Cell therapeutics for the Insulin-Dependent Diabetes Mellitus

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Introduction

- Type I diabetes is caused by the autoimmune destruction of pancreatic β-cells.
- Many studies have shown that transplantation of undifferentiated cells could normalize hyperglycemia of diabetic animals.
- These studies have shown that cell transplantation might lower the blood glucose level of the recipient **via supporting the regeneration of recipient β-cells** rather than providing insulin synthesized by donor cells.
- However, regenerated β-cells in type I diabetic patient can again be damaged by autoimmune attacks.
- Recruitment of therapeutic cells that directly regulate the blood glucose level of the patient following transplantation is an important issue in treating type I diabetes.

Sources of Therapeutic Cells

- **Embryonic stem cells (ESC)**

- **Fetal stem cells**
  - Amniotic fluid-derived cells

- **Adult stem cells (Tissue-specific stem cells)**
  - Bone marrow-derived hematopoietic stem cells (HSC) & mesenchymal stem cells (MSC)
  - Umbilical cord blood-derived HSC & MSC
  - Truncal and abdominal adipose-derived cells
  - Amniotic membrane-derived MSC
  - Epidermal stem cells
  - Tooth stem cells

- **Induced pluripotent stem cells (iPC)**

Requirements for the Therapeutic Cells

- **Safety**
  - Non-tumorigenicity
  - No side effect

- **Stability throughout lifespan**
  - Long term survival
  - Long term functional

- **Consistency after preservation**
  - Cryopreservation

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Table 1. Transplantation of human adult stem cells into diabetic animals.

Human cell source for transplantation	In vitro differentiation	Effects in diabetic animal model	Mouse blood insulin (human insulin)	Reference
Neural progenitor cell line	O	Normoglycemia	? (32pg/ml human c-peptide)	Hori et al. (2005)
Placenta-derived multipotent cells	O	Normoglycemia	?	Chang et al. (2007)
PDX-1 gene transfected BM-MSC	O	Reverse hyperglycemia	?	Karnieli et al. (2007)
PDX-1 gene transfected BM-MSC	O	Reverse hyperglycemia	?	Li et al. (2007)
PDX-1, NeuroD1, Ngn3 gene transfected BM-MSC	O	Normoglycemia	? (1.6ng/ml human c-peptide)	Zhao et al. (2008)
Umbilical cord stem cells	O	Reverse hyperglycemia	? (115pg/ml human insulin)	Chao et al. (2008)
UCB mononuclear cells	X	Lowered hyperglycemia	?	Ende et al. (2004)
BM-MSC	X	Reverse hyperglycemia	?	Lee et al. (2006)
PB insulin-producing cells	X	Reduced hyperglycemia	? (12pmol/L human c-peptide)	Zhao et al., (2007)

Materials and Methods

- Isolation of human adult stem cells from eyelid adipose (hEA) and amnion (hAM)
- RT-PCR
- Immunocytochemistry & immunohistochemistry
- Differentiation of stem cells into insulin-secreting cells in vitro
- ELISA for the insulin and c-peptide
- Dithiozone staining
- Therapeutic effect tests by transplantation of insulin-secreting cells into STZ-induced diabetic mice

Results

1. Differentiation of hEA and hAM into Insulin-Secreting Cells In Vitro

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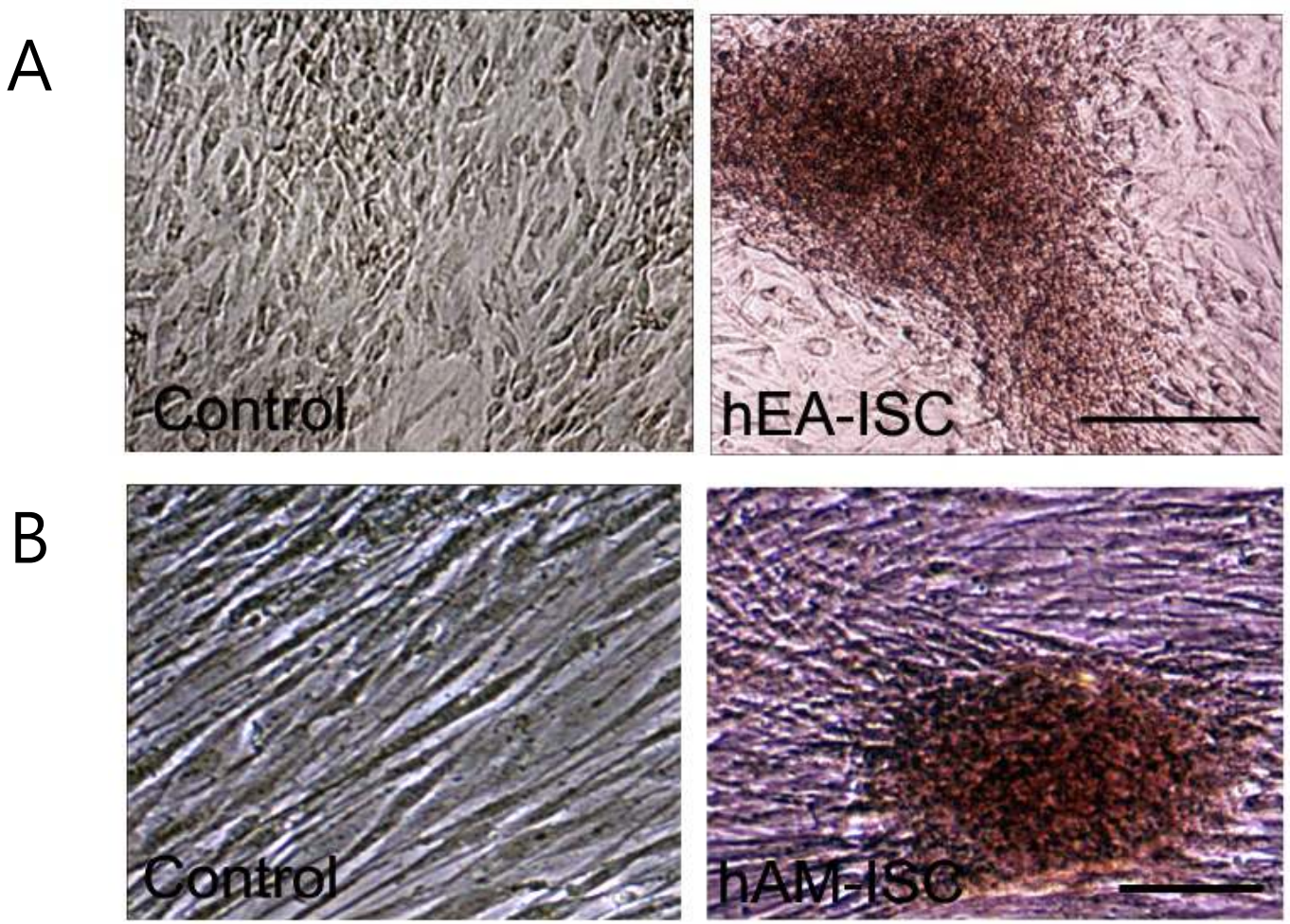

Fig.1. Dithizone staining of hEA and hAM before and after differentiation culture in insulinogenic medium. (A) hEA, (B) hAM.

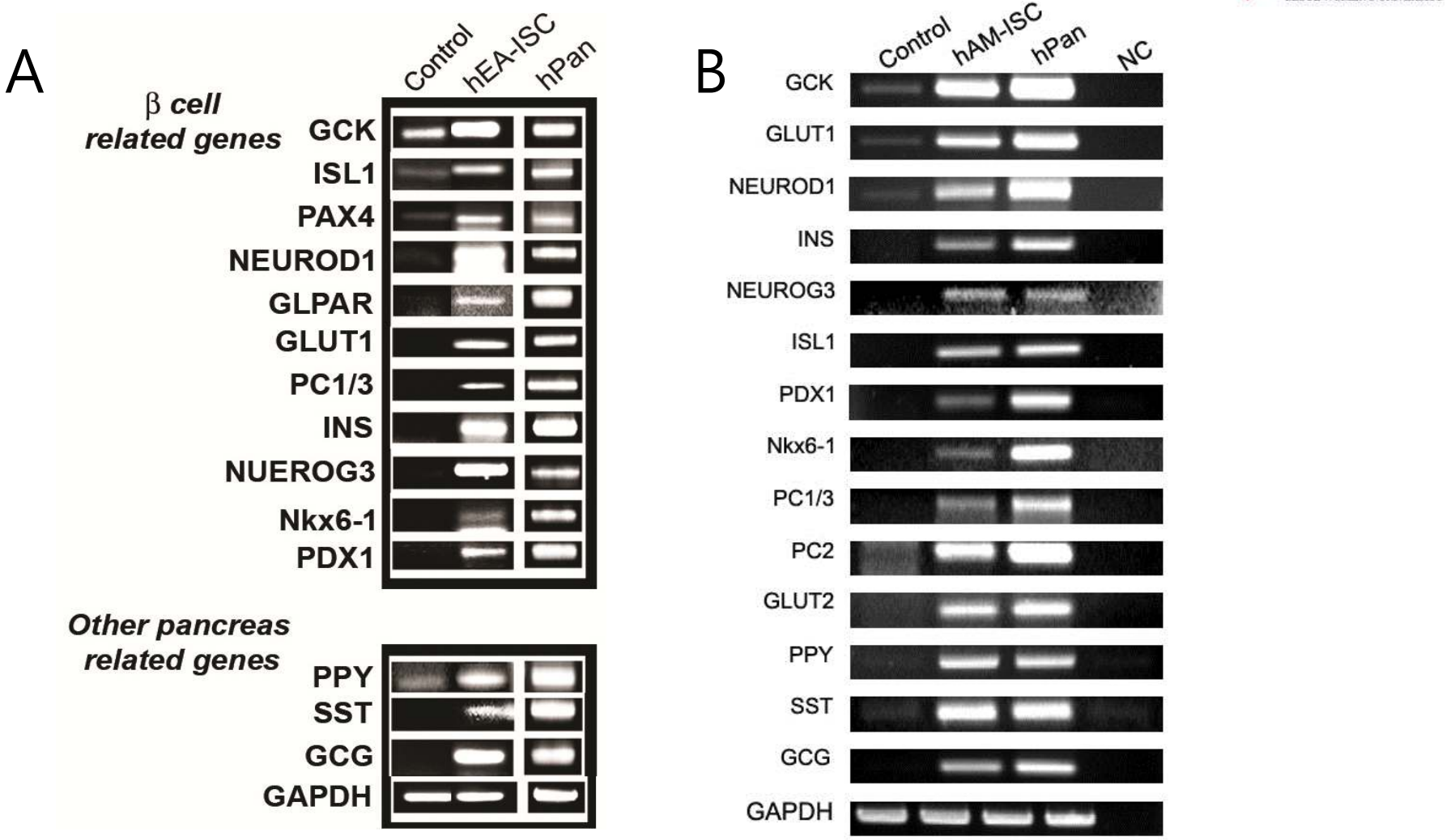

Fig.2. Pancreatic β-cell-related gene expression of hEA and hAM before and after differentiation culture in insulinogenic medium. (A) hEA, (B) hAM.

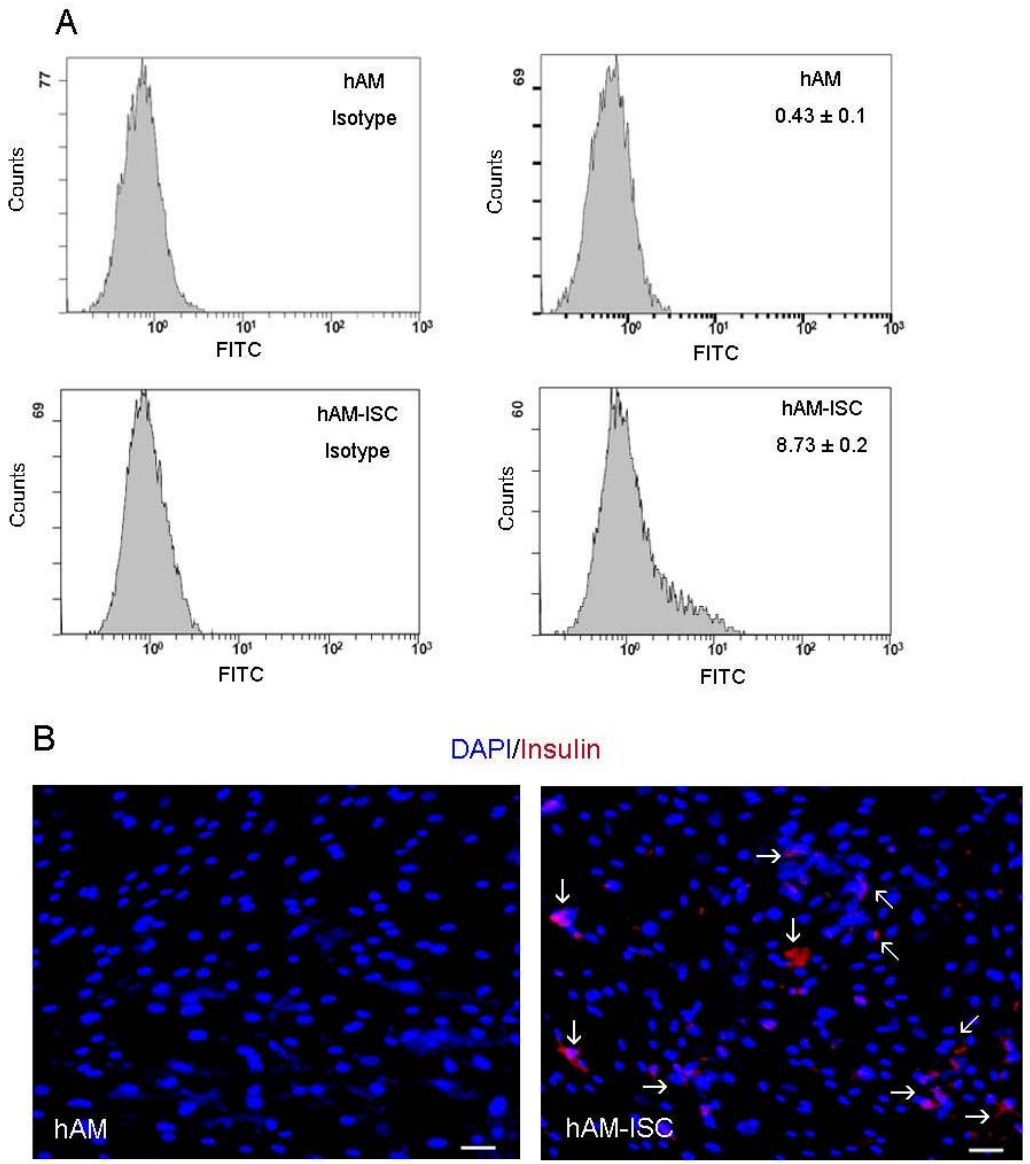

Fig.3. Differentiation efficiency of hAMs into hAM-ISCs. (A) FACS analyses of undifferentiated hAMs and hAM-ISCs. (B) Arrows indicate hAM-ISCs stained with anti-human insulin antibody. Scale bar = 100 μm.

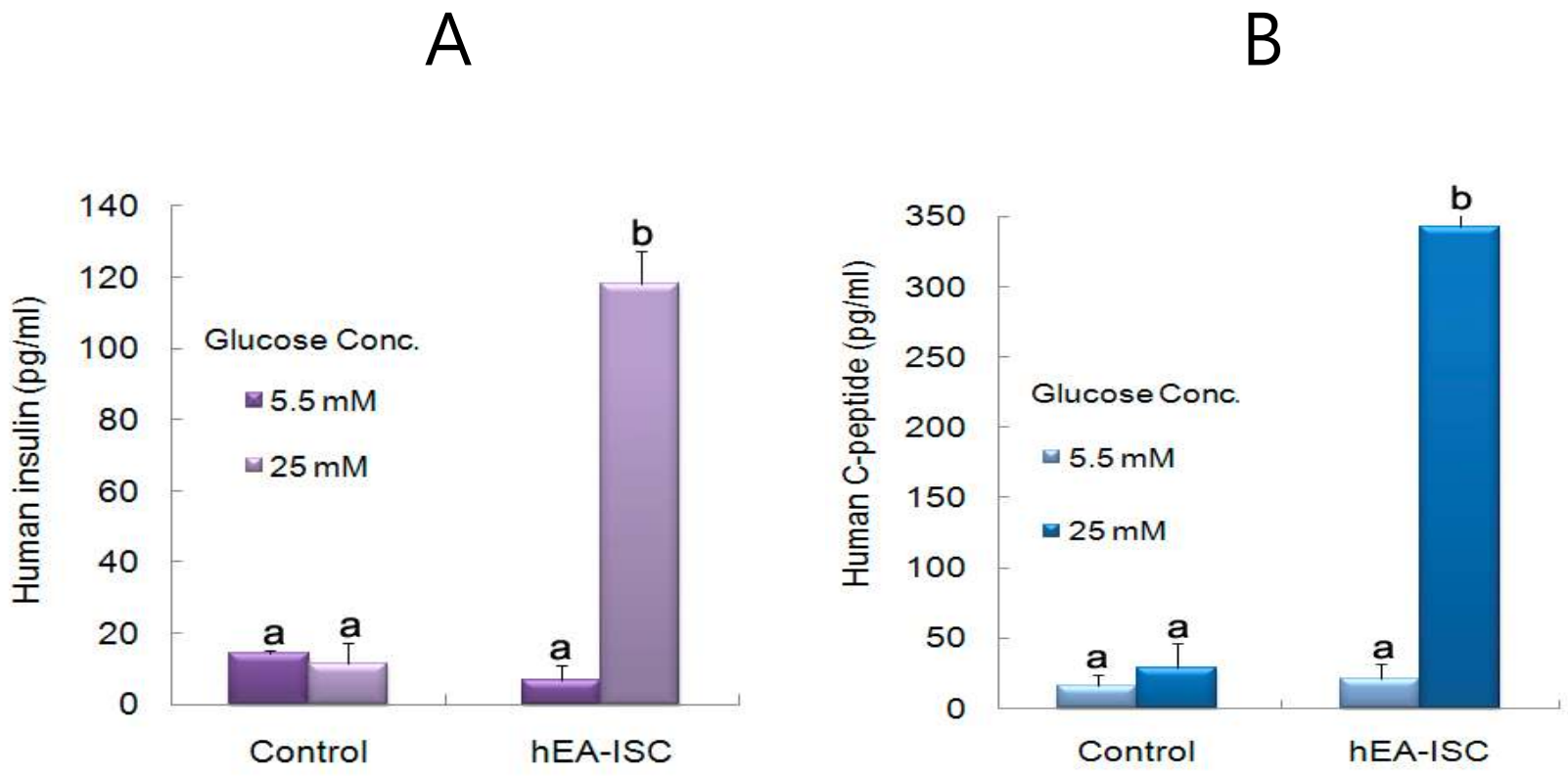

Fig.4. ELISA analyses of insulin and c-peptide secreted from hEA-ISC in response to low or high glucose concentration.
(A) Human insulin ELISA, (B) Human c-peptide ELISA.

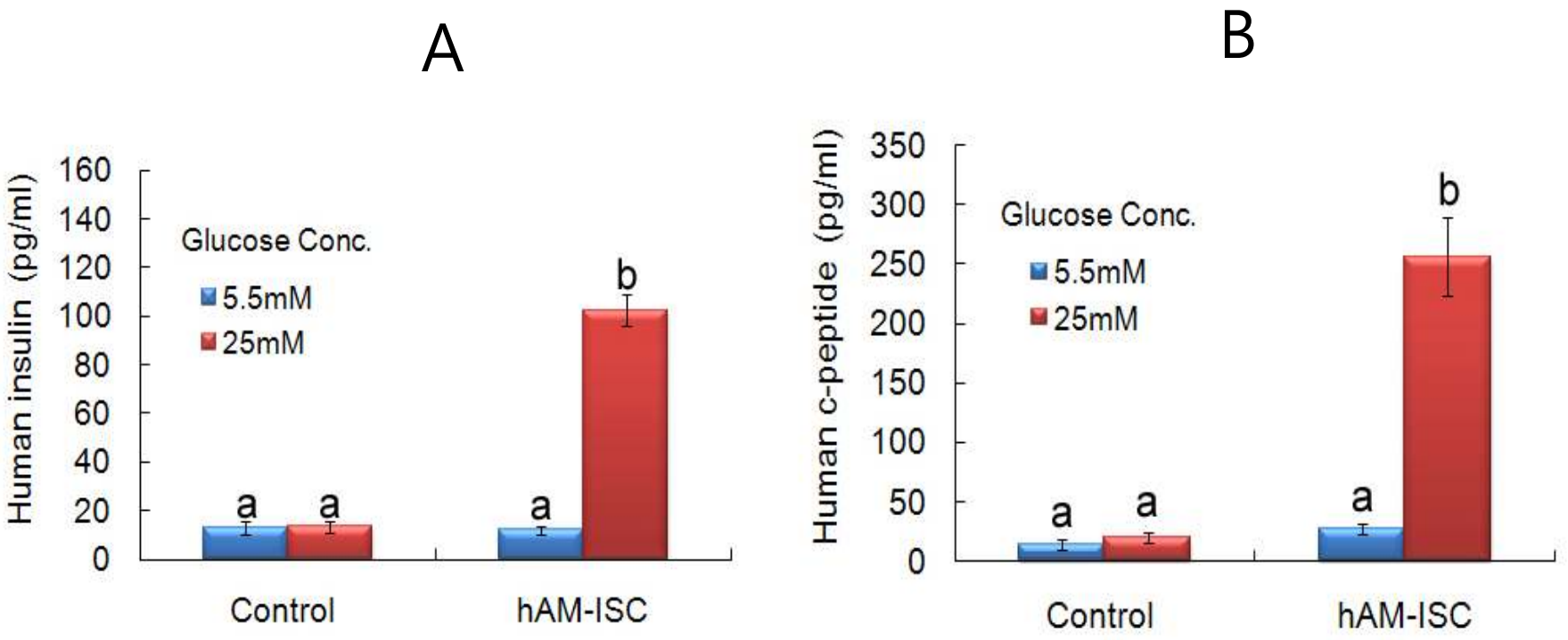

Fig.5. ELISA analyses of insulin and c-peptide secreted from hAM-ISC in response to low or high glucose concentration.
(A) Human insulin ELISA, (B) Human c-peptide ELISA.

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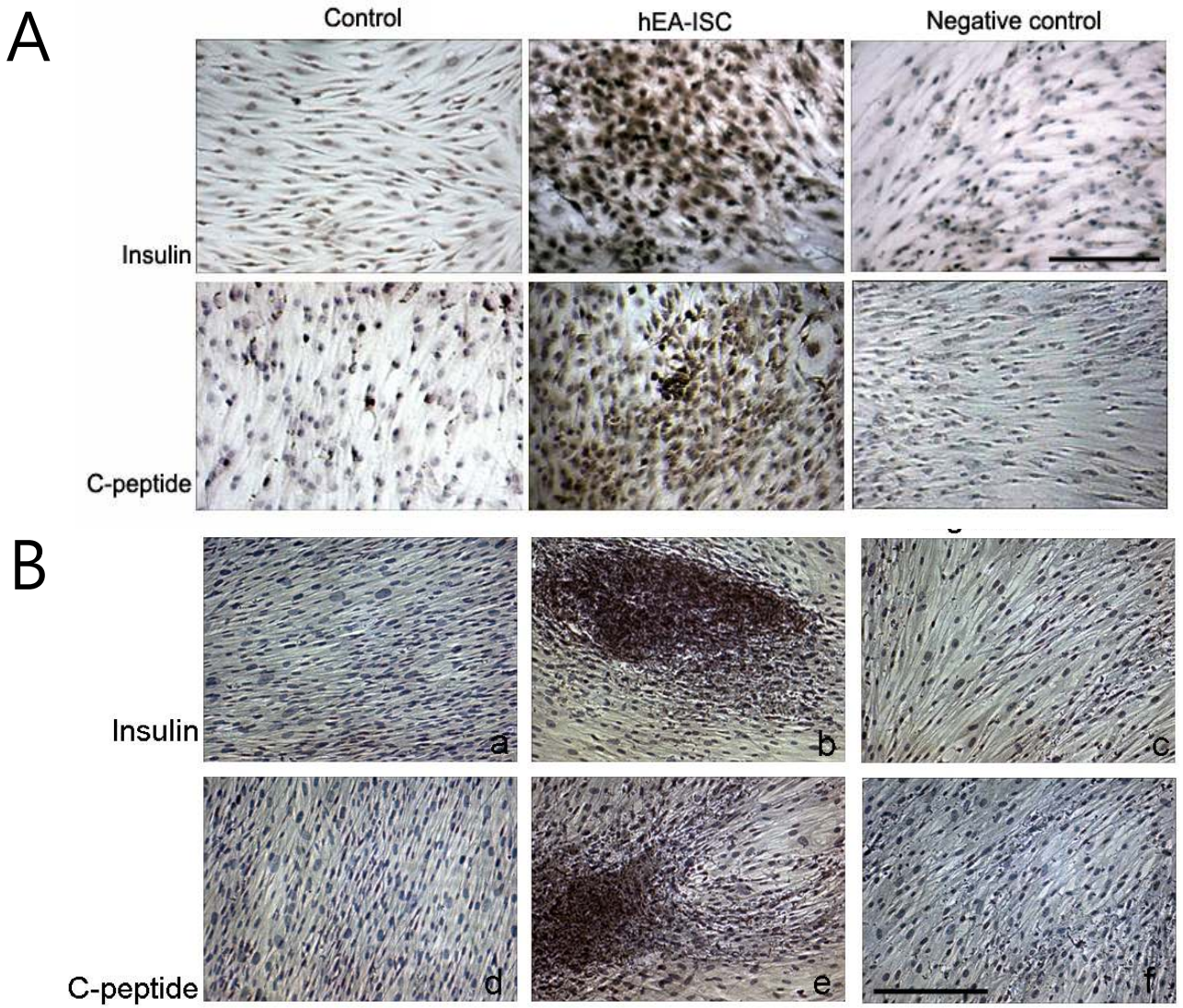

Fig.6. Immunocytochemistry of hEA and hAM before and after differentiation culture in insulinogenic medium. (A) hEA, (B) hAM .

Conclusion

- Stem cells isolated from human eyelid adipose and amniotic membrane can functionally differentiate into insulin-secreting cells in vitro.

Results

2. Normalization of Hyperglycemia in Diabetic Mice by Transplantation of hEA-ISC or hAM-ISC

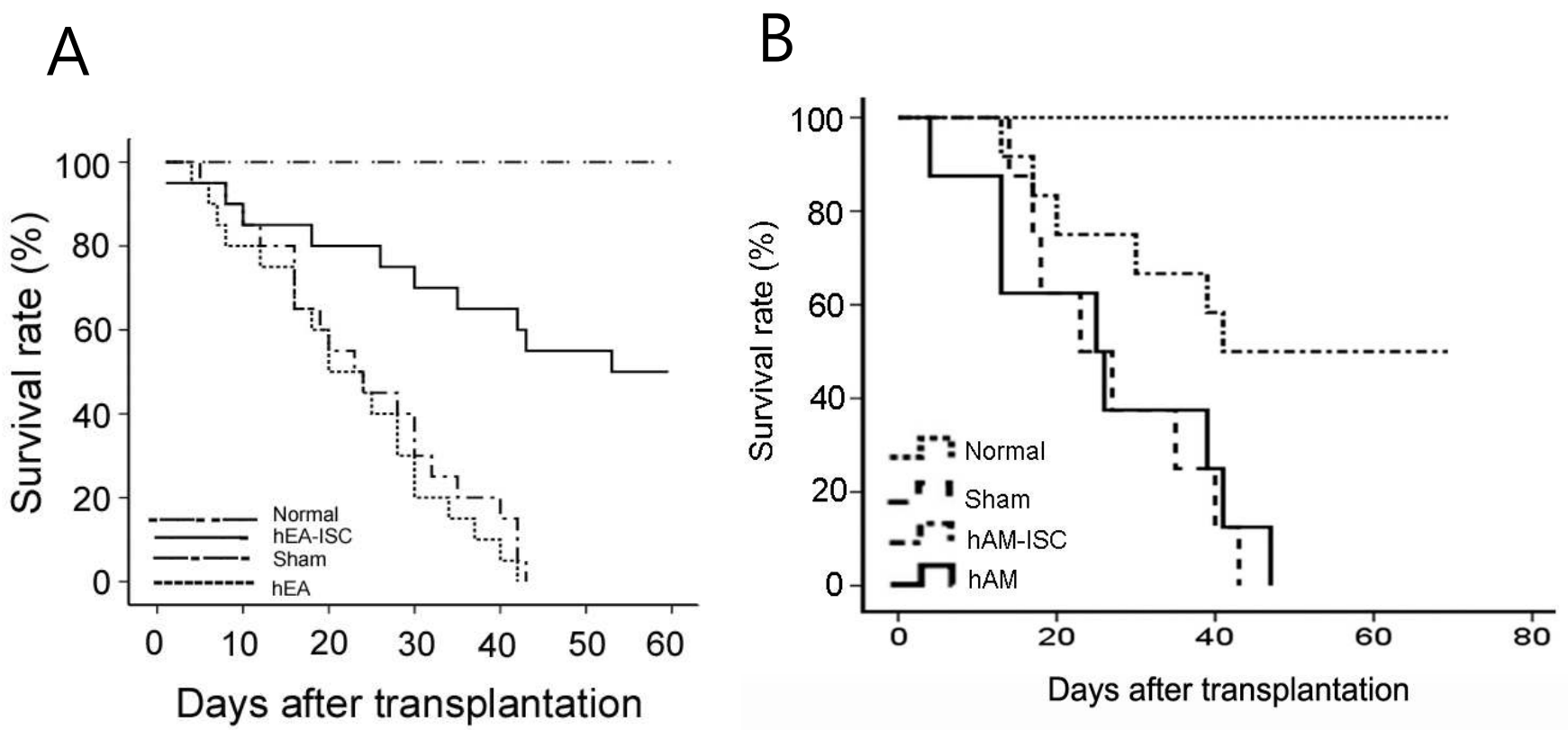

Fig.6. Survival rate of mice following cell transplantation.
(A) hEA-ISC transplant group, (B) hAM-ISC transplant group.
Normal, normal mice; hEA-ISC, hEA-ISC transplant group; Sham, saline injection group; hEA, hEA transplant group; hAM-ISC, hAM-ISC transplant group; hAM, hAM transplant group.

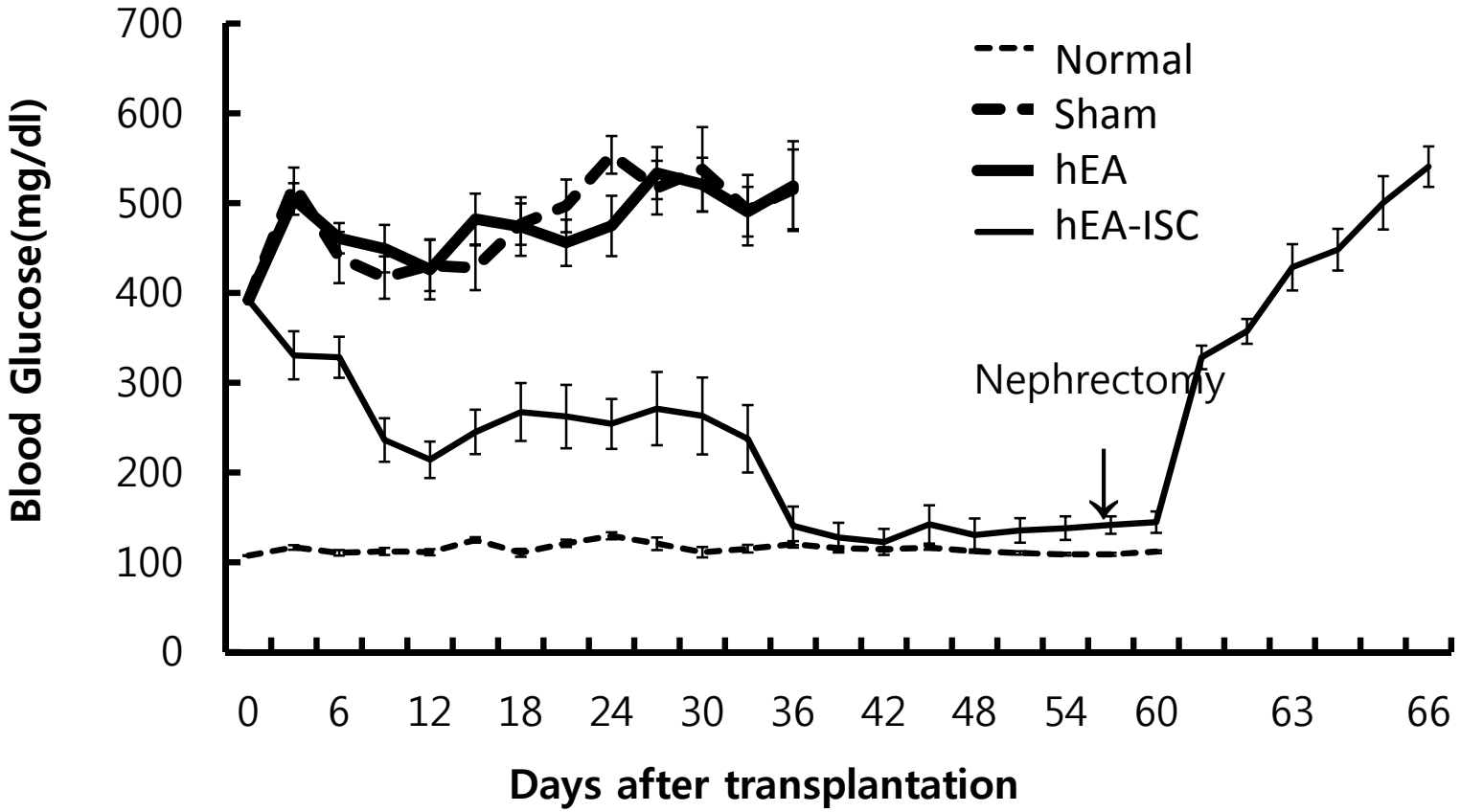

Fig.7A. Blood glucose levels of mice following transplantation with saline, hEA or hEA-ISC.
Normal, normal mice; Sham, saline-injected mice; hEA, hEA-transplanted mice; hEA-ISC, hEA-ISC-transplanted mice.

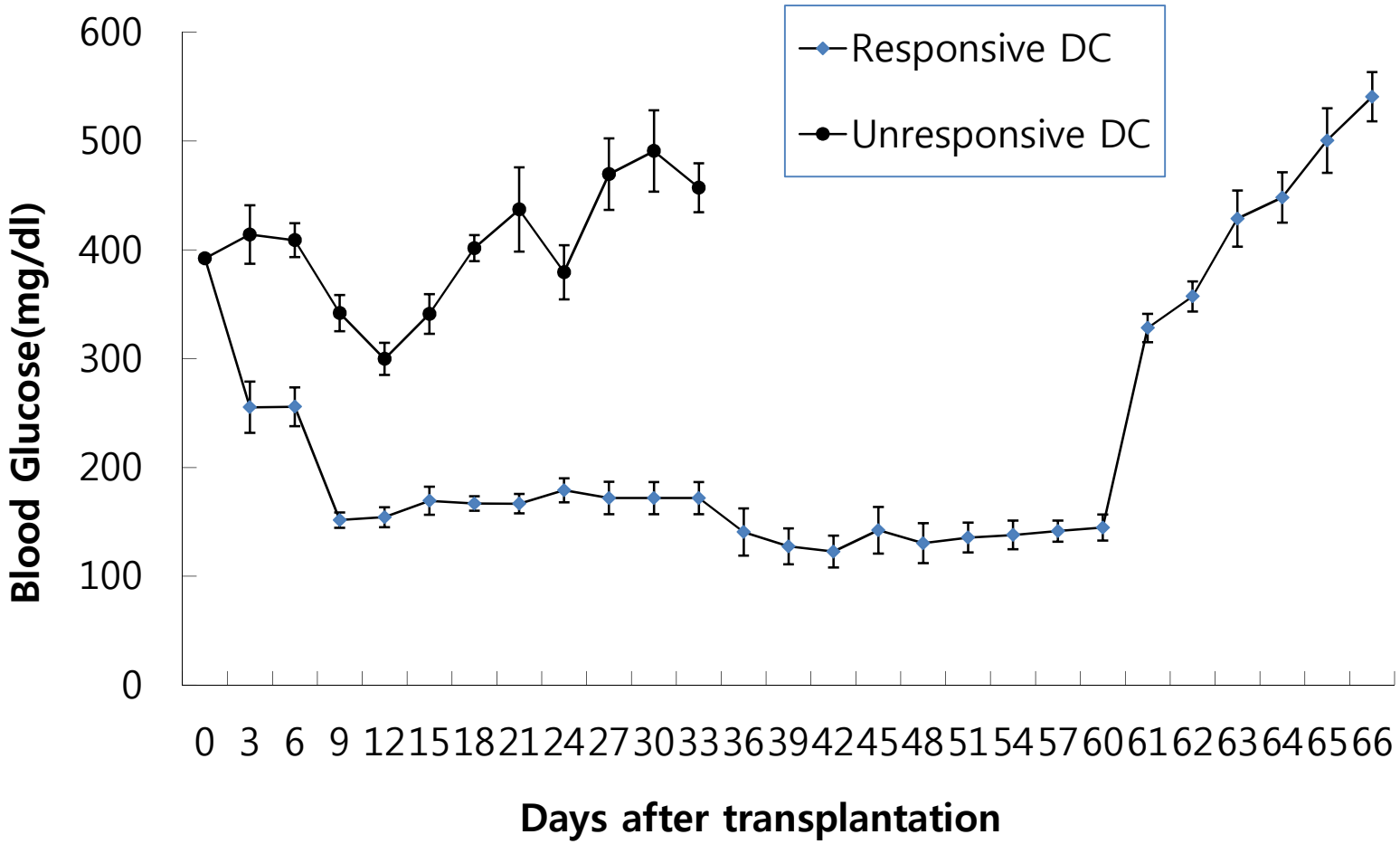

Fig.7B. Blood glucose levels of mice following transplantation with hEA-ISC. Responsive DC, mice with decreasing blood glucose level within 3 days after transplantation; Unresponsive DC, mice with maintaining high blood glucose level after transplantation.

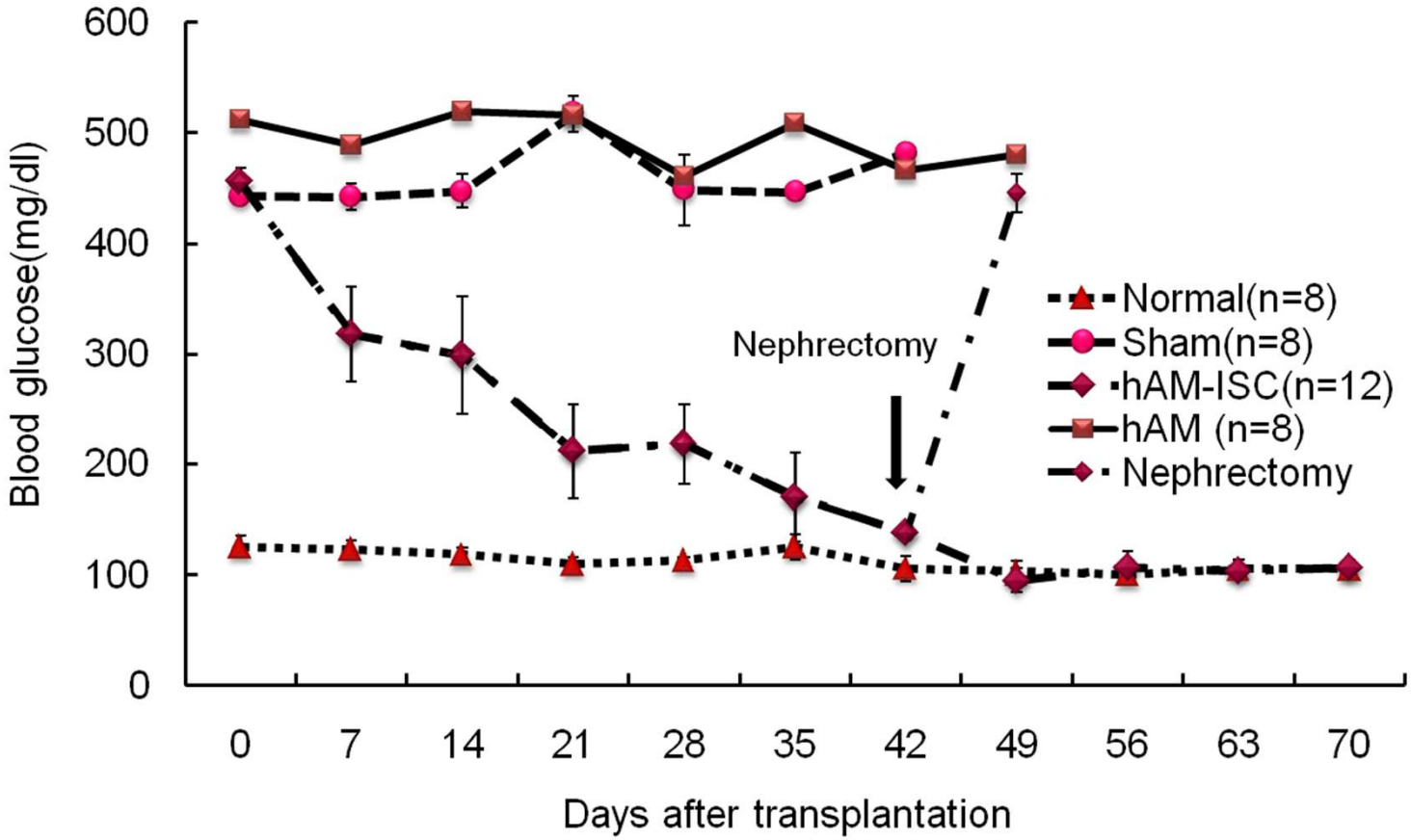

Fig.8. Blood glucose levels of mice following transplantation with saline, hAM or hAM-ISC.
Normal, normal mice; Sham, saline-injected mice; hEA, hEA-transplanted mice; hEA-ISC, hEA-ISC-transplanted mice.

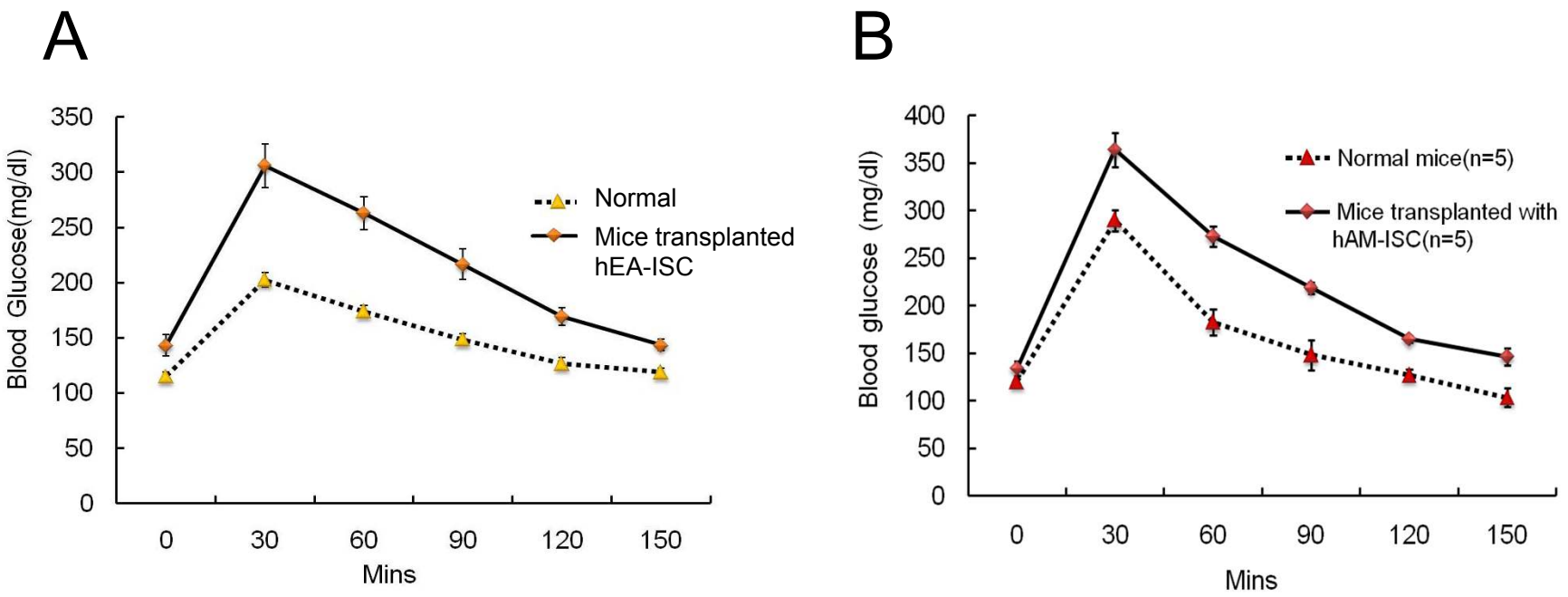

Fig.9. Glucose tolerance test in normal mice and mice transplanted with hEA-ISC or hAM-ISC 8 weeks before.
Normal, normal mice; Sham, saline-injected mice; hEA, hEA-transplanted mice; hEA-ISC, hEA-ISC-transplanted mice.

Conclusion

- Transplantation of insulin-secreting cells generated in vitro can normalize the blood glucose level of recipient mice with hyperglycemia.

Results

3. Regulation of Mouse Blood Glucose Level by Human Insulin Released from Transplanted hEA-ISC or hAM-ISC

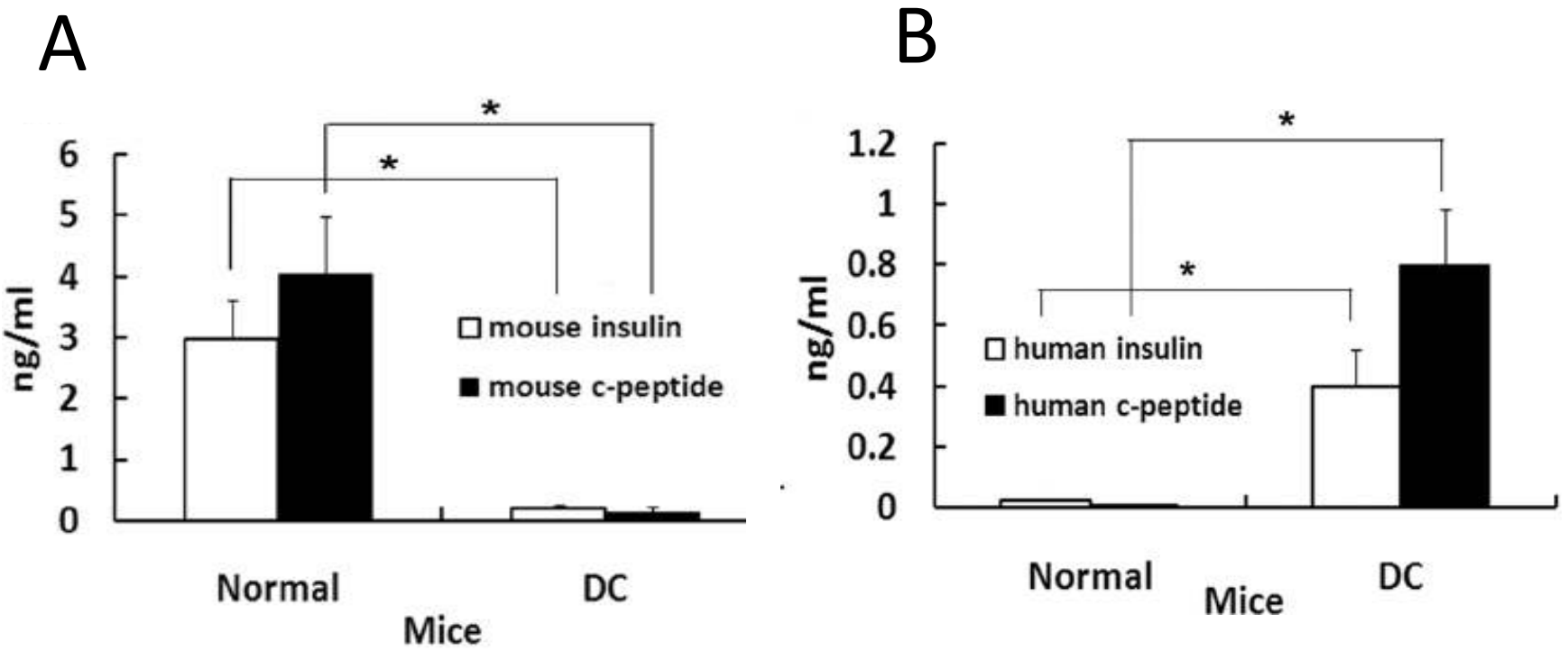

Fig.10. ELISA analyses of insulin and c-peptide.

(A) **Mouse** insulin and c-peptide levels in sera of normal mice and mice transplanted with hEA-ISC.

(B) **Human** insulin and c-peptide levels in sera of normal mice and mice transplanted with hEA- ISC.

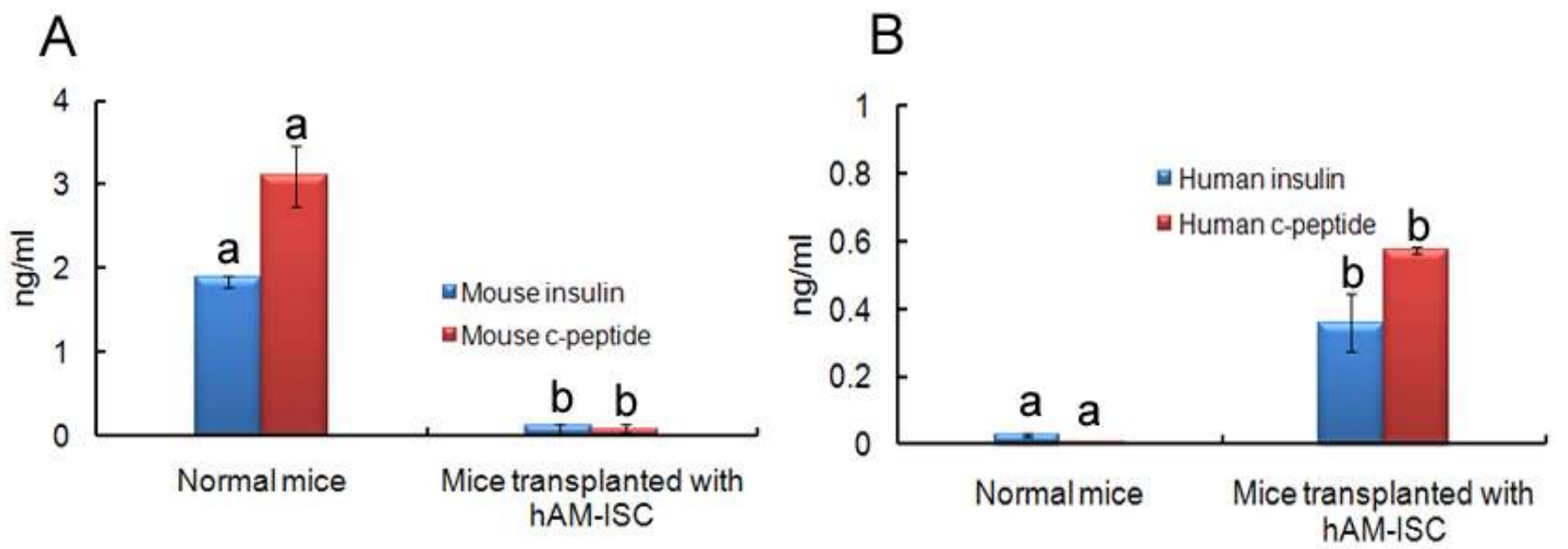

Fig.11. ELISA analyses of insulin and c-peptide.

(A) **Mouse** insulin and c-peptide levels in sera of normal mice and mice transplanted with hAM-ISC.

(B) **Human** insulin and c-peptide levels in sera of normal mice and mice transplanted with hAM- ISC.

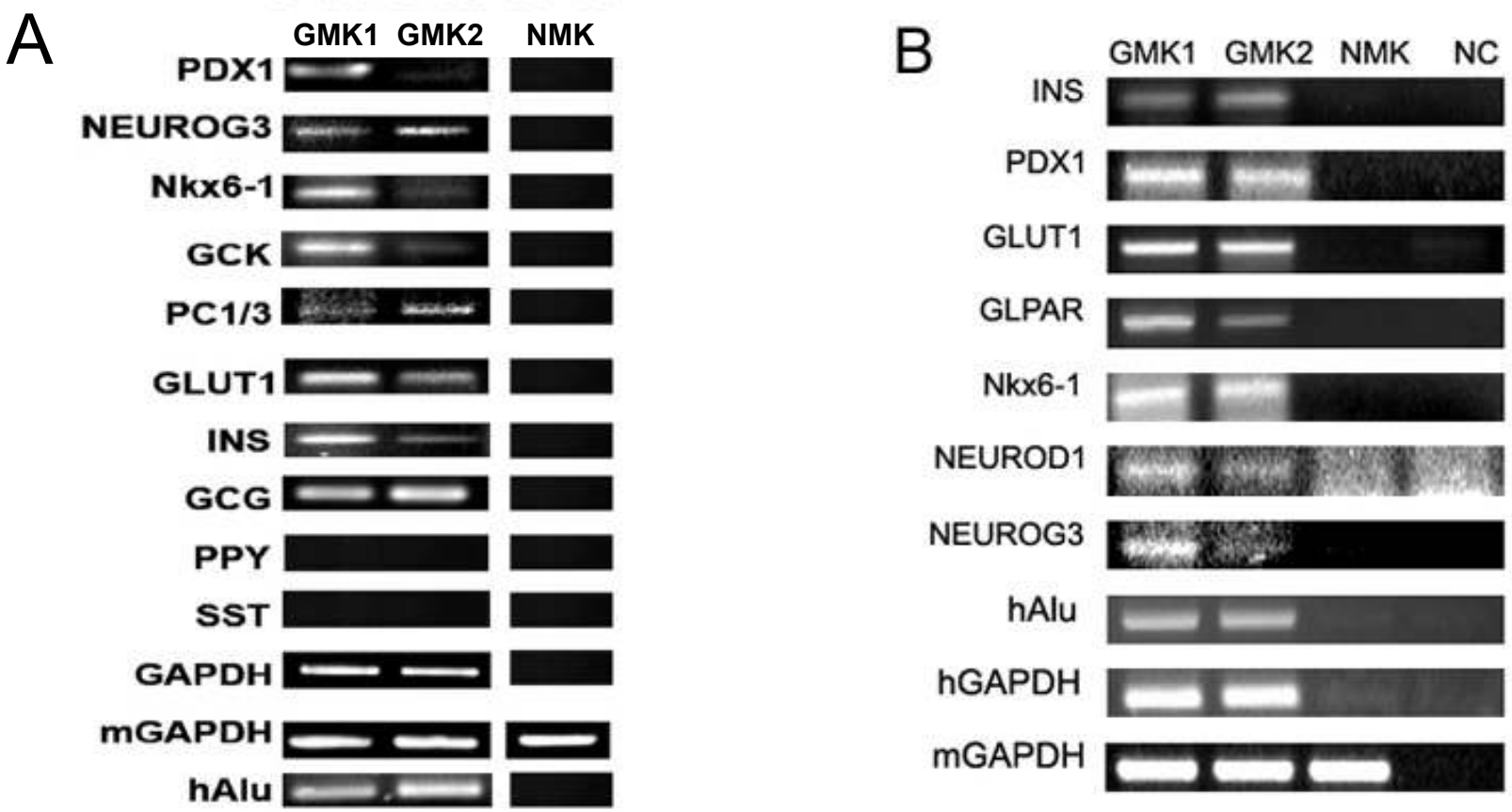

Fig.12. Expression of human β-cell-related genes in graft-bearing kidney.
(A) Graft-bearing kidney transplanted with hEA-ISC,
(B) Graft-bearing kidney transplanted with hAM-ISC.
GMK, graft-bearing mice kidney; NMK, normal mice kidney, NC, negative control.

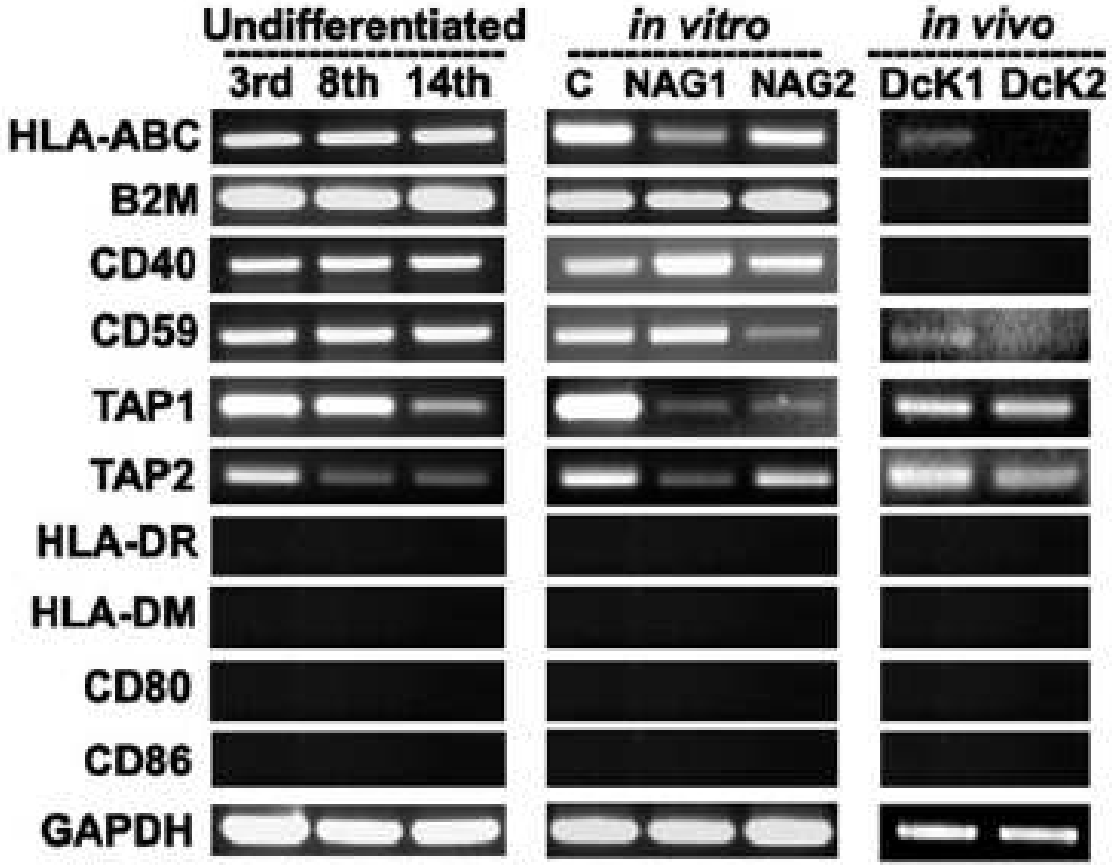

Fig.13. Expression of human immune-related genes by hEA and hEA-ISC in vitro or by kidney transplanted with hEA-ISC in vivo.
DcK; graft-bearing kidney 1 year after transplantation in vivo.

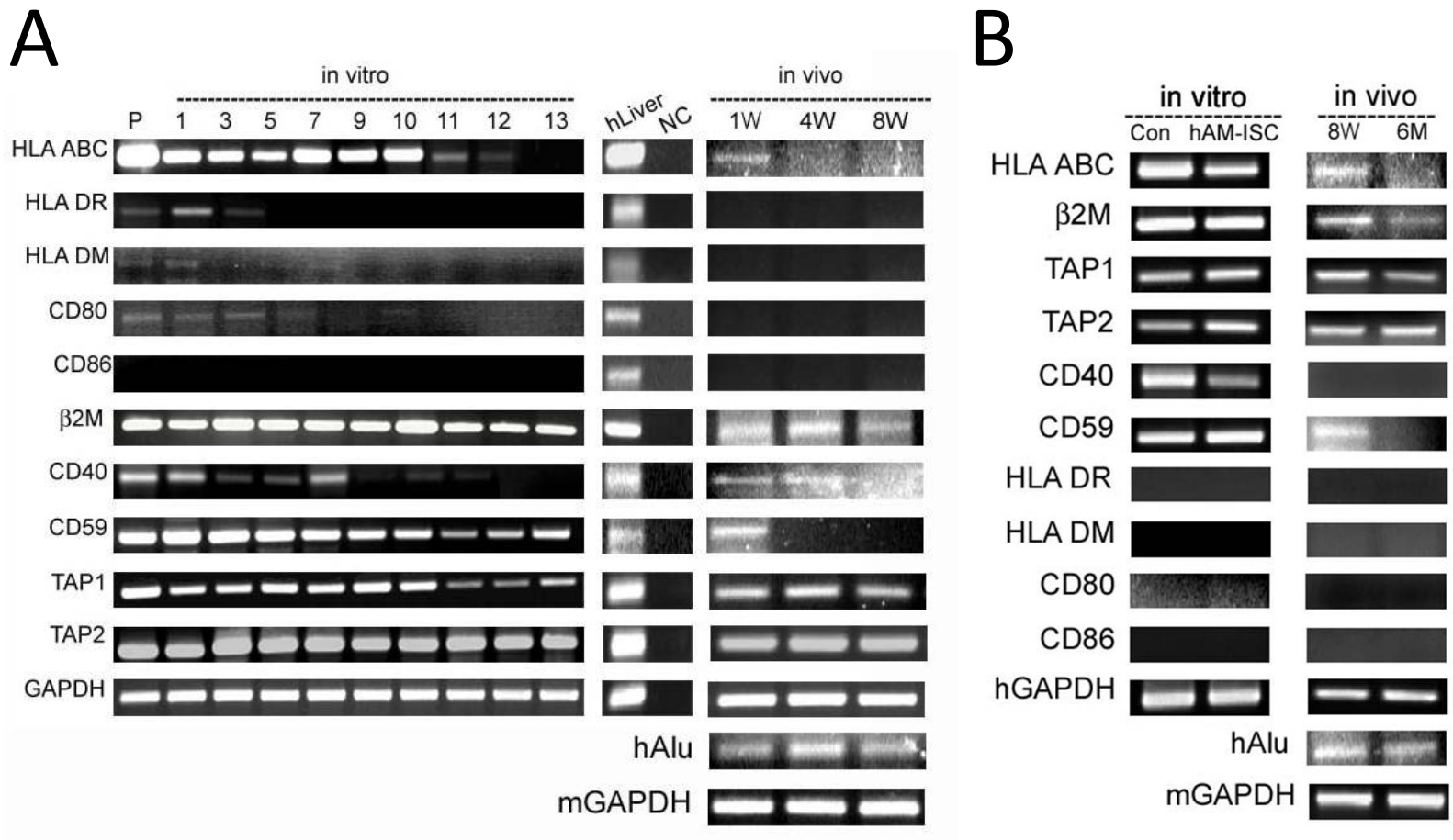

Fig.14. Expression of human immune-related genes by hAM and hAM-ISC in vitro or in vivo.

(A) Gene expression by hAM during in vitro and in vivo, (B) Gene expression by hAM-ISC in vitro or by kidney transplanted with hAM-ISC in vivo.

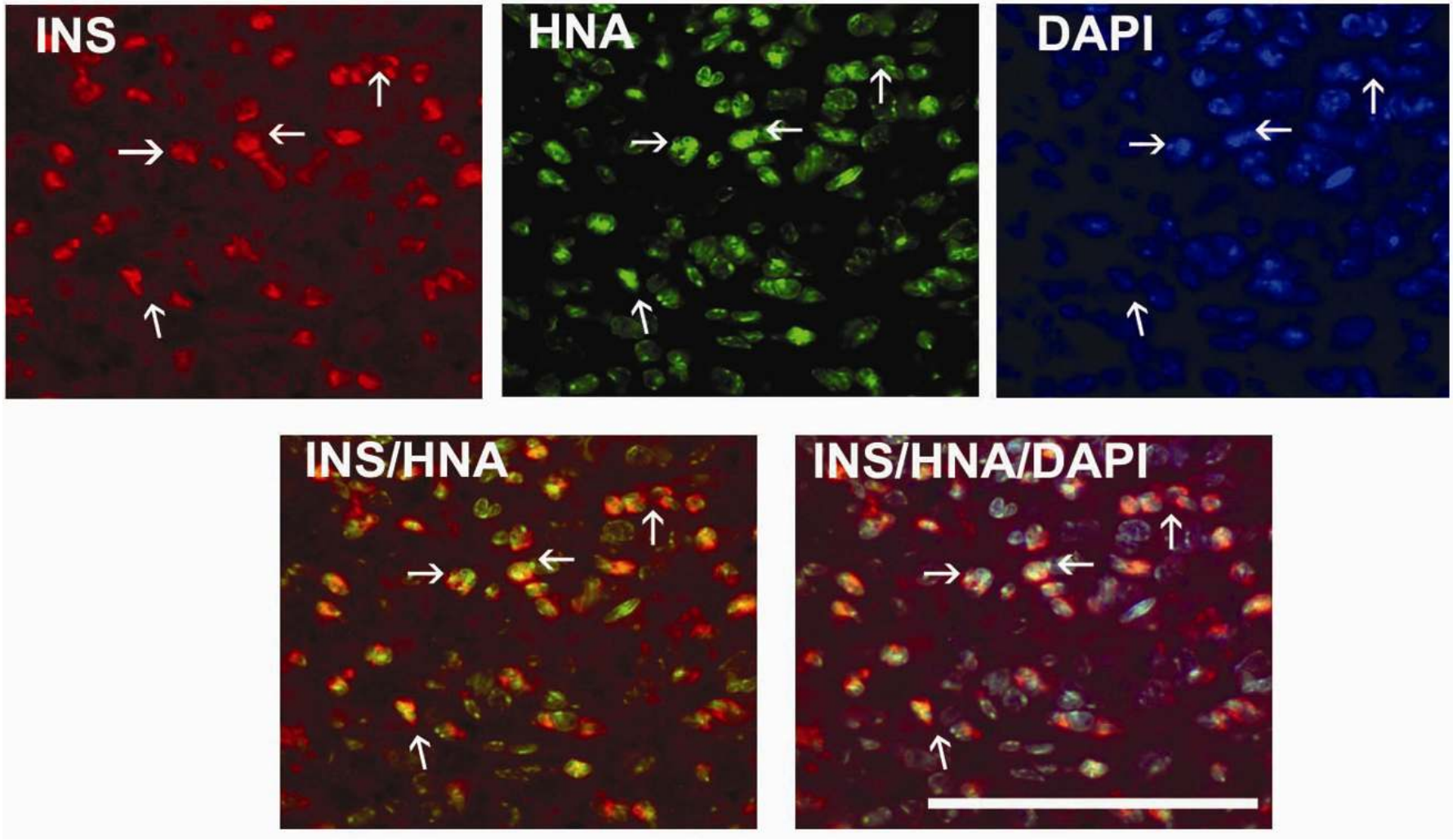

Fig.15. Immunofluoroscence staining for human insulin and human cell nuclei in mouse kidney transplanted with hEA-ISC.

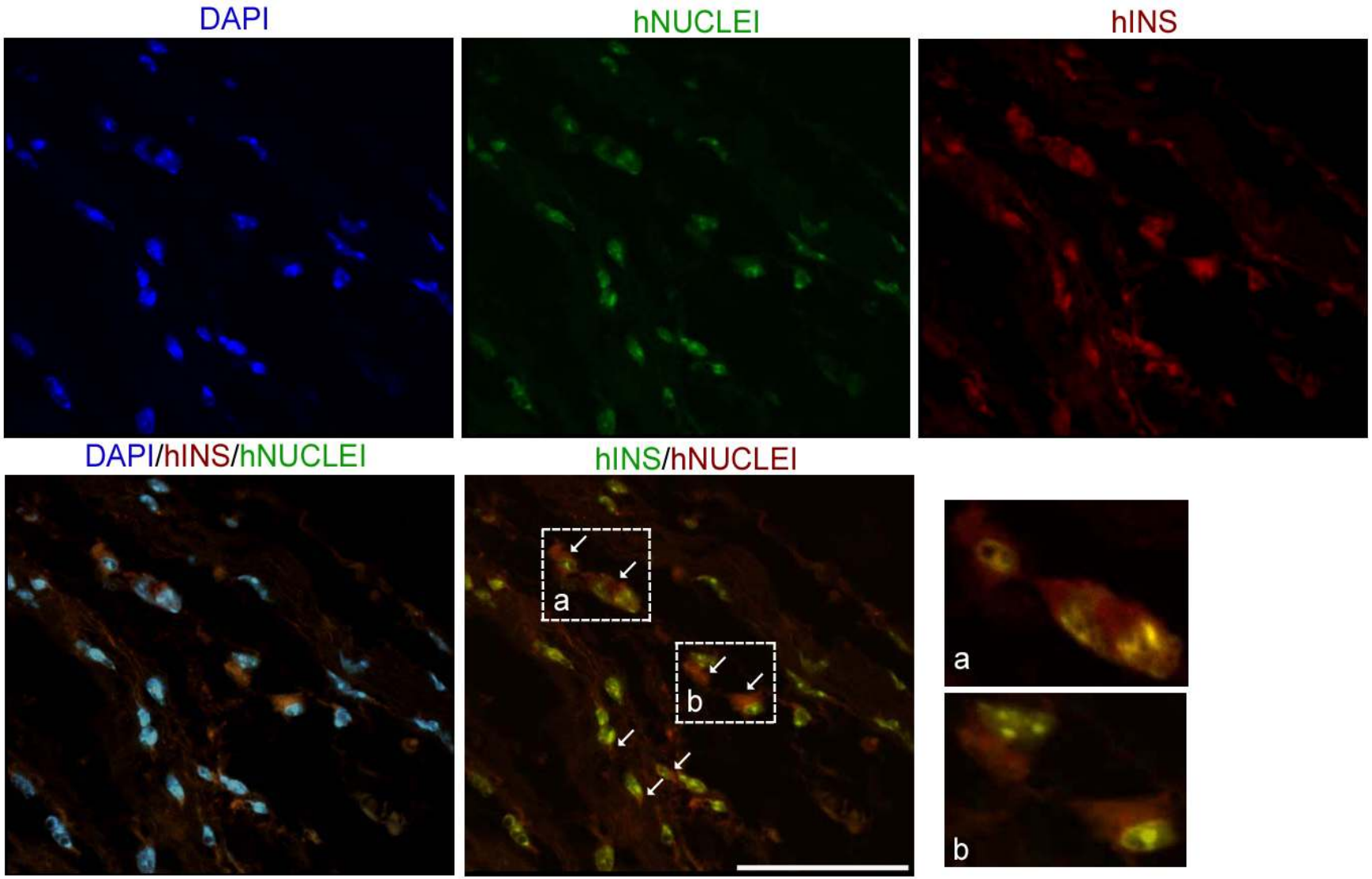

Fig.16. Immunofluroscence staining for human insulin and human cell nuclei in mice kidney transplanted with hAM-ISC.

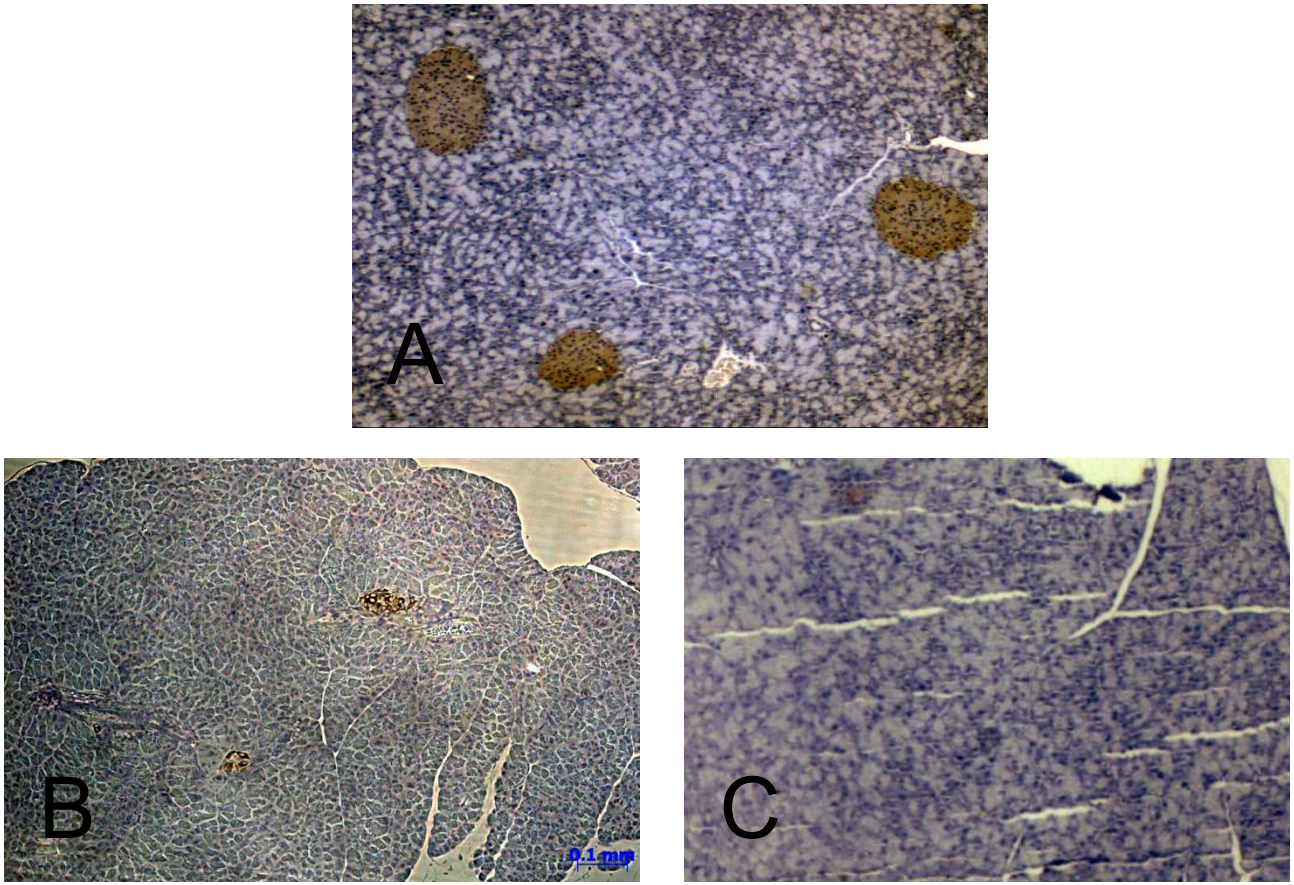

Fig. 17. Immunohistochemistry of pancreata section from normal and cell transplanted mice .
(A) Pancreas of normal mouse, (B) Pancreas of mouse transplanted with hEA-ISC, (C) Pancreas of mouse transplanted with hAM-ISC.

Conclusion

- Normalization of blood glucose level in diabetic mice following transplantation of insulin-secreting cells is due to human insulin released from transplanted human cells but not by mouse insulin from their own β-cells.

- Human adult stem cells such as hEA and hAM, whether differentiated or not, can tolerate immune response in mice.

SUMMARY

- hEA and hAM can functionally differentiate into insulin-secreting cells in vitro.
- hEA-ISC and hAM-ISC can successfully engraft into the kidneys of the immunocompetent mice, overcoming the graft rejection.
- hEA-ISC and hAM-ISC can directly regulate blood glucose level of diabetic mice by releasing human insulin, rather than supporting the regeneration of mouse β-cells.
- Therapeutic effects of hEA-ISC can persist as long as one and half year.

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Further Research

- Will the autologous and allogenic cells function in human body without immunosuppression?
- Where is the appropriate transplantation site in body?
- How many cells are needed?
- Will the single transplantation function permanently?

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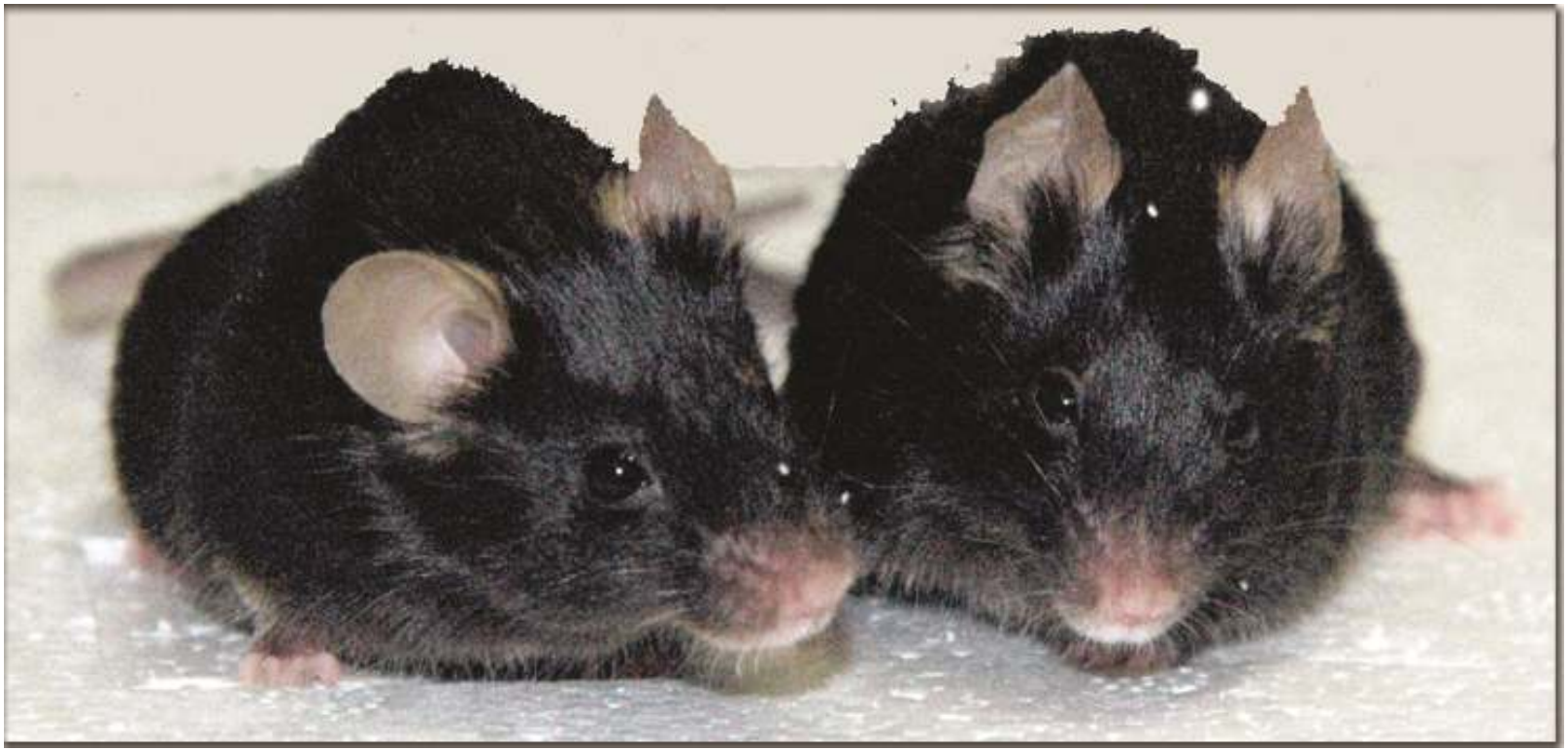

Fig. Normal mouse (left) and mouse (right) recovered from diabetes by transplantation of HEACs before 1 and half years.

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Supplementary Results

Stem Cell Properties of hEA

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A

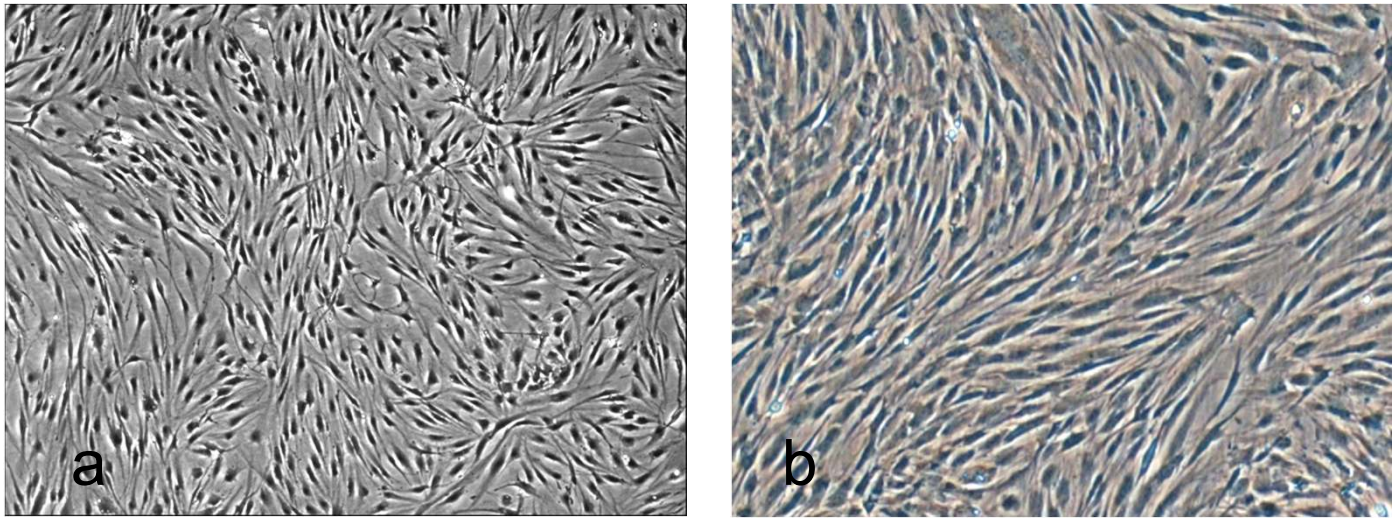

Fig 1. Morphology of hEA and BM-MSC.

(a) Human eyelid adipose tissue-derived stem cells (hEA).
(b) Human bone marrow-derived mesenchymal stem cells (BM-MSC).

B

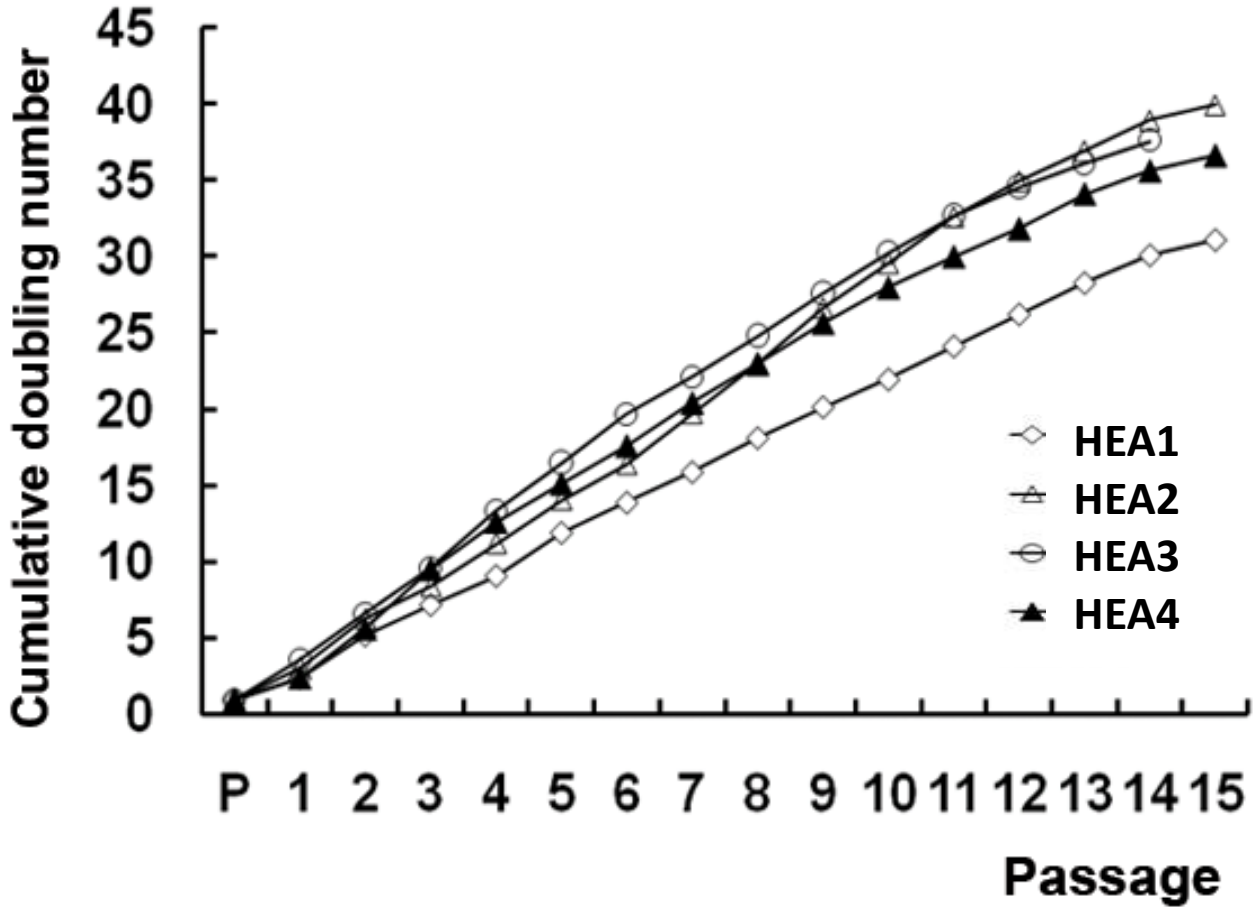

Fig 1. (B) Cumulative doubling number of hEA throughout *ex vivo* expansion.

C

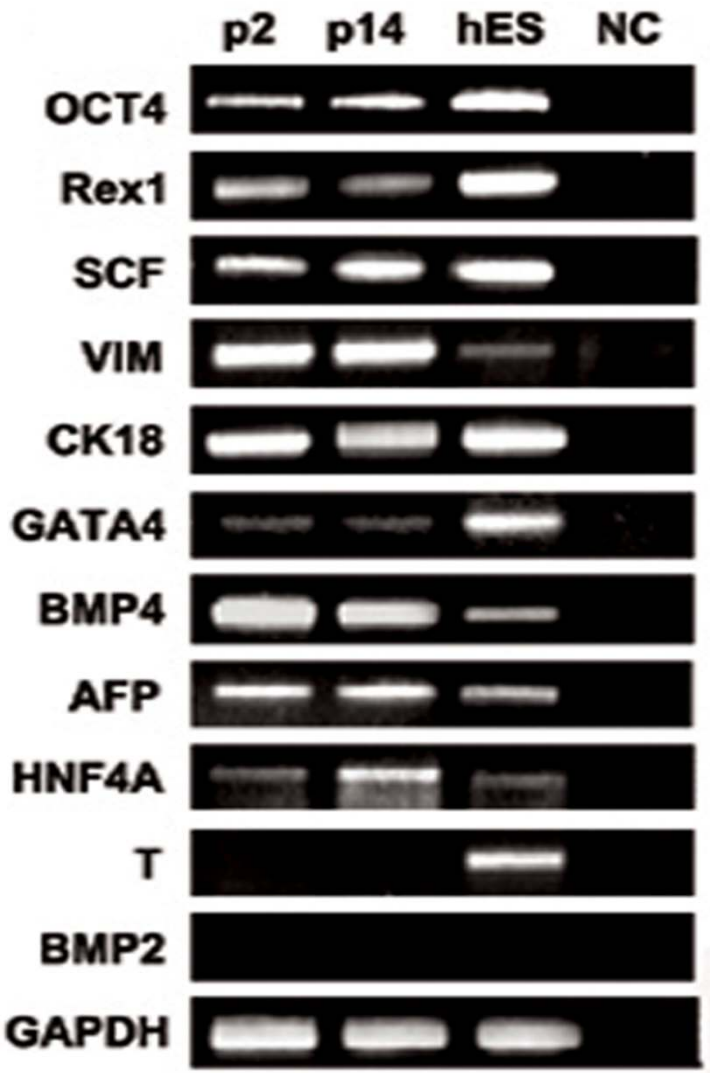

Fig 1. (C) Expression of stem cell-specific genes by hEA and hES. NC, negative control.

D

Fig 1. (D) Differentiation potential of hEA at p4 into adipocytes, osteoblasts, and chondroblasts.

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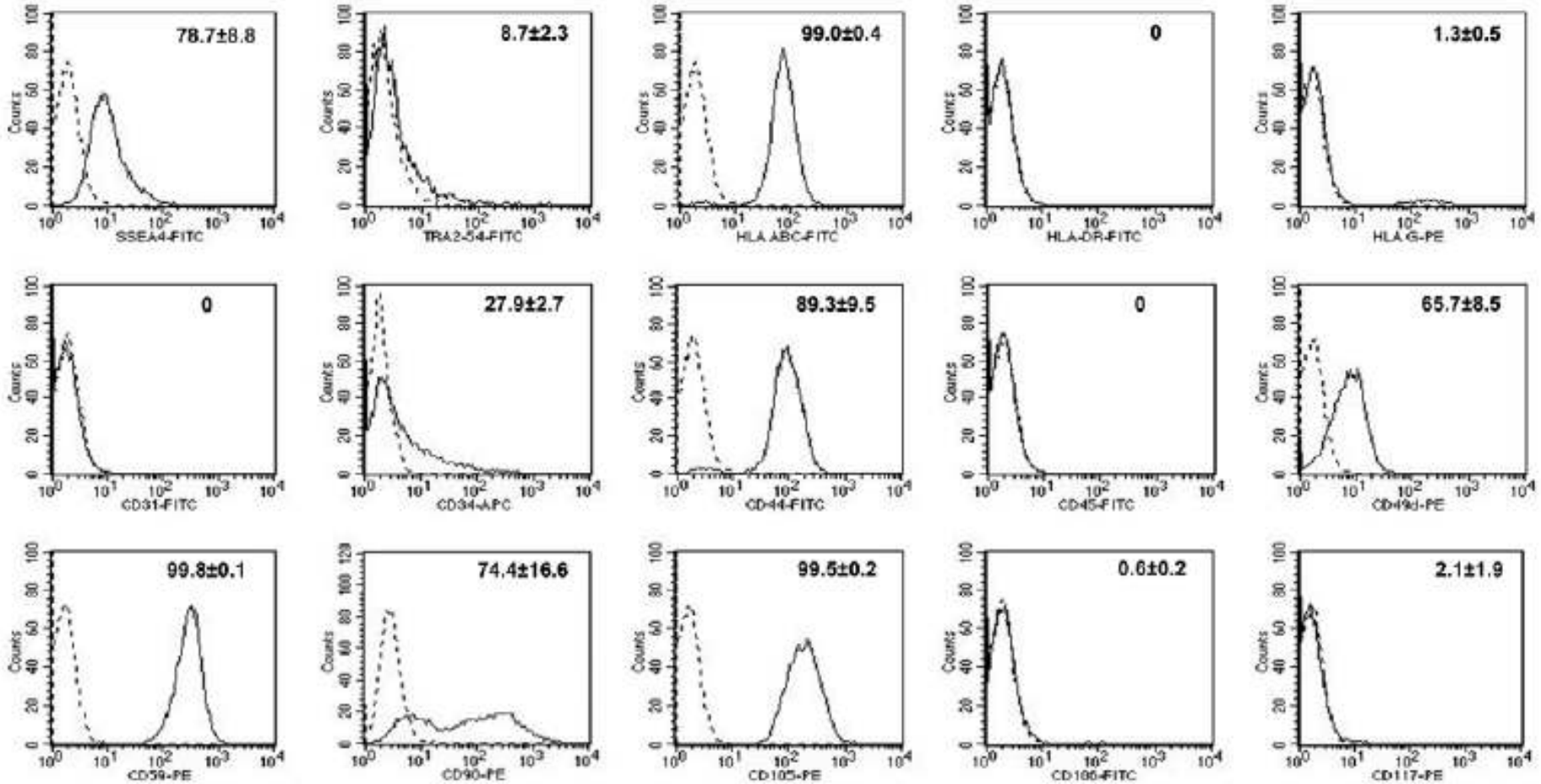

Fig.2. Flow cytometric analyses of hEA.

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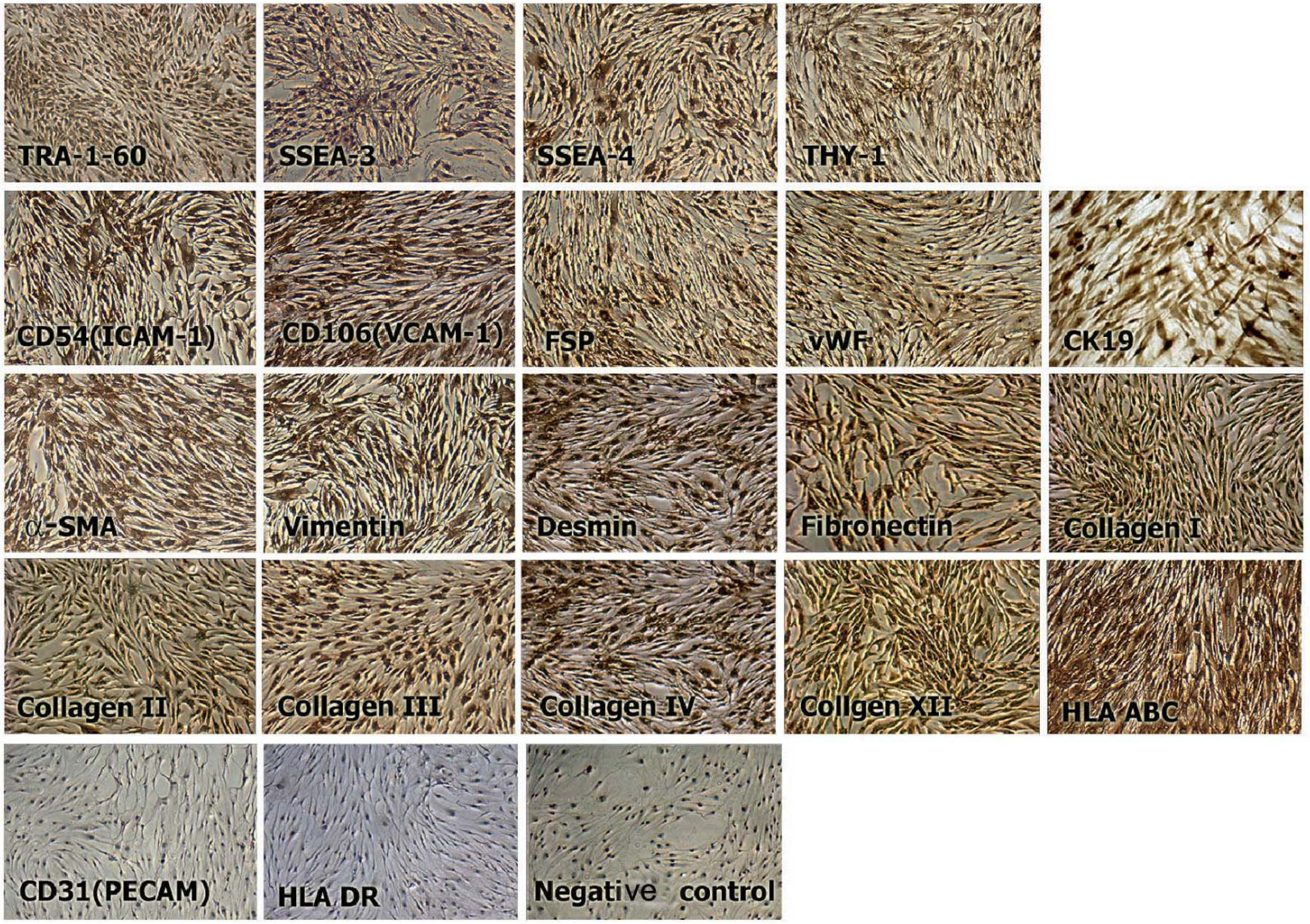

Fig.3. Immunocytochemical properties of hEA.

Table 1. Immunophenotypes of hEA.

Antigen	Expression
SSEA-4 (ESC marker)	+
TRA-2 (ESC marker)	±
CD31 (PECAM)	-
CD34 (HSC, cell adhesion)	+
CD44 (HCAM)	+
CD45 (HSC marker)	-
CD49d (Integrin α_4 subunit)	+
CD59 (Negative regulator of complement activation)	+
CD90 (Thy-1, MSC marker)	+
CD105 (MSC marker)	+
CD106 (Immunoglobulin superfamily)	-
CD117 (germ cell marker)	±
HLA-ABC	+
HLA-DR	-
HLA-G	-

A

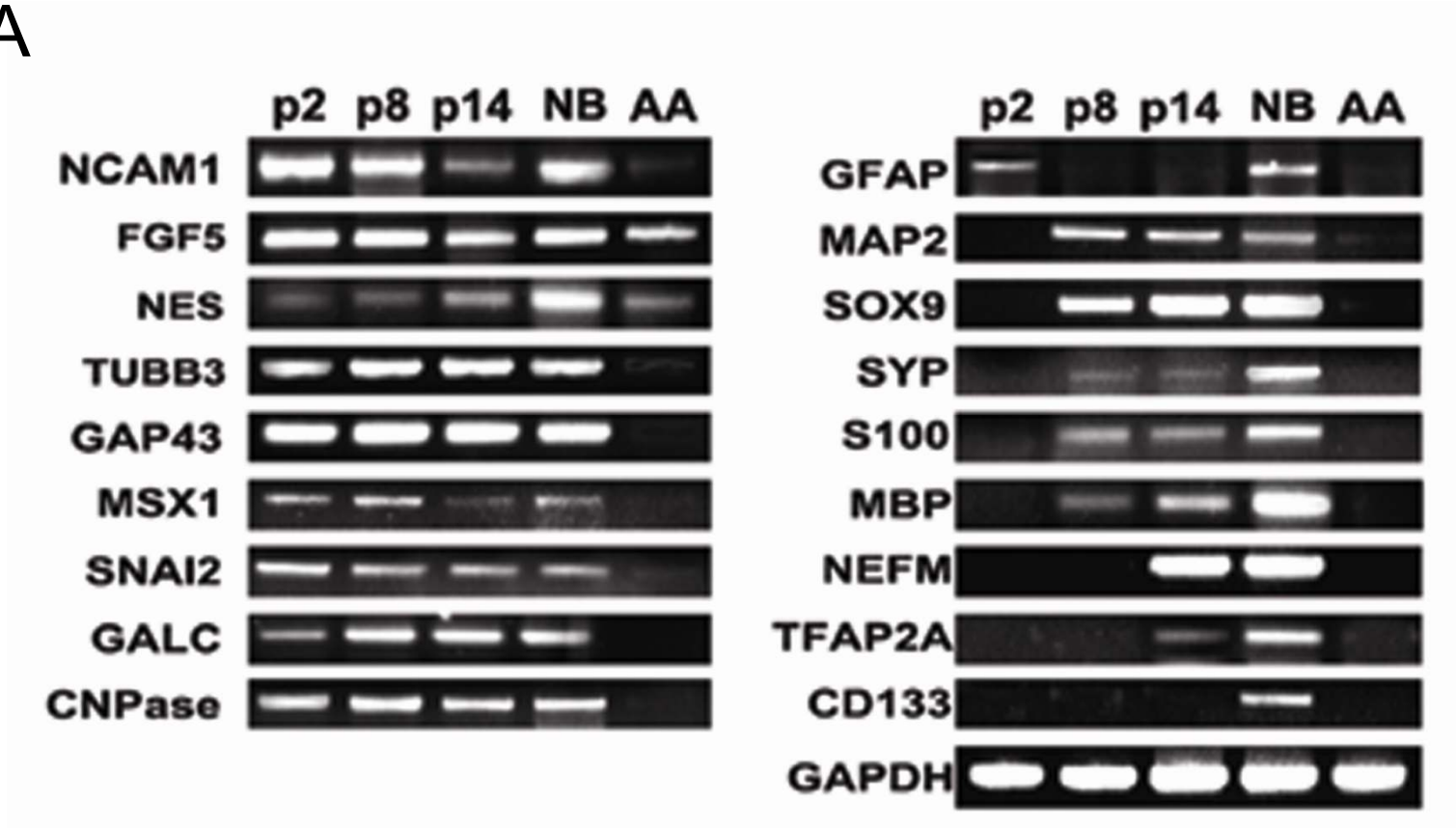

Fig.4. Neural properties of hEA.

(A) Expression of neuron-related genes by HEA at p2, p8, and p14. NB, neuroblastoma cells. AA, human abdominal adipose-derived stem cells at p3.

B

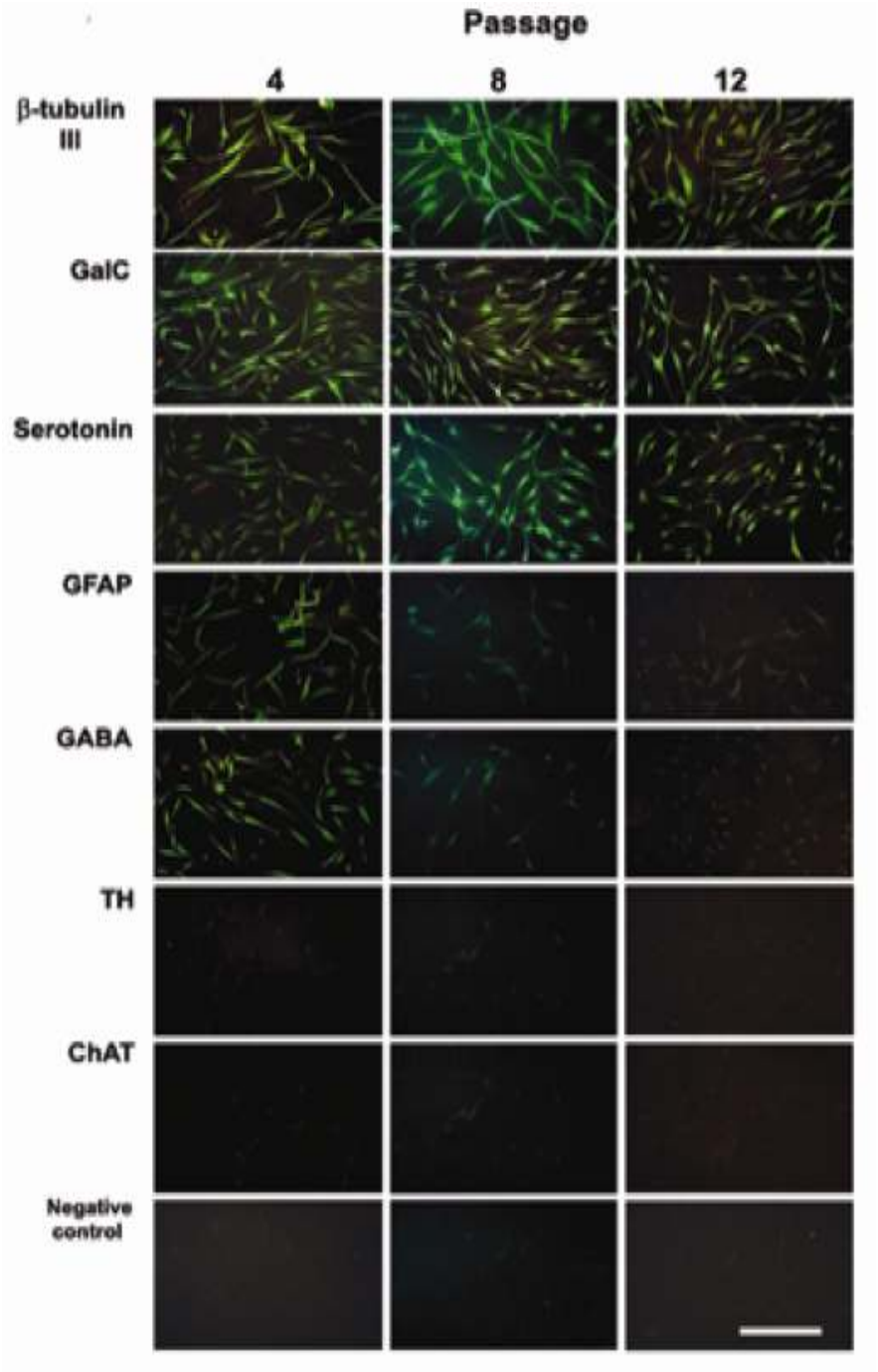

Fig.4. (B) Immunocytochemistry of hEA at p4, p8, and p12.

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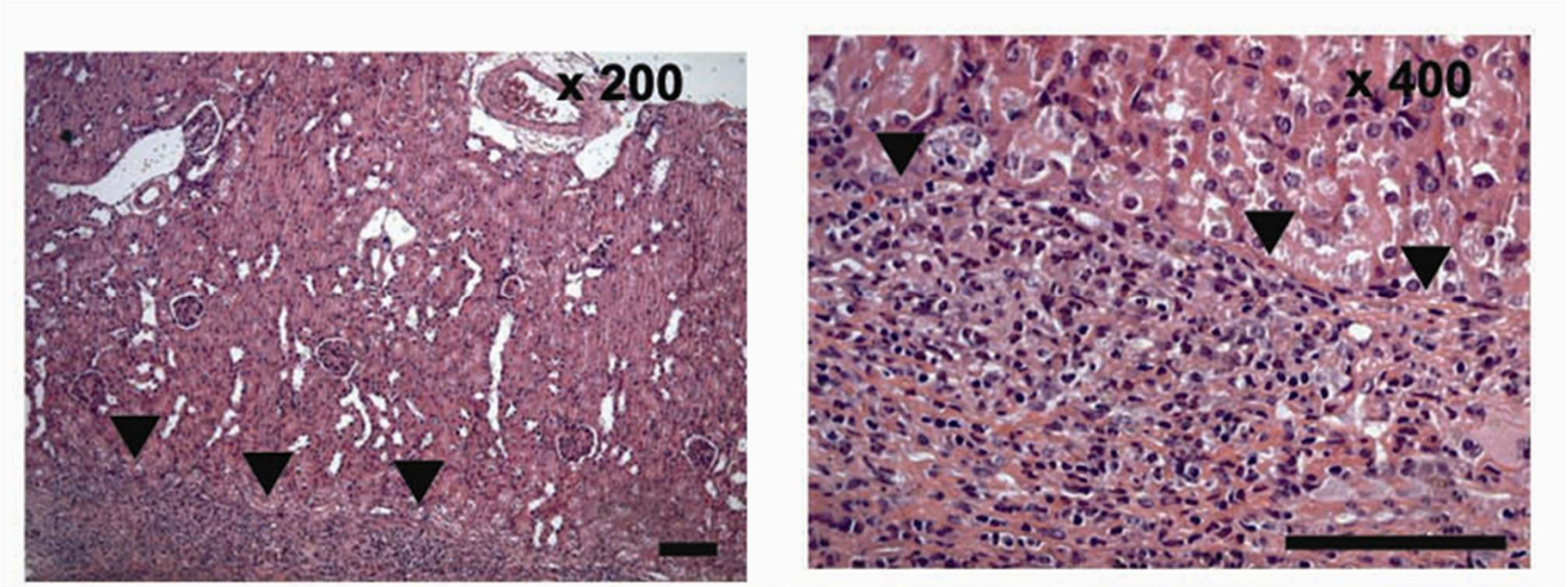

Fig.5. H&E stained kidneys of DC mice.

Arrowheads indicate boundary between human and mouse cells.

J Neurol (2002) 249 [Suppl 3]: III/33 – III/35
DOI 10.1007/s00415-002-1307-y

WORKSHOP W1

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Current state of stem cell research for the treatment of Parkinson's disease

stitution therapies, including treatment with L-DOPA (L-3,4-dihydroxyphenylalanine, levodopa) and a peripheral decarboxylase inhibitor and/or a catechol-O-methyl-transferase inhibitor, dopamine agonists and the selective monoamine oxidase type B (MAO-B) inhibitor selegiline.

An alternative approach for symptomatic PD therapy is fetal dopamine neuron transplantation [3]. This approach remains experimental, however, and technical and ethical difficulties in obtaining sufficient and appropriate donor fetal brain tissue have limited its application. To date more than 300 patients have been transplanted with embryonic ventral mesencephalic neurons worldwide. Transplanted human cells can survive up to 12 years following transplantation and can provide significant clinical effects improvements in patients with advanced PD with some patients no longer requiring

Problems associated with stem cells and demands for use in the treatment of Parkinson's disease

It is hoped that research on stem cells may reveal methods for producing an infinite supply of dopamine neurons for transplant into patients. The problem is controlling cell growth and differentiation. A patient with advanced PD died 23 months following intrastriatal transplantation of embryonic mesencephalic dopamine neurons [8]. No surviving tyrosine hydroxylase-immunoreactive neurons were found at autopsy. Instead, pieces of bone, hair, cartilage, and squamous epithelium were evident at multiple sites in the ventricles. Death was probably caused by obstruction of the fourth ventricle with secondary brainstem compression due to growth from the cell suspension implant (see [8]).

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Donor-Derived Brain Tumor Following Neural Stem Cell Transplantation in an Ataxia Telangiectasia Patient

N Amariglio, A Hirshberg, B W Scheithauer, Y Cohen, R Loewenthal, L Trakhtenbrot, N Paz, M Koren-Michowitz, D Waldman, L Leider-Trejo, A Toren, S Constantini, G Rechavi

Background

Neural stem cells are currently being investigated as potential therapies for neurodegenerative diseases, stroke, and trauma. However, concerns have been raised over the safety of this experimental therapeutic approach, including, for example, whether there is the potential for tumors to develop from transplanted stem cells.

Methods and Findings

A boy with ataxia telangiectasia (AT) was treated with intracerebellar and intrathecal injection of human **fetal neural stem cells**. **Four years after** the first treatment he was diagnosed with a **multifocal brain tumor**. The biopsied tumor was diagnosed as a glioneuronal neoplasm. We compared the tumor cells and the patient's peripheral blood cells by fluorescent in situ hybridization using X and Y chromosome probes, by PCR for the amelogenin gene X- and Y-specific alleles, by MassArray for the ATM patient specific mutation and for several SNPs, by PCR for polymorphic microsatellites, and by human leukocyte antigen (HLA) typing. Molecular and cytogenetic studies showed that the tumor was of nonhost origin suggesting it was derived from the transplanted neural stem cells. Microsatellite and HLA analysis demonstrated that the tumor is derived from at least two donors.

Conclusions

This is the first report of a human brain tumor complicating neural stem cell therapy. The findings here suggest that neuronal stem/progenitor cells may be involved in gliomagenesis and provide the first example of a **donor-derived brain tumor**. Further work is urgently needed to assess the safetv of these therapies.

Am J Pathol. 2005 Jun;166(6):1781-91

Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells.

Fujikawa T, Oh SH, Pi L, Hatch HM, Shupe T, Petersen BE.

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Embryonic stem (ES) cells have been proposed to be a powerful tool in the study of pancreatic disease, as well as a potential source for cell replacement therapy in the treatment of diabetes. However, data demonstrating the feasibility of using pancreatic islet-like cells differentiated from ES cells remain controversial. In this study we characterized ES cell-derived insulin-expressing cells and assessed their suitability for the treatment of type I diabetes. ES cell-derived insulin-stained cell clusters expressed insulin mRNA and transcription factors associated with pancreatic development. The majority of insulin-positive cells in the clusters also showed immunoreactivity for C-peptide. Insulin was stored in the cytoplasm and released into the culture medium in a glucose-dependent manner. **When the cultured cells were transplanted into diabetic mice, they reversed the hyperglycemic state for approximately 3 weeks, but the rescue failed due to immature teratoma formation**. Our studies demonstrate that reversal of hyperglycemia by transplantation of ES cell-derived insulin-producing cells is possible. However, the risk of teratoma formation would need to be eliminated before ES cell-based therapies for the treatment of diabetes are considered.