Newsletter

"OZB Highlights" 2010 - Volume 2 Number 1

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OZ BIOSCIENCES
The art of delivery systems

Sucessful DNA transfection in mouse hematopoietic cells with DreamFect™ (cell lines) and PolyMag (primary cells)

Khurana S. et al. Hepatocyte nuclear factor-4 induces transdifferentiation of hematopoietic cells into hepatocytes. *Journal of biological chemistry. 2010; 285:4725-4731.*

Hepatocyte Nuclear Factor-4 (HNF4) is a transcription factor that belongs to the superfamily of the nuclear hormone receptors. In mammals, it is represented by two paralogs : HNF4α and HNF4γ. If the latter one's functions are not well characterized, HNF4α is better known. Its mutations have been associated with maturity onset of diabetes of the young and in murine models, its gene disruption leads to embryonic death at day 6,5. Moreover, HNF4α is indispensable for hepatic specification and controls epithelial morphology.

In this paper Khurana *et al.* demonstrated the role of HNF4α as a molecular switch in the transdifferentiation mechanisms of hematopoietic cells into hepatocytes. Transfection experiments with **DreamFect™** (Lipofection) or **PolyMag** (Magnetofection™) have been used to study the loss and gain of function either in hematopoietic cell line (murine myeloid cells - 32Dcl3) or in primary cells purified from a lineage-depleted oncostatin M receptor β-expressing (Lin$^-$OSMRβ^+) mouse bone marrow cells.

To assess the role of HNFα in cell transdifferentiation into hepatocytes, a full length form of HNF4α (FL-HNFα) was transfected into 32Dcl3 cells using **DreamFect™**. The numerous experiments that followed this transfection method allowed the authors to observe : - a distinct epithelial morphology, - the overexpression of c-Met, a characteristic receptor of hepatocytes, - the upregulation of albumin-related genes, - the myeloid colony-forming ability, and the presence of hepatic markers in FL-HNF4α transfected cells. These results suggested that ectopic expression of HNF4α caused transdifferentiation of 32D cells into hepatocyte-like cells.
To confirm that HFN4α is indispensable for this process, Lin$^-$OSMRβ^+ cells were transfected with a dominant negative form (DN-HNF4α) using **PolyMag**. Thus, the DN-HNF4α transfectant expressed considerably lower amounts of hepatocyte specific gene markers confirming the obligatory role of HNF4α during transdifferentiation.

Deciphering the HNF4α mechanisms is of paramount importance because transdifferentiation is thought to be involved in tissue regeneration. Using **DreamFect™** and **PolyMag**, the authors have unravelled the molecular basis for the conversion of Bone Marrow derived stem cells into hepatocytes by highlighting the molecular switch function of HNF4α in transdifferentiation.

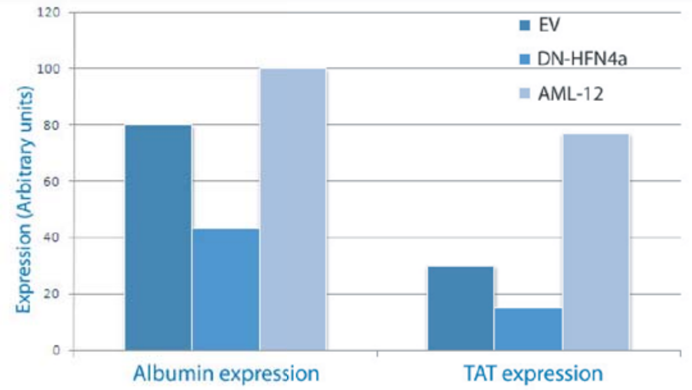
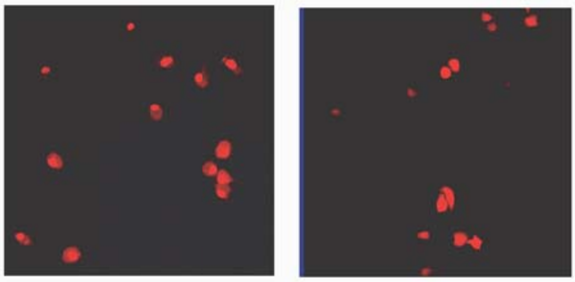


Figure 1: Relative transcription levels of Albumin and Tyrosine Amino Transferase genes
Lin$^-$OSMRβ^+ cells transfected with EV (vector control), DN-HFN4α plasmid (test sample) and AML-12 cells (positive control).



Primary hepatocytes   32Dcl3 Cells

Figure 2: Transfected cells with PolyMag (Primary hepatocytes) and with DreamFect (32Dcl3 cells).

We are grateful to Dr. Khurana, for kindly providing these pictures.

Other successful DreamFect™ transfections on cell lines:
- 3T3-L1, P815, SH-SY5Y, SHP-77, U2-OS cells: Grohman M. *et al.* BMC Cancer. 2009; **9**:301.
- MKN45 cells: Imamura Y. *et al.* Annals of Surgical Oncology. 2010; **17**:643-652.
- HeLa cells: Uehara R. *et al.* PNAS. 2009; **106**:6998-7003.

Other sucessful transfections with PolyMag on primary cells:
-Primary human epithelial corneal cells: Ueta M. *et al.* Brain Research Bulletin. 2010; **81**:219-228.
-Primary T lymphocytes (CD4+): Cho ML. *et al.* Immunology letters. 2009; **123**: 21-30.
-Primary spermatozoa duroc boar: Kim TS. *et al.* Reprod. Dom. Anim. 2009; doi: 10.1111/j.1439-0531.2009.01516.x.

Simultaneous and selective DNA co-transfection in primary hippocampal neurons with CombiMag or NeuroMag

Application note: Christophe Pellegrino and Igor Medina
INMED/INSERM Unité 29, 163 Route de Luminy, 13009 Marseille, France

Primary cultures of rat hippocampal neurons

Neurons from 18-day rat embryos were dissociated using trypsin and plated on coverslips coated with polyethyleneimine at a density of 70 000 cells/cm^2 in minimal essential medium (MEM) supplemented with 10% NU serum (BD Biosciences), 0.8% glucose, 1mm sodium pyruvate, 2mm glutamine, and 10 IU/ml penicillin-streptomycin as previously described (Buerli, Pellegrino et al.)[1]. On days 7, 10 and 13 of culture incubation, one-half of the medium was changed to MEM supplemented with 2% B27 supplement (Invitrogen). Transfections were performed after 14 D.I.V.

Procedure for co-transfection in the same neurons (Fig.1)

This procedure allows you to transfect 2 or 3 different plasmid DNA into the same neurons. For instance, for a shRNA approach you can have a pGFP + a control shRNA, pGFP + a shRNA of interest and pGFP+ a shRNA of interest + plasmid encoding the targeted protein as rescue.
This procedure is an adaptation of the previous protocol, published in 2007 in Nature Protocols (Buerli, Pellegrino et al.)[1].

The protocol is divided in several steps: For a 35mm well plate

i) Prepare a 1,5mL microtube containing: 300µL of Opti-MEM® (Invitrogen), 1µg of the desired DNA (for example GFP encoding vector) and 1µg of the the second DNA of interest (RFP encoding vector in this case), 7µL of Lipofectamine™ 2000 (Invitrogen), 2µl of **CombiMag** (OZ Biosciences), then after vortexing incubate 20 minutes at room temperature.
Notes: ***NeuroMag*** *can be used (3.5µL/µg DNA) instead of* ***CombiMag*** *+ Lipofectamine™ 2000*

ii) After the incubation time, add the mixture on culture dish. Incubate 30-35 min in the incubator on the magnetic plate, then replace the media by a warmed new one and return to the incubator until assay.

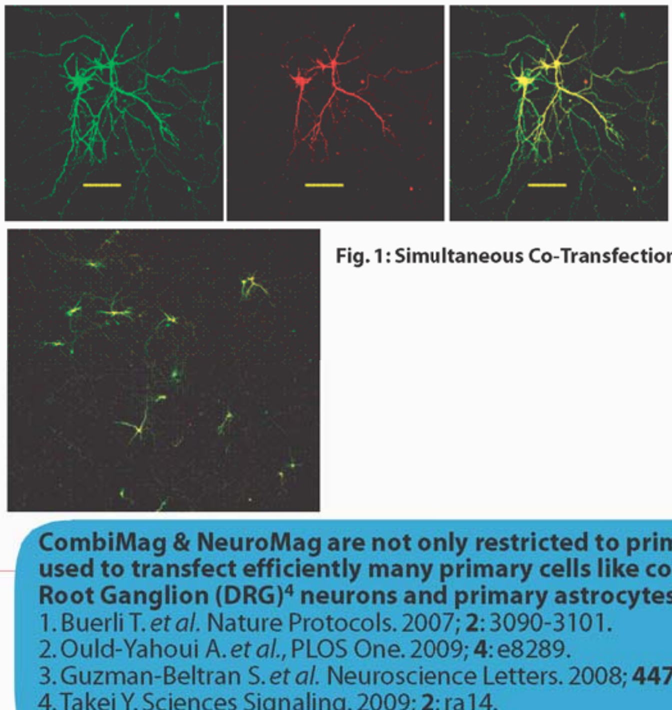


Fig. 1: Simultaneous Co-Transfection

Procedure for co-transfection in separate neurons (Fig.2)

This procedure as above allows you to transfect two or three different plasmid DNA into different neurons cultured on the same dish. For a shRNA approach, in his case you can have on the same dish transfected neurons with pRFP as controls and pGFP+ shRNA of interest or any other combinations.
This procedure is an adaptation of the previous protocol, published in 2007 in Nature Protocols (Buerli, Pellegrino et al.)[1].

The protocol is divided in several steps: For a 35mm well plate

i) Prepare a 1,5mL microtube containing: 150µl of Opti-MEM® (Invitrogen), 1µg of the desired DNA (for example GFP encoding vector), 3.5µl of Lipofectamine™ 2000 (Invitrogen), 1µl of **CombiMag** (OZ Biosciences), then after vortexing incubate 20 minutes at room temperature.
Notes: ***NeuroMag*** *can be used (3.5µL/µg DNA) instead of* ***CombiMag*** *+ Lipofectamine™ 2000.*

ii) In a second 1,5 mL microtube, prepare the same mixture but instead of GFP encoding vector add the second DNA of interest (RFP encoding vector in this case). Vortex and incubate also 20 minutes at room temperature.

iii) After the incubation time, pool together the two tubes and add immediately on culture dish. Incubate 30-35 min in the incubator on the magnetic plate, then replace the media by a warmed new one and return to the incubator until assay.

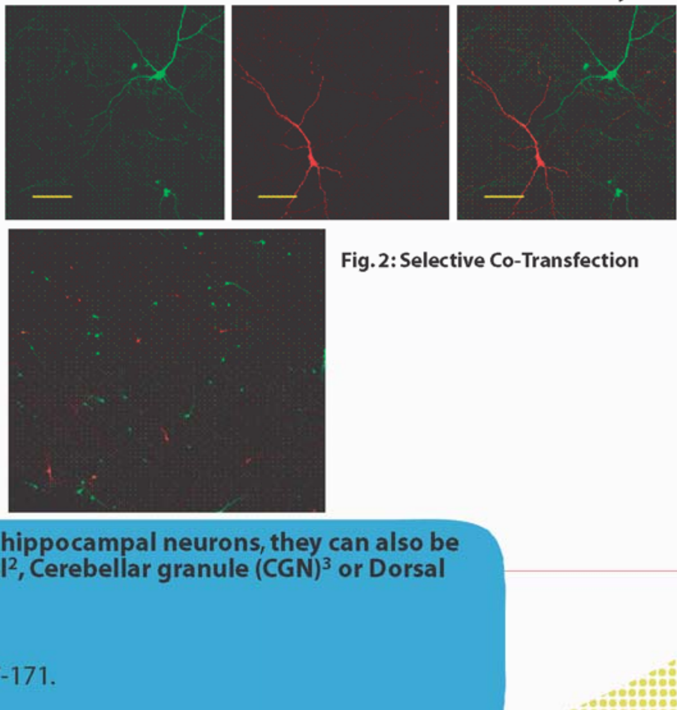


Fig. 2: Selective Co-Transfection

CombiMag & NeuroMag are not only restricted to primary hippocampal neurons, they can also be used to transfect efficiently many primary cells like cortical[2], Cerebellar granule (CGN)[3] or Dorsal Root Ganglion (DRG)[4] neurons and primary astrocytes[5]:

1. Buerli T. *et al.* Nature Protocols. 2007; **2**: 3090-3101.
2. Ould-Yahoui A. *et al.*, PLOS One. 2009; **4**: e8289.
3. Guzman-Beltran S. *et al.* Neuroscience Letters. 2008; **447**: 167-171.
4. Takei Y. Sciences Signaling. 2009; **2**: ra14.
5. Pickard M. *et al.* Nanomedicine. 2010; **5**: 217-232.

Infection of immature haematopoietic cells (KLS$^+$) favored and enhanced with ViroMag

Naka K. et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature. 2010;* 463: *676-680.*

FOXO is a protein that belongs to the O subclass of the forkhead family of transcription factors. This protein likely functions as a trigger for apoptosis and are suppressed by Akt signaling. In Chronic Myeloid Leukaemia (CML) Akt signaling is activated by a genetic disorder that generates BCR-ABL production leading to a deregulation of CML cells apoptosis. Although CML treatment (like imatinib) inhibits the tyrosine kinase leading to efficient leukemia cells death it does not deplete the leukaemia-initiating cells that drive the recurrence of CML.

In this paper, Naka K. *et al.*, first proved that in KLS$^+$ cells (Leukaemia Initiating Cells, LICs) FOXO3a remained active in the nucleus whereas most KLS$^-$ cells (non-LICs) showed the expected cytoplasmic localization of Foxo3a. They then showed with a Foxo3a-deficient CML mouse model that FOXO3a is essential for the long-term maintenance of leukaemia-initiating potential. For this purpose Naka K. *et al.* establish a CML-like MPD mouse model. They infected KLS$^+$ cells with MSCV-BCR-ABL-ires-GFP using **ViroMag** (Magnetofection^{TM}) and then transplanted the transduced cells intravenously into lethally irradiated (9.5 Gy) C57BL/6 congenic mice.

ViroMag allowed the authors to increase virus infection and concentrate the virus onto cells. This model corresponds to the first bone marrow transplanted (BMT) recipient group. To generate second and third BMT recipient group they transplanted of Foxo3a$^{+/+}$ and Foxo3a$^{-/-}$ LICs from previous BMT mice into a new set of recipients. The first and second Foxo3a$^{+/+}$ and Foxo3a$^{-/-}$ group showed the same CML symptoms and mortality. However, the third Foxo3a$^{-/-}$ LIC recipient group did not developed ALL or CML 40 days after the BMT compared to Foxo3a$^{+/+}$ LIC group suggesting that FOXO3a deficient LICs lose their potential to generate malignancies.

Using purified foxo3a$^{-/-}$ or foxo3a$^{+/+}$ KLS$^+$ cells, they showed that Foxo3a is required for LIC survival because it mediates suppression of apoptosis. To assess the role of TGF-β-FOXO axis they also displayed in vitro that TGF-β signalling pathway controls FOXO3a localization (either in nucleus or in cytoplasm) in CML LICs suggesting that TGF-β-FOXO inhibition might represent a highly useful adjunct to TKI therapy.

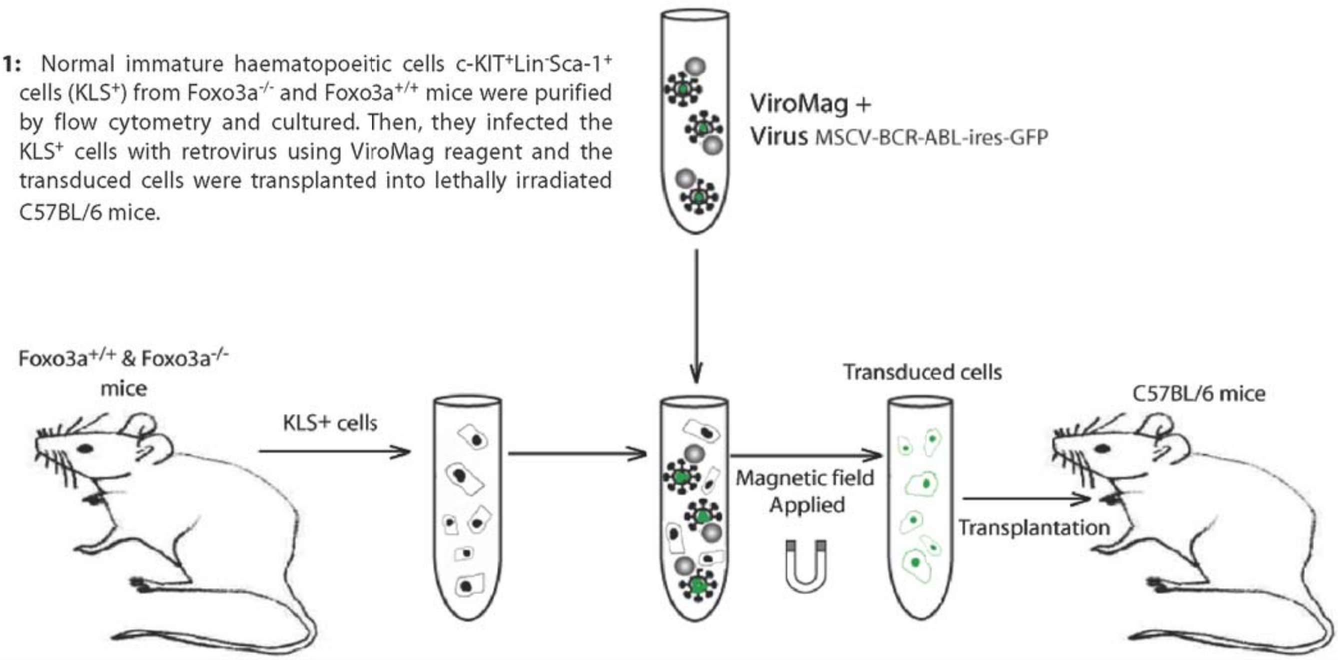


Figure 1: Normal immature haematopoeitic cells c-KIT$^+$Lin$^-$Sca-1$^+$ cells (KLS$^+$) from Foxo3a$^{-/-}$ and Foxo3a$^{+/+}$ mice were purified by flow cytometry and cultured. Then, they infected the KLS$^+$ cells with retrovirus using ViroMag reagent and the transduced cells were transplanted into lethally irradiated C57BL/6 mice.

Other successful applications of ViroMag and ViroMag R/L:

-Primary T lymphocytes (CD4+) PBMC and splenocytes:

Sacha JB. *et al.* Nature Protocols. 2010; **5**,2:239-246.

Greene JM. *et al.* Journal of Virology. 2010; 2010; doi:10.1128/JVI.02028-09.

Bolton DL. *et al.* Journal of Immunology. 2010; 184 doi/10.4049/jimmunol.0902413.

Minang JT. *et al.* Journal of Immunology. 2010; 184 doi/10.4049/jimmunol.0902410.

Vojnov L. *et al.* Journal of Virology. 2010; **84**, 2 : 753-764.

-Ph1-positive leukemia cell lines (KPON): Miyazaki K. *et al.* Blood. 2009; 113:4702-4710.

For more information:

DreamFect™ DNA and siRNA transfection reagent:
http://www.ozbiosciences.com/dreamfect.html

PolyMag, magnetic assisted transfection reagent for DNA and siRNA:
http://www.ozbiosciences.com/polymag.html

CombiMag, magnetic assisted transfection reagent:
http://www.ozbiosciences.com/combimag.html

NeuroMag, magnetic assisted transfection reagent for neurons:
http://www.ozbiosciences.com/neuromag.html

ViroMag, magnetic assisted transduction reagent:
http://www.ozbiosciences.com/viromag.html

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