Arch. Immunol. Ther. Exp. (2015) 63:377–384
DOI 10.1007/s00005-015-0340-3

ORIGINAL ARTICLE

Transdifferentiation of Bone Marrow Mesenchymal Stem Cells into the Islet-Like Cells: the Role of Extracellular Matrix Proteins

Marta Pokrywczynska[1] · Marzena Anna Lewandowska[2,3] · Sandra Krzyzanowska[1] · Arkadiusz Jundzill[1] · Marta Rasmus[1] · Karolina Warda[1] · Maciej Gagat[4] · Aleksander Deptula[5] · Anna Helmin-Basa[6] · Marcin Holysz[7] · Maciej Nowacki[1] · Lukasz Buchholz[1] · Magdalena Bodnar[8] · Andrzej Marszalek[8,9] · Alina Grzanka[4] · Wojciech Jozwicki[10] · Jacek Michalkiewicz[6] · Tomasz Drewa[1,11]

Received: 12 August 2014 / Accepted: 16 April 2015 / Published online: 10 May 2015

Abstract Pancreatic islet implantation has been recently shown to be an efficient method of treatment for type 1 diabetes. However, limited availability of donor islets reduces its use. Bone morrow would provide potentially unlimited source of stem cells for generation of insulin-producing cells. This study was performed to evaluate the influence of extracellular matrix proteins like collagen, laminin, and vitronectin on bone marrow mesenchymal stem cells (BM-MSCs) transdifferentiation into islet-like cells (ILCs) in vitro. To our knowledge, this is the first report evaluating the importance of vitronectin in transdifferentiation of BM-MSCs into ILCs. Rat BM-MSCs were induced to ILCs using four-step protocol on plates coated with collagen type IV, laminin type I and vitronectin type I. Quantitative real-time PCR was performed to detect gene expression related to pancreatic β cell development. The induced cells expressed islet-related genes including: neurogenin 3, neurogenic differentiation 1, paired box 4, NK homeobox factor 6.1, glucagon, insulin 1 and insulin 2. Laminin but not collagen type IV or vitronectin enhanced expression of insulin and promoted formation of islet-like structures in monolayer culture. Laminin triggered transdifferentiation of BM-MSCs into ILCs.

Keywords Mesenchymal stem cells · Islet-like cells · Collagen · Laminin · Vitronectin · Transdifferentiation

Introduction

Pancreatic islet implantation has been recently shown to be an efficient method of treatment for brittle type 1 diabetes. However, lack of organ donors, immune rejection and side effects of immunosuppressive treatment limits its

✉ Marta Pokrywczynska
marta.pokrywczynska@interia.pl

[1] Chair of Regenerative Medicine, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Karlowicza 24, 85-092 Bydgoszcz, Poland

[2] Department of Molecular Oncology and Genetics, Innovative Medical Forum, Franciszek Łukaszczyk Oncology Center, Bydgoszcz, Poland

[3] Department of Thoracic Surgery and Tumors, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

[4] Department of Histology and Embryology, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

[5] Department of Microbiology, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

[6] Department of Immunology, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

[7] Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznań, Poland

[8] Department of Clinical Pathomorphology, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

[9] Department of Pathology, Poznań University of Medical Sciences, Poznań, Poland

[10] Department of Tumor Pathology and Pathomorphology, Franciszek Łukaszczyk Oncology Center, Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

[11] Department of Urology, Nicolaus Copernicus Hospital, Toruń, Poland

application (Pepper et al. 2013). It was indicated that embryonic stem cells and induced pluripotent stem cells can be differentiated in pancreatic-like cells in vitro (Boyd et al. 2008; Tateishi et al. 2008). However, using embryonic stem cells is associated with ethical dilemmas and risk of teratoma formation (Boyd et al. 2008). Another studies showed that also, pancreatic ductal cells, hepatic oval cells, umbilical cord blood stem cells and neural progenitor cells can be used for generation of insulin-producing cells (Bonner-Weir et al. 2000; Ende et al. 2004a, b; Hori et al. 2005; Ramiya et al. 2000; Yang et al. 2002). Nevertheless, generation of insulin-producing cells from these sources in large scale, realistic for clinical application is nebulous (Pokrywczynska et al. 2013b). Bone marrow is easily accessible and has abundant source of mesenchymal stem cells capable to transdifferentiate into a variety of lineages. Several authors indicated that bone marrow mesenchymal stem cells (BM-MSCs) can transdifferentiate in vitro and in vivo into the insulin-producing cells (Hisanaga et al. 2008; Zhang et al. 2010).

Extracellular matrix proteins play an important role in cell proliferation, differentiation and apoptosis. Pancreatic extracellular matrix consists of collagen type IV, laminin, fibronectin and vitronectin. Collagen type IV and laminin are present in basement membrane, fibronectin is found in both basement membrane and stroma, while vitronectin is detected in pancreatic epithelia (Cirulli et al. 2000; Shimoyama et al. 1995; Van Deijnen et al. 1994). In this study we evaluated for the first time if combination of extracellular matrix proteins such as collagen, laminin, and vitronectin affect the pancreatic cell transdifferentiation in vitro.

Materials and Methods

BM-MSCs Isolation and Expansion

All procedures were performed under protocols approved by Nicolaus Copernicus University Ethics Committee (no. 27/2011). Bone marrow was obtained from the femurs and tibias of 20 male Wistar rats. Mesenchymal stem cells were isolated according to the procedure described previously (Pokrywczynska et al. 2013a). BM-MSCs were cultured in medium consisting of Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 (PAA, Austria) supplemented with 10 % fetal bovine serum (FBS; PAA, Austria), basic fibroblast growth factor (bFGF; 10 ng/ml; Sigma, Germany), penicillin (100 U/ml; PAA, Austria), streptomycin (100 μg/ml; PAA, Austria) and amphotericin B (5 μg/ml; PAA, Austria) until third passage.

Analysis of BM-MSCs Phenotype

BM-MSCs from third passage were subjected to antigen analysis by flow cytometry. Detached cells were washed and resuspended in phosphate-buffered saline (PBS; PAA, Austria). Approximately 0.5×10^6 cells were incubated with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies against CD11b, CD29, CD31, CD34, CD44, CD45 and CD90 (BD, USA; Santa Cruz Biotechnology, USA) for 30 min. FITC- or PE-conjugated IgG_1, IgG_{2A}, IgM and IgA (BD, USA) were used as an isotype controls. Data were analyzed by collecting 3×10^4 events on a FACS Canto (BD Biosciences, USA).

Analysis of Multipotential Character of BM-MSCs

Differentiation of BM-MSCs in adipogenic, osteogenic and chondrogenic lineages was induced by culture in appropriate differentiation media according to manufacturer's instruction (Invitrogen, USA). Negative control cells were maintained in DMEM/Ham's F-12 supplemented with 10 % FBS and antibiotics. Adipogenesis was measured by the accumulation of neutral lipids in fat vacuoles, stained with Oil-Red-O. Osteogenesis was confirmed using alizarin red staining (Millipore, Germany). Chondrogenic differentiation was evaluated by anti-collagen type II immunocytochemical staining (anti-collagen II clone 6B3, 1:100, 16 h, 4 °C; Millipore, Germany).

Transdifferentiation of BM-MSCs into Pancreatic-Like Cells

BM-MSCs at third passage were seeded in density of 3×10^4 cells/cm^2 onto six-well plates uncoated (negative control and differentiation control) or coated with collagen type IV (differentiation collagen) (8.3 μg/cm^2, Sigma, Germany), laminin type I (differentiation laminin) (1.6 μg/cm^2, Sigma, Germany), vitronectin type I (differentiation vitronectin) (50 ng/cm^2; BD, Pharmingen, USA) and all proteins together (differentiation collagen–laminin–vitronectin). When cells reached 80 % confluence they were induced to transdifferentiate into islet-like cells (ILCs). Transdifferentiation of rat BM-MSCs into ILCs was carried out by four stages according to the protocol described previously by Zhang et al. (2010). In stage I, undifferentiated BM-MSCs were cultured for 6 days in DMEM-high glucose (DMEM-HG; 25 mmol/l glucose, Sigma, Germany), 20 % fetal calf serum (Sigma, Germany), and 0.1 mmol/l β-mercaptoethanol (Sigma, Germany). In stage II, cells were cultured for 6 days in DMEM-HG, 10 ng/ml

bFGF (Invitrogen, USA), 10 ng/ml epidermal growth factor (EGF; Sigma, Germany), 2 % B27 supplement (Invitrogen, USA), 0.5 % bovine serum albumin (BSA; Sigma, Germany), and 0.1 mmol/l β-mercaptoethanol. In stage III, cells were cultured for 6 days in DMEM-HG, 10 ng/ml EGF, 10 ng/ml hepatocyte growth factor (HGF; Invitrogen, USA), 10 mmol/l nicotinamide (Sigma, Germany), 2 % B27, 0.5 % BSA and 0.1 mmol/l β-mercaptoethanol. In stage IV, cells were cultured for 4 days in DMEM-low glucose (5.6 mmol/l glucose; Sigma, Germany), 10 ng/ml EGF, 10 nmol/l exendin-4 (Sigma, Germany), 10 ng/ml betacellulin (Sigma, Germany), 25 mmol/l zinc acetate (Sigma, Germany), 2 % B27, 0.5 % BSA, and 0.1 mmol/l β-mercaptoethanol. The medium was changed every 2 days. BM-MSCs cultured in serum-free medium (MSC Nutri Stem Xeno Free Medium, Biological Industries, Israel) served as a negative control.

RNA Extraction, Reverse Transcription and Real-Time PCR

Total RNA was extracted from cells after each differentiation stage by High Pure RNA Isolation Kit (Roche Diagnostics, Germany) and reverse transcribed to cDNA by Transcription High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Germany) according to manufacturer's instructions. Approximately, 1 μg of each RNA sample was used to synthesize cDNA. Gene expression related to pancreatic endocrine development was determined by SYBR green-based quantitative real-time polymerase chain reaction (qRT-PCR) LC 480 SYBR Green Master (Roche Diagnostics, Germany). The amplification was carried out in total volume of 25 μl containing 0.5 μM each primer, 12.5 μl SYBR Master, and 2 μl cDNA under following PCR conditions: one cycle at 95 °C (10 min); 45 cycles of denaturation (95 °C, 10 s), annealing (60 °C, 20 s) and extension (72 °C, 5 s); one cycle of melting curves: 95 °C (5 s), 40 °C (1 min), 97 °C (5–10 continuous acquisitions/1 °C) and final cooling step to 40 °C (10 s).

Specific primers were designed using Beacon Designer software. All primers were purchased from Oligo (IBB PAN, Poland). The primer sequences used for qRT-PCR are listed in Table 1. The expression of target and reference genes was analyzed by RT-qPCR (reverse transcription with real-time quantitative PCR) using Light Cycler 480II System (Roche Diagnostics). All experiments were performed at least in triplicate and additionally included samples without reverse transcriptase and samples without RNA as a negative control. The series of five cDNA mixture dilutions (1, 0.5, 0.25, 0.125 and 0.0625) were prepared to produce standard curve for each target and reference gene. Based on standard curve the amplification reaction efficiency was measured separately for each gene of interest. The relative quantification of target gene expression was calculated based on E-method algorithm (Roche Diagnostics), which is more precise than commonly used ΔΔCt algorithm. All results were normalized to expression of two reference housekeeping genes (PDGD and HPRT) and compared to appropriate control experiments.

Statistical Analysis

Statistical differences between gene expressions were determined by one-way ANOVA followed by least statistical differences (equal variances) or Tamhane's (different variances) post hoc multiple-comparison test. Statistically significant differences were taken at $p < 0.05$.

Results

Characterization of BM-MSCs

Flow cytometry confirmed homogenous BM-MSCs phenotype. BM-MSCs from the third passage were positive for the CD29 (99.83 ± 0.09), CD44 (90.63 ± 2.18) and CD90 (99.35 ± 0.59) markers and negative for typical endothelial and hematopoietic markers CD11b (0.74 ± 0.51), CD31 (0.20 ± 0.10), CD34 (0.12 ± 0.01) and CD45 (1.03 ± 1.20) (Fig. 1). BM-MSCs were able to differentiate into adipocytes, osteoblasts and chondrocytes after cultivation in respective media as confirmed by Oil-red-O, alizarin red and anti-collagen type II immunocytochemical staining, respectively (Fig. 2). Controls showed negative results.

Morphological Changes of BM-MSCs during Transdifferentiation into Pancreatic-Like Cells

Undifferentiated BM-MSCs displayed typical fibroblast-like, spindle-shaped morphology through 22 days of culture. During transdifferentiation process cells changed their phenotype into epithelial-like. There were no differences in cell morphology between cells seeded on coated vs uncoated with extracellular matrix protein plates after first, second and third stage of transdifferentiation. However, after fourth stage of transdifferentiation small clusters began to form on plates coated with extracellular matrix proteins. This process was most marked on plates coated with laminin where cells formed islet-like clusters (Fig. 3).

Table 1 Rat gene-specific primers

Gene	Primer sequence (5′-3′)	Product size (bp)
Pdx1	Forward: CGTTCATCTCCCTTTCCCGT	106
	Reverse: GGTCCTCTTATTCTCCTCCG	
Ngn3	Forward: AAGCAGAGGAGAGCCGTAG	129
	Reverse: AGACGCAACACTGGATTAGG	
NeuroD1	Forward: TAACAACAGGAAGTGGAAACAT	140
	Reverse: CTCCTTCTTGTCTGCCTCG	
Pax4	Forward: CCAGTGTATCCTCTATCAATC	132
	Reverse: GGGGAGCCTCACAGTTAC	
Nkx6.1	Forward: CGGAGAGTCAGGTCAAGGTC	183
	Reverse: CGTCGTCAGAGTTCGGGTC	
Glut-2	Forward: CGGCTGTCTCTGTGCTGC	131
	Reverse: CATCCAGGTGAACTTATCCAG	
Gcg	Forward: ACTCCCGCCGTGCTCAAG	124
	Reverse: CTCACATCACTGGTAAAGGTC	
Ins1	Forward: TACAATCATAGACCATCAGCA	108
	Reverse: TGGGCAGGCTTGGGCTCC	
Ins2	Forward: GTGTGTGGGGAGCGTGGA	232
	Reverse: GGTGGACAGGGTAGTGGTG	
HPRT	Forward: GTTCTTTGCTGACCTGCTG	129
	Reverse: ACTTTTATGTCCCCCGTTGA	
PBGD	Forward: ATTATCCTGGCTGTGGCTG	136
	Reverse: TATCCTGGTCCTTGGCTCG	

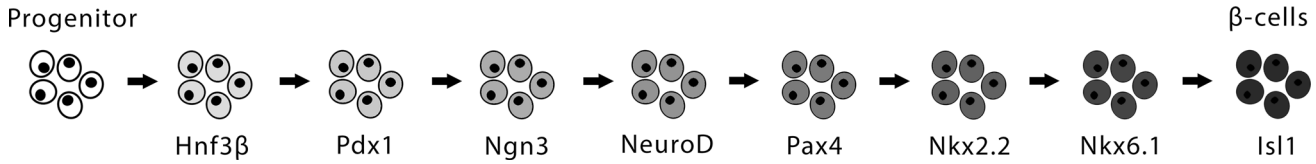


Fig. 1 Representative phenotype of bone marrow mesenchymal stem cells. Negative (CD11b, CD31, CD34, CD45) and positive (CD29, CD44, CD90) expression of cell surface markers analyzed by flow cytometry. *FITC* fluorescein isothiocyanate, *PE* phycoerythrin

Expression of Pancreatic-Related Genes

To determine whether BM-MSCs had undergone pancreatic transdifferentiation, gene expression of transcription factors and hormones related to pancreatic differentiation was assessed using qRT-PCR. The expression of pancreatic-related hormone genes was not detected in undifferentiated BM-MSCs. Unexpectedly, undifferentiated BM-MSCs expressed genes that are essential for differentiation of pancreatic cells in vivo including neurogenic differentiation 1 (NeuroD1) and NK homeobox factor 6.1 (Nkx6.1). Cells undergoing transdifferentiation expressed genes characteristic for endocrine cell development including: NeuroD1, Nkx6.1, Glucagon (Gcg), Insulin 1 (Ins1) and Insulin 2 (Ins2). Gene expression of pancreatic and duodenal homeobox 1 (Pdx1) was not detected while Neurogenin 3 (Ngn3), paired box 4 (Pax4) and glucose transporter 2 was incoherent in all stages of transdifferentiation (Fig. 4). Extracellular matrix proteins, especially collagen and laminin strengthen the transdifferentiation process (Figs. 5, 6, 7). However, combining all extracellular matrix proteins together had opposite outcome.

Discussion

Transdifferentiation of BM-MSCs into β cells in vitro cannot be achieved in a single step, but requires a series of transition steps replicating pancreatic organogenesis (Fig. 8). Formation of β cells requires activation of cascade of transcription factors including Hnf3β, Pdx1, Ngn3, NeuroD1, Pax4, Nkx2.2 and Nkx6.1 (Schwitzgebel et al. 2000).

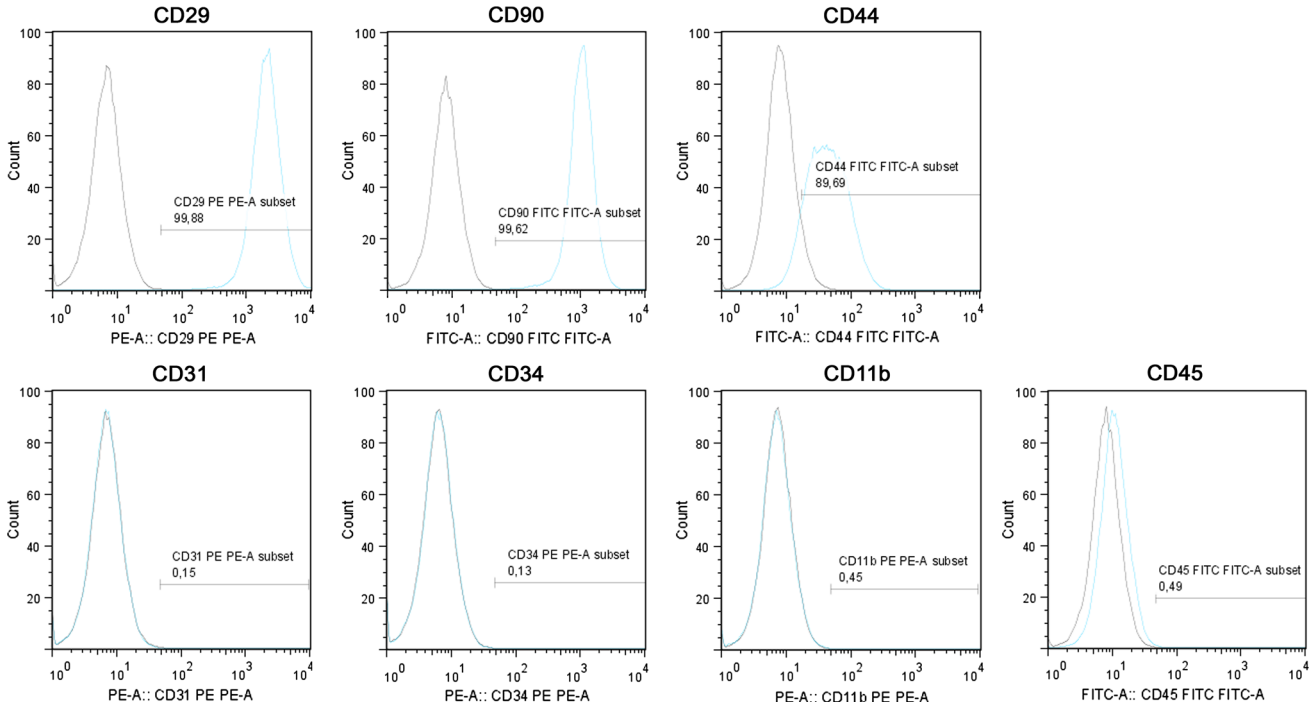


Fig. 2 Differentiation potential of BM-MSCs: positive anti-collagen type II staining after chondrogenic induction (**a**), a positive Oil-Red-O staining after adipogenic induction (**b**) positive staining after alizarin red staining after osteogenic induction (**c**). Light microscope, *scale bar* 50 μm

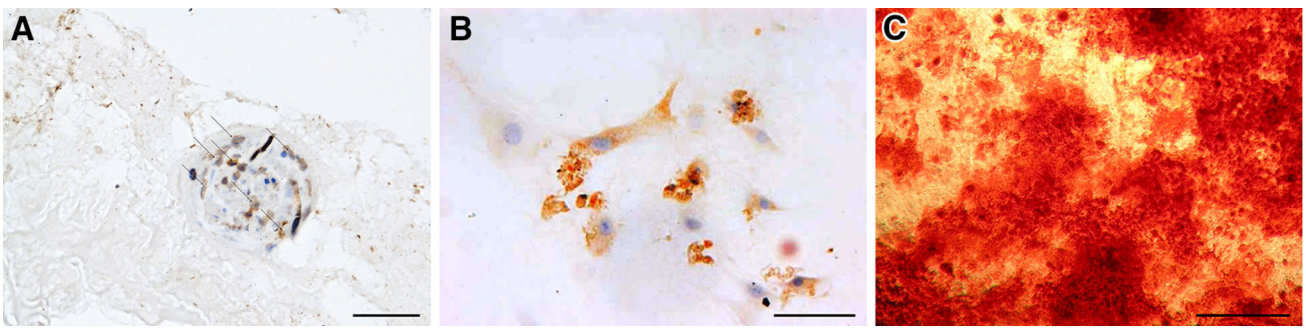


Fig. 3 Morphological changes of bone marrow mesenchymal stem cells undergoing transdifferentiation into the ILCs. Light microscope, objective magnification: ×10. *NEG-CON* negative control, *D-CON* differentiation control, *D-COL* differentiation collagen, *D-LM* differentiation laminin, *D-COL-LM-VN* differentiation collagen–laminin–vitronectin

In the present study, we found that undifferentiated BM-MSCs spontaneously express endocrine pancreas genes such us NeuroD1 and Nkx6.1. It was also proved by other authors who found that BM-MSCs express Ngn3 and Nkx6.1 (Chang et al. 2008; Moriscot et al. 2005). These findings make BM-MSCs very attractive type of cells for generation of insulin-producing cells in vitro. It is very interesting because Ngn3 and NeuroD1 are neighboring transcription factors of insulin-secreting cells' differentiation.

Using protocol described previously by Zhang et al. (2010) we were able to induce transdifferentiation of BM-MSCs into insulin-expressing cells. However, in contrast to Zhang et al. (2010), induced ILCs did not express Pdx1. Moreover, the ILCs after four-step induction process expressed higher level of NeuroD1 compared to Ins1 or Ins2 indicating that the induced cells are at the initial stage toward the β cells.

It was previously indicated that, extracellular matrix proteins affect β cell differentiation, proliferation or even insulin secretion (Hulinsky et al. 1995). The distribution of extracellular matrix proteins differs considerably between adult and fetal islet tissues. Vitronectin and their receptors are significantly higher expressed in fetal β cells than their

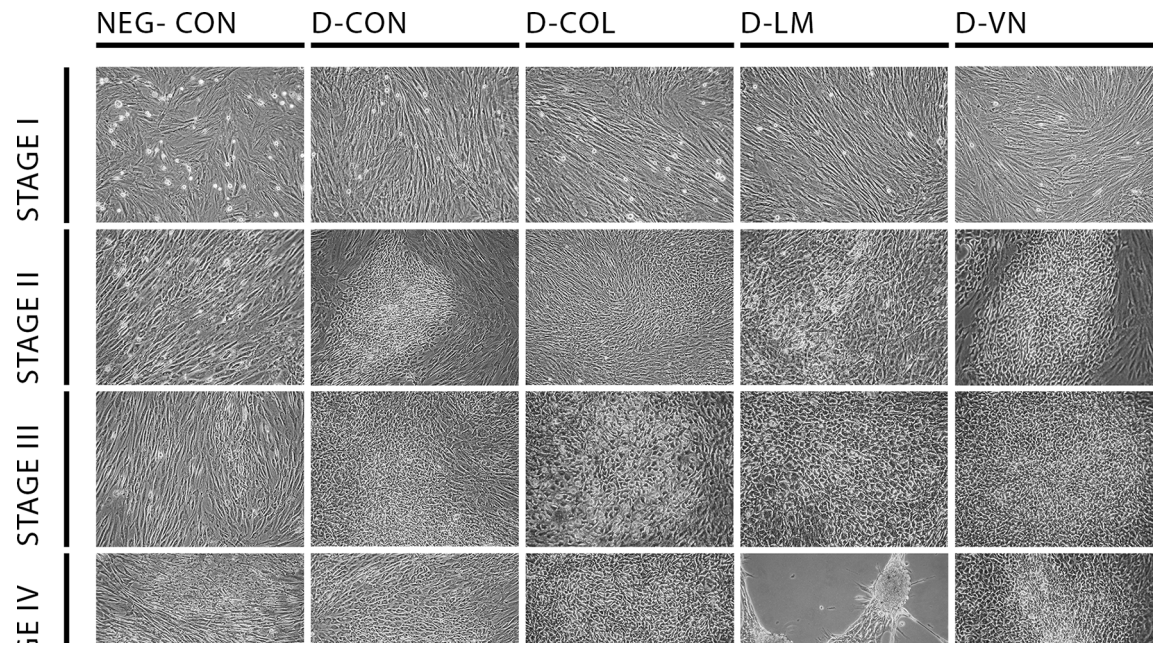


Fig. 4 Matrix diagram illustrating presence of pancreatic genes expression in BM-MSCs undergoing transdifferentiation into the ILCs: negative expression marked with *white*, any positive expression marked with *gray*. *NEG-CON* negative control, *D-CON* differentiation control, *D-COL* differentiation collagen, *D-LM* differentiation laminin, *D-COL-LM-VN* differentiation collagen–laminin–vitronectin, *NeuroD1* neurogenic differentiation 1, *Nkx6.1* NK homeobox factor 6.1, *Ngn3* neurogenin 3, *Pax4* paired box 4, *Glut-2* glucose transporter 2, *Pdx1* pancreatic and duodenal homeobox 1

Protocol	Stage	Pdx1	Ngn3	NeuroD1	Pax4	Nkx6.1	Glut-2	Gcg	Ins1	Ins2
NEG-CON	I									
	II									
	III									
	IV									
D-CON	I									
	II									
	III									
	IV									
D-COL	I									
	II									
	III									
	IV									
D-LM	I									
	II									
	III									
	IV									
D-VN	I									
	II									
	III									
	IV									
D-COL-LM-VN	I									
	II									
	III									
	IV									

Fig. 5 Quantitative RT-PCR analysis of NeuroD1 expression in bone marrow mesenchymal stem cell-derived ILCs after first stage of transdifferentiation. Gene transcripts of cells differentiated on plates coated with collagen (D-COL), laminin (D-LM), vitronectin (D-VN) and all extracellular matrix proteins (D-COL–LM–VN) were compared with gene transcripts of cells differentiated on uncoated plates (D-CON) or undifferentiated cells (NEG-CON). Relative levels of gene expression were normalized to the HPRT and PBG mRNA levels (internal control). Data are presented as mean ± SD from three replicates. D-COL vs all $p < 0.05$, D-CON vs D-COL-LM-VN $p < 0.05$

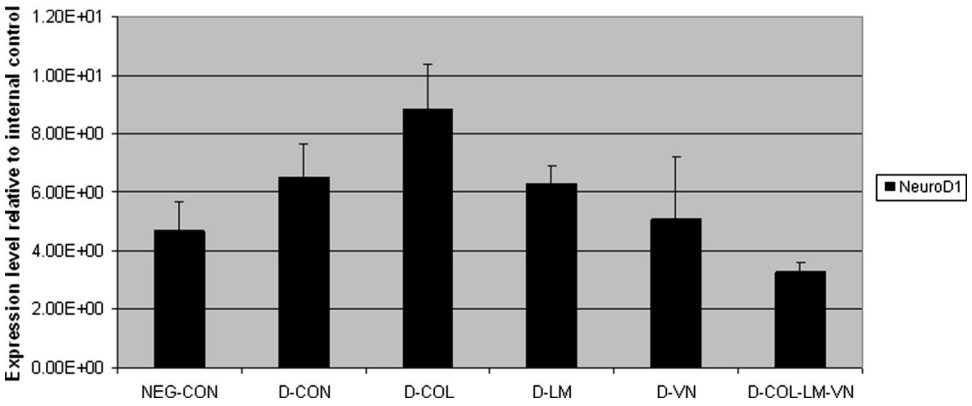


Fig. 6 Quantitative RT-PCR analysis of Nkx6.1 expression in BM-MSC-derived ILCs after third and fourth stage of transdifferentiation. Gene transcripts of cells differentiated on plates coated with collagen (D-COL), laminin (D-LM), vitronectin (D-VN) and all extracellular matrix proteins (D-COL–LM–VN) were compared with gene transcripts of cells differentiated on uncoated plates (D-CON) or undifferentiated cells (NEG-CON). Relative levels of gene expression were normalized to the HPRT and PBG mRNA levels (internal control). Data are presented as mean ± SD from three replicates. NEG-CON (stage III) vs all $p < 0.05$, D-CON (stage III) vs all $p < 0.05$, D-LM (stage IV) vs NEG-CON and D-VN $p < 0.05$

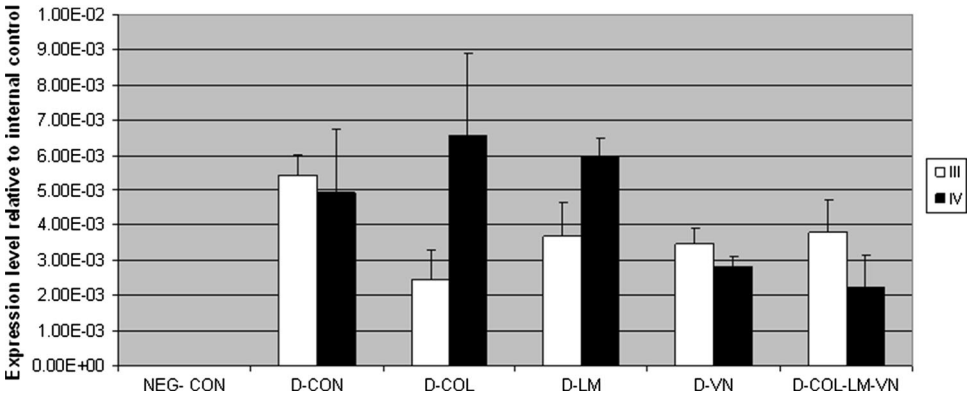
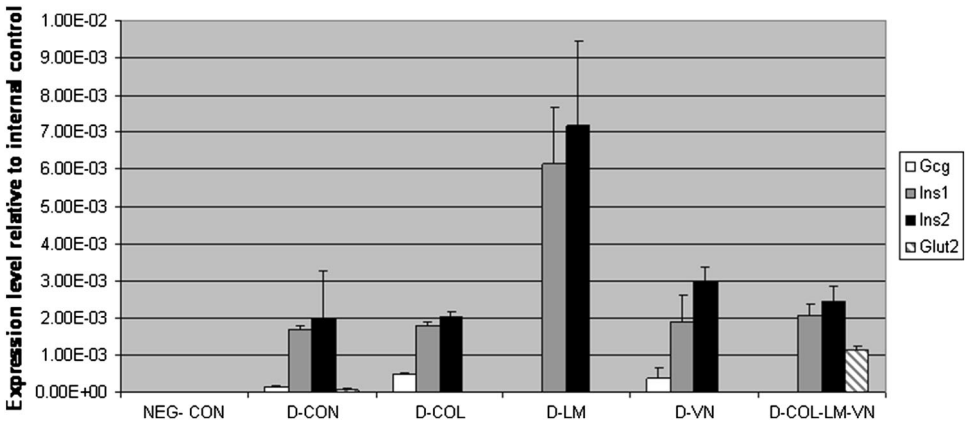


Fig. 7 Quantitative RT-PCR analysis of expression of pancreatic hormones: glucagon (Gcg), insulin 1 (Ins1) and insulin 2 (Ins2) and glucose transporter 2 (Glut-2) in BM-MSC-derived ILCs after stage IV of transdifferentiation. Gene transcripts of cells differentiated on plates coated with collagen (D-COL), laminin (D-LM), vitronectin (D-VN) and all extracellular matrix proteins (D-COL–LM–VN) were compared with gene transcripts of cells differentiated on uncoated plates (D-CON) or undifferentiated cells (NEG-CON). Relative levels of gene expression were normalized to the HPRT and PBG mRNA levels (internal control). Data are presented as mean ± SD from three replicates. Gcg: D-COL vs NEG-CON and D-CON and D-LM and D-COL–LM–VN $p < 0.05$, D-VN vs NEG-CON and D-LM and D-COL–LM–VN $p < 0.05$. Ins1: NEG-CON vs D-LM $p < 0.05$. Ins2: NEG-CON vs D-COL $p < 0.05$

Fig. 8 Model of the cascade of transcription factors controlling β cell development according to Schwitzgebel et al. (2000)



adult counterparts (Cirulli et al. 2000). These data suggest that vitronectin may play an important role in generation of insulin-producing cells in vitro. However, our study did not confirm it. The pancreatic gene expression profile was comparable in transdifferentiated cells regardless of whether the cells were seeded on plates coated or uncoated with vitronectin.

Collagen type IV enhanced significantly expression of NeuroD1 and Gcg but did not enhance expression of Ins1 and Ins2, while laminin increased significantly Nkx6.1 and Ins1 expression. These results are consistent with the findings of Lin et al. (2010) who demonstrated that laminin has great ability to stimulate differentiation of BM-MSCs into insulin-producing cells. ILCs expressed very low or negative level of glucagon compared to insulin, suggesting that generated cells display rather β- than α-cell phenotype.

In conclusion, our study demonstrated that laminin enhance expression of insulin and promotes formation of islet-like structures in monolayer culture. Further studies are required to determine if these cells secrete insulin in response to glucose and normalize glucose in vivo. The vitronectin did not stimulate differentiation of BM-MSCs into insulin-producing cells in vitro.

Acknowledgments This work was supported by Ludwik Rydygier Medical College in Bydgoszcz, Nicolaus Copernicus University in Torun, Faculty of Medicine, Young Scientist Grant MN-12/WL.

Conflict of interest The authors declare no potential conflict of interest.

References

Bonner-Weir S, Taneja M, Weir GC et al (2000) In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA 97:7999–8004

Boyd AS, Wu DC, Higashi Y et al (2008) A comparison of protocols used to generate insulin-producing cell clusters from mouse embryonic stem cells. Stem Cells 26:1128–1137

Chang CF, Hsu KH, Chiou SH et al (2008) Fibronectin and pellet suspension culture promote differentiation of human mesenchymal stem cells into insulin producing cells. J Biomed Mater Res A 86:1097–1105

Cirulli V, Beattie GM, Klier G et al (2000) Expression and function of alpha(v)beta(3) and alpha(v)beta(5) integrins in the developing pancreas: roles in the adhesion and migration of putative endocrine progenitor cells. J Cell Biol 150:1445–1460

Ende N, Chen R, Reddi AS (2004a) Effect of human umbilical cord blood cells on glycemia and insulitis in type 1 diabetic mice. Biochem Biophys Res Commun 325:665–669

Ende N, Chen R, Reddi AS (2004b) Transplantation of human umbilical cord blood cells improves glycemia and glomerular hypertrophy in type 2 diabetic mice. Biochem Biophys Res Commun 321:168–171

Hisanaga E, Park KY, Yamada S et al (2008) A simple method to induce differentiation of murine bone marrow mesenchymal cells to insulin-producing cells using conophylline and betacellulin-delta4. Endocr J 55:535–543

Hori Y, Gu X, Xie X et al (2005) Differentiation of insulin-producing cells from human neural progenitor cells. PLoS Med 2:e103

Hulinsky I, Harrington J, Cooney S et al (1995) Insulin secretion and DNA synthesis of cultured islets of Langerhans are influenced by the matrix. Pancreas 11:309–314

Lin HY, Tsai CC, Chen LL et al (2010) Fibronectin and laminin promote differentiation of human mesenchymal stem cells into insulin producing cells through activating Akt and ERK. J Biomed Sci 17:56

Moriscot C, de Fraipont F, Richard MJ (2005) Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation in vitro. Stem Cells 23:594–603

Pepper AR, Gala-Lopez B, Ziff O et al (2013) Current status of clinical islet transplantation. World J Transplant 3:48–53

Pokrywczynska M, Jundzill A, Bodnar M et al (2013a) Do mesenchymal stem cells modulate the milieu of reconstructed bladder wall? Arch Immunol Ther Exp 61:483–493

Pokrywczynska M, Krzyzanowska S, Jundzill A et al (2013b) Differentiation of stem cells into insulin-producing cells: current status and challenges. Arch Immunol Ther Exp 61:149–158

Ramiya VK, Maraist M, Arfors KE et al (2000) Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. Nat Med 6:278–282

Schwitzgebel VM, Scheel DW, Conners JR et al (2000) Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development 127:3533–3542

Shimoyama S, Gansauge F, Gansauge S et al (1995) Altered expression of extracellular matrix molecules and their receptors in chronic pancreatitis and pancreatic adenocarcinoma in comparison with normal pancreas. Int J Pancreatol 18:227–234

Tateishi K, He J, Taranova O et al (2008) Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 283:31601–31607

Van Deijnen JH, Van Suylichem PT, Wolters GH et al (1994) Distribution of collagens type I, type III and type V in the pancreas of rat, dog, pig and man. Cell Tissue Res 277:115–121

Yang L, Li S, Hatch H et al (2002) In vitro trans differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc Natl Acad Sci USA 99:8078–8083

Zhang Y, Shen W, Hua J et al (2010) Pancreatic islet-like clusters from bone marrow mesenchymal stem cells of human first-trimester abortus can cure streptozocin-induced mouse diabetes. Rejuvenation Res 13:695–706