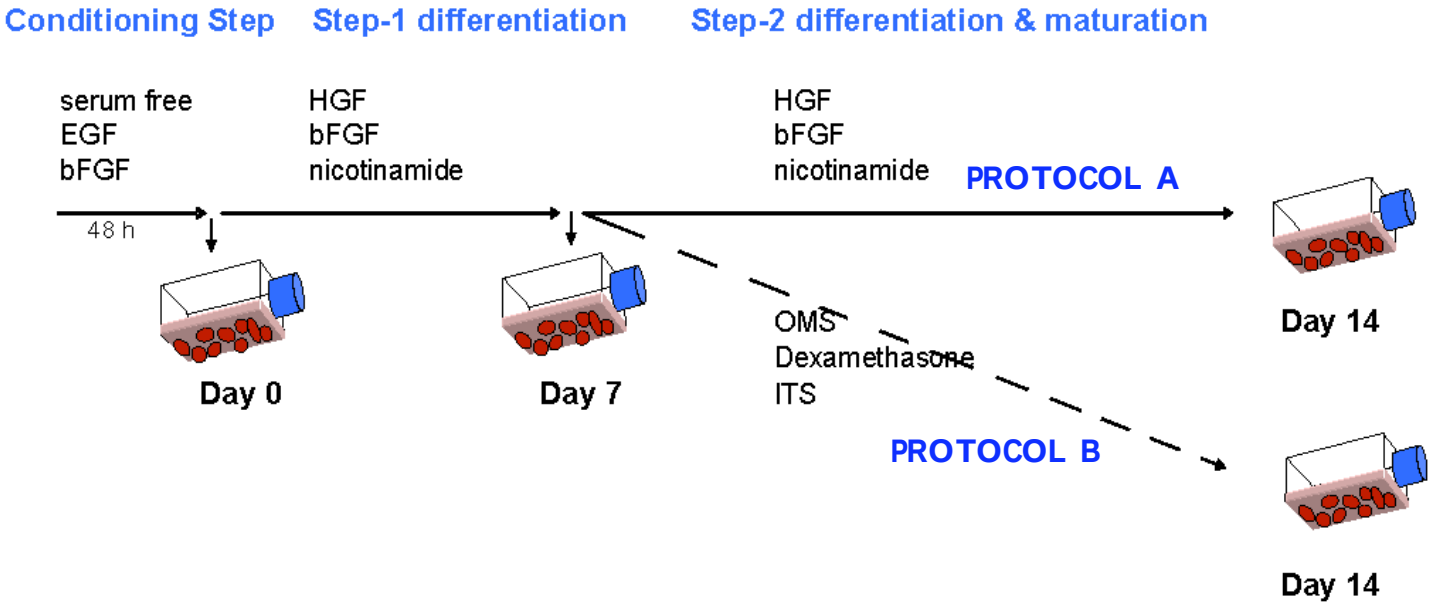
**Fragments were digested with collagenasa I (1mg/ml) in HBSS at 37°C for 60 min.**

**Cells were washed, pelleted and resuspended in DMEM-low glucose supplemented with 15% AB human serum.**

**Cells were plated and cultured for 48 hours , and then subcultured at a density of 5-10x$10^3$ cells/$cm^2$, (passage 1)**

**Monolayers were subcultured (passage 2) and used for differentiation assays when cell monolayer reached 85% confluency**

# METHODS: differentiation protocol



# METHODS: transduction with adenoviral vector cEBPβ

- CCAAT/enhancer-binding protein (C/EBP) are a family of liver-enriched transcription factors, which play an important role in regulating the transcription of multiple hepatic genes.

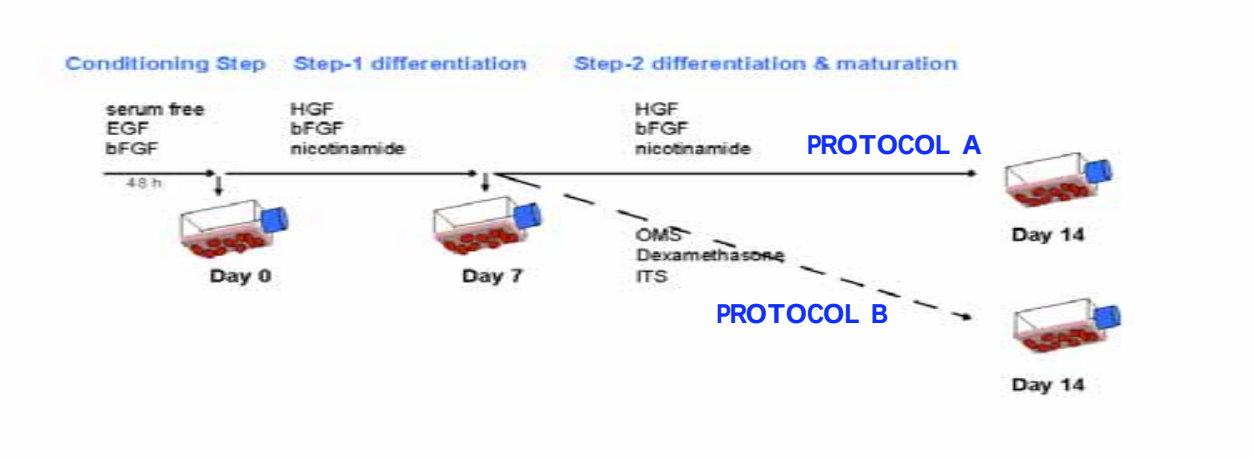
- To check the role of C/EBPb, ADSCs were transduced after 7 days of culture with increasing doses of adenoviruses (C/EBPβ) for 120min at a MOI (multiplicity of infection) ranging from 3 to 15 PFUs (plaque formit units) per cell (MOI).

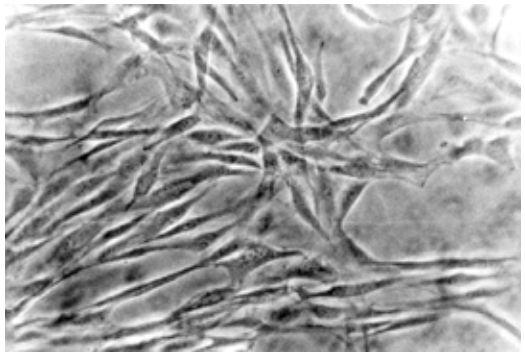
- Cells were washed with PBS and free medium was added. 48 hours post-transduction cells were analyzed or harvested for analysis and frozen in liquid nitrogen.

# RESULTS

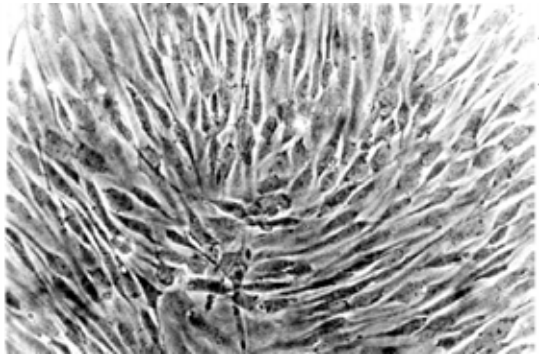
- **Morphologic changes**
- **RT-PCR of hepatic gene expression**
- **Role of cEBPβ factor in MSC to hepatic transition**

# RESULTS: morphologic changes

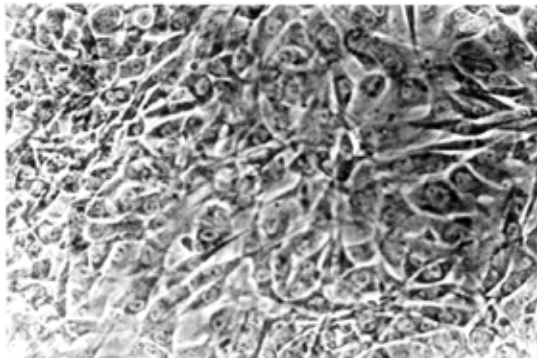


**Fibroblastic-like morphology**

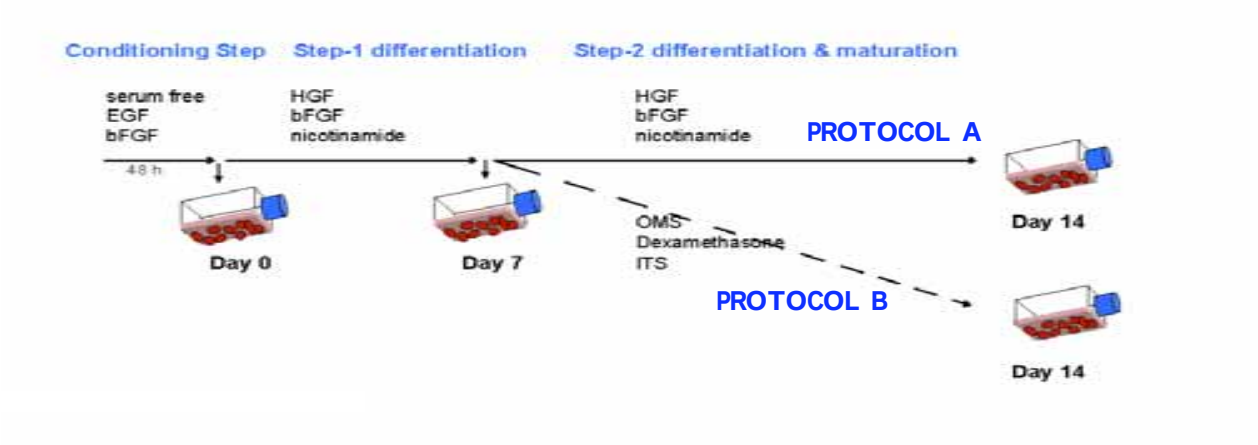


**Broadened flattened shape**



**Polygonal shape**

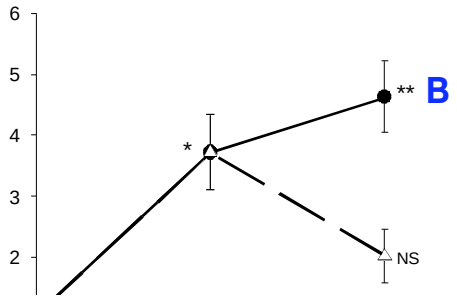
# RESULTS: RT-PCR analysis of hepatic gene expression




A

A

B

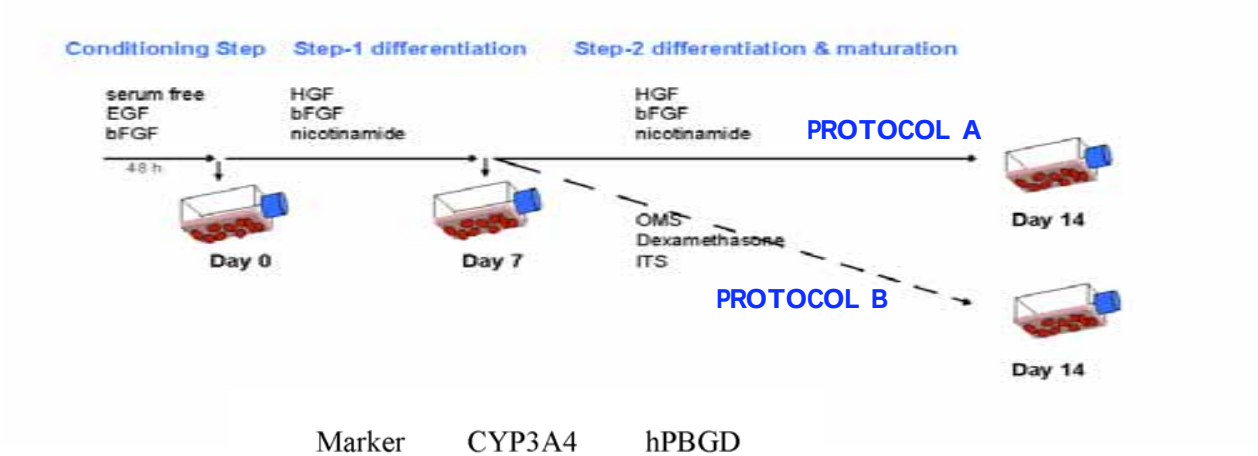



B

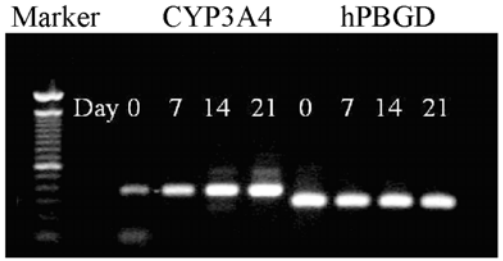
**Expression of mRNA for albumin**

**Expression of mRNA for TTR**

# RESULTS: RT-PCR analysis of hepatic gene expression




A




**Expression of mRNA for CYP3A4**

B

A

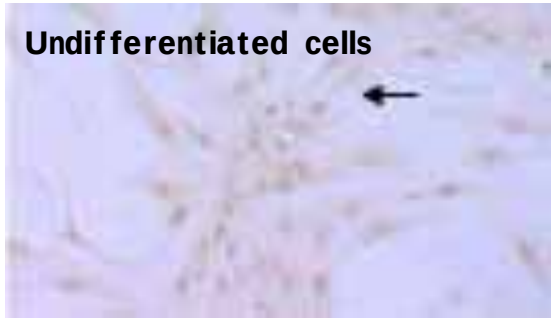
B

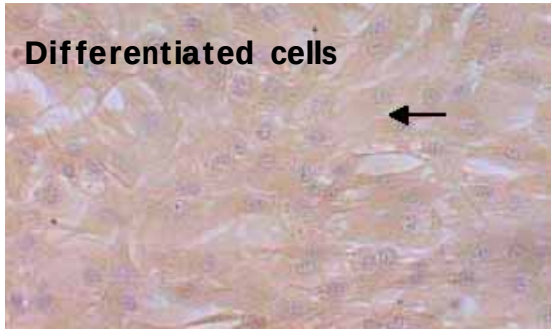
**Expression of mRNA for CYP2E1**

**Expression of mRNA for cEBPβ**

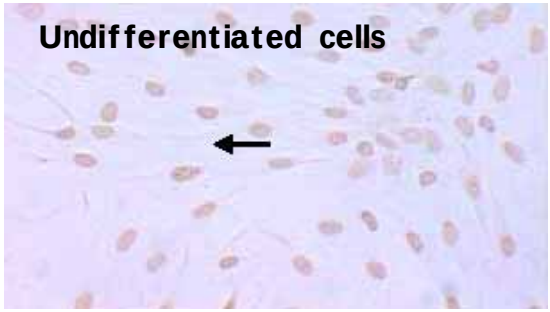
# RESULTS: Immunohistochemical analysis

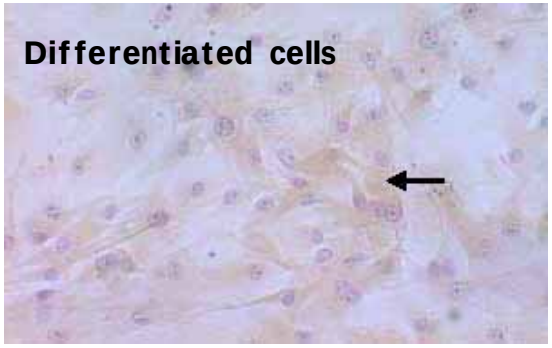
**ALBUMIN**



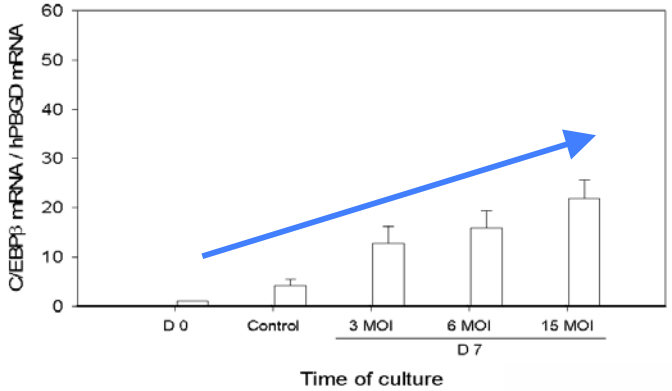

**ALPHA-FETOPROTEIN**



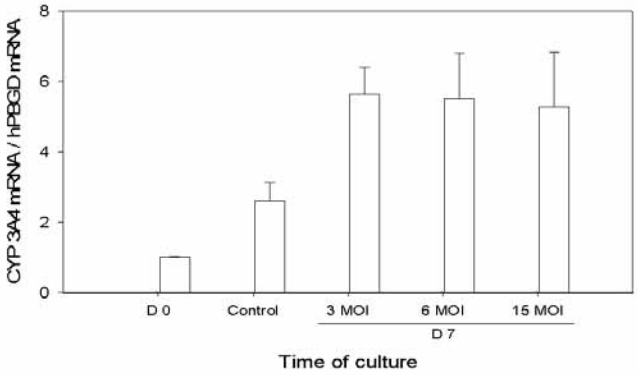



# RESULTS: transduction with adenoviral vectors

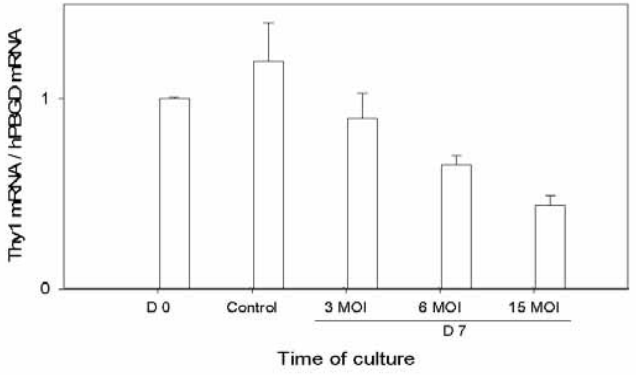
To investigate the relevance of the increase in the expression of C/EBPβ during the transdifferentiation of ADSCs, we transduced undifferentiated cells with adenoviral vector C/EBPβ.




•Adenoviral transduction cause a dose-dependent increase in the level of C/EBP β.




•An up-regulation of CYP3A4 was observed




•A down-regulation of the mesenchymal marker THY 1 was observed

# CONCLUSIONS

**Mesenchymal stem cells from adipose tissue can be induced to hepatogenic transdifferentiation *in vitro*.**

**ADSCs have a long culture survival period and a high proliferation capacity.**

**Therefore, adipose tissue may be an ideal source of large amounts of autologous stem cells, and may become a promising alternative for hepatocyte regeneration, liver cell transplantation or preclinical drug testing**

# THANK YOU