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Multipotential nestin and Isl-1 positive mesenchymal stem cells isolated from human pancreatic islets

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Abstract

Mesenchymal cells in the developing pancreas express the neural stem cell marker nestin and the transcription factor islet-1 (Isl-1). Using defined culture conditions we isolated on a single cell basis nestin producing cells from human pancreatic islets. These cells were immortalized with lentiviral vectors coding for telomerase and mBmi. They are positive for Isl-1 and nestin and have the potential to adopt a pancreatic endocrine phenotype with expression of critical transcription factors including Ipf-1, Isl-1, Ngn-3, Pax4, Pax6, Nkx2.2, and Nkx6.1 as well as the islet hormones insulin, glucagon, and somatostatin. In addition, they can be differentiated into human albumin producing cells in vivo when grafted into a SCID mouse liver. In accordance with a mesenchymal phenotype, the cells were also able to adopt adipocytic or osteocytic phenotypes in vitro. In conclusion, cultured pancreatic islets contain nestin and Isl-1 positive mesenchymal stem cells with multipotential developmental capacity.

Keywords: Human; Pancreatic islet; Nestin; Mesenchymal stem cells; ABCG2; Isl-1; Differentiation; Diabetes; Insulin; Albumin

The development of normal pancreas is the result of close interaction between mesenchymal and epithelial cells that form the initial buds. Signals from mesenchymal cells direct pancreatic development towards endocrine or exocrine fate [1]. Mesenchymal cells of the developing pancreas express islet 1 (Isl-1) [2], an essential transcriptional factor for the generation of endocrine cells and nestin [3], a neural stem cell marker [4] that was also identified within adult pancreatic islets [5]. Fate and function of pancreatic mesenchymal cells in postnatal life are unknown. They could represent a population of dormant mesenchymal stem cells (MSC) with the potential to differentiate into pancreatic endocrine or even hepatic phenotype given the appropriate stimuli [5]. Accordingly in recent in vitro

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studies nestin positive cells isolated from fetal human pancreas could be differentiated into insulin producing cells and cured diabetes in an animal model [6,7].

Here we demonstrate that multipotent mesenchymal cells can be found in primary cultures of human islets. We further describe the isolation and immortalization of single cell derived nestin and Isl-1 positive cells from adult human islets of Langerhans. These cells display a mesenchymal phenotype and can be induced to differentiate towards a pancreatic endocrine, an adipocytic or an osteocytic phenotype in vitro and a hepatic phenotype in vivo.

Research design and methods

Cell culture studies. Human islet tissue was obtained from the Islet Transplantation Center in Geneva University Hospital, (Geneva Switzerland). Nestin positive islet derived progenitor cells were isolated as described previously [5]. Briefly, islets were washed and cultured in expansion medium (RPMI 1640 medium containing 10% fetal bovine serum, antibiotics, sodium pyruvate (1 mM), Hepes (10 mM), and β-mercaptoethanol (50 mM)). Within several days, nestin positive cells grew out from islets. These cells were expanded in medium containing 20 ng/ml each of FGF2 and EGF. After two passages, cells were re-suspended in a single cell suspension and subjected to a single cell deposition treatment by a FACS (Becton–Dickinson, Palo Alto, CA). EGF was from Sigma (Buchs, Switzerland). All other reagents were from Invitrogen/Gibco BRL (Basel, Switzerland).

Cell transduction using lentiviral vectors. HLOX vectors used for immortalization (pLOX-CW-Bmi1 and pLOX-TERT-iresTK) have been described previously [8,9], and express the murine Bmi-1 cDNA [10] (GenBank No. M64067) and the human hTERT cDNA (GenBank No. AF018167) followed by the thymidine kinase of Herpes simplex virus type 1 (HSV1-TK), respectively, in a pLox backbone. The vector used for transcription activated cell sorting (TRACS) was constructed as follows. Briefly, the neuron-specific GFP transgene described by Yamaguchi et al. [11] and comprising the rat nestin promoter, the EGFP cDNA (Clontech), the neuron-specific second intron of the rat nestin gene, and the chicken β-globin polyadenylation signal was inserted in opposite orientation into a CCL-LoxP HIV-1 derived vector. Detailed maps and sequences of the lentiviral vectors used in this study can be obtained at http://www.medecine.unige.ch/~salmon.

Production of HIV derived vectors pseudotyped with the VSV G envelope protein was achieved by transient co-transfection of three plasmids into 293T epithelial cell line as previously described [12]. Titers of LV stocks were determined by transduction of target cells and measuring of the fraction of transgene-expressing cells by flow cytometry (for GFP-expressing vectors) or number of integrated proviral genomes by quantitative PCR (for Bmi-1 or hTERT-expressing vectors). Details on titration techniques are available at http://www.medecine.unige.ch/~salmon. The functionality of the nestin-GFP LV was assessed by transduction of the nestin-expressing NFSK-1 human neuronal cell line (ATCC CRL-2060). The tissue-specificity of the nestin-GFP cassette in the context of the lentiviral vector was confirmed by GFP expression in NFSK cells and absence of GFP expression in HeLa cells (data not shown). Using a ubiquitous promoter (PGK or EF1), NFSK and HeLa cells were shown to be equally transduceable by LVs (data not shown). Titers of nestin-GFP LV stocks as determined by FACS on NFSK cells were thus considered equivalent to titers determined on HeLa cells. BC11 cells were transduced by lentiviral vectors at multiplicities of infection (MOI) ranging from 0.5 to 2.

Reverse transcription and polymerase chain reaction. Total RNA from human islets, cell cultures, and liver samples was extracted with TRIzol reagent (Lucerna Chemie AG, Switzerland). RNA (1 μg) was then reverse transcribed using Omniscript (Qiagen, Basel, Switzerland) and amplified by polymerase chain reaction (PCR) using *Taq* PCR core kit (Qiagen). Negative controls without reverse transcriptase enzyme were run in parallel to exclude possible contamination. Human gene-specific, intron spanning primers are listed in Table 1. PCR products were separated and visualized on agarose gels containing 0.5 μg/ml ethidium bromide (EtBr, Bio-Rad Laboratories AG, Reinach, Switzerland). One hundred bp molecular ruler (Bio-Rad) was run as size reference. PCR product identity was confirmed by nucleotide sequencing (Microsynth AG, Balgach, Switzerland).

Differentiation of immortalized BC11 cells. For differentiation into an endocrine phenotype, 90% confluent BC11 cells were trypsinized and seeded in low attachment plates (Vitaris, Baar, Switzerland) containing serum-free DMEM-F12 medium (17.5 mM) supplemented with B-27

Table 1
Primer sequences for RT-PCR

Gene	Sense primer	Antisense primer	Amplicon (bp)	Accession No.	Cycles
Nestin	5′-CGTTGGAACAGAGGTTGGAG-3′	5′-TAAGAAAGGCTGGCACAGGT-3′	396	BC032580	40
ABCG2	5′-CACAGGTGGAGGCAAATCTT-3′	5′-TCCAGACACACCACGGATAA-3′	322	AY289766	40
Thy-1	5′-GTCCTTTCTCCCCCAATCTC-3′	5′-GGGAGACCTGCAAGACTGTT-3′	239	NM_033209	40
SCF	5′-GGTGGCAAATCTTCCAAAAG-3′	5′-TCTTTCACGCACTCCACAAG-3′	222	BC074725	40
c-Kit	5′-GGCATCACGGTGACTTCAAT-3′	5′-GGTTTGGGGAATGCTTCATA-3′	244	L04143	40
Isl-1	5′-GTTACCAGCCACCTTGGAAA-3′	5′-TTCCCACTTTCTCCAACAGG-3′	240	BC031213	40
Insulin	5′-GGCTTCTTCTACACACCCAAG-3′	5′-CATCTCTCTCGGTGCAGGA-3′	248	AY138590	40
Glucagon	5′-CCCAAGATTTTGTGCAGTGGTT-3′	5′-CAGCATGTCTCTCAAATTCATCGT-3′	80	NM_002054	40
Somatostatin	5′-GATGCCCTGGAACCTGAAGA-3′	5′-CCGGGTTTGAGTTAGCAGATCT-3′	82	BC032625	40
Ipf-1	5′-CCTTTCCCATGGATGAAGTC-3′	5′-TTGTCCTCCTCCTTTTTCCA-3′	269	AF035259	40
Ngn-3	5′-CTATTCTTTTGCGCCGGTAGA-3′	5′-CTCACGGGTCACTTGGACAGT-3′	73	NM_020999	40
Pax-4	5′-TCTCCTCCATCAACCGAGTC-3′	5′-GTTGGAAAACCAGACCCTCA-3′	299	AF043978	40
Pax-6	5′-TGCGACATTTCCCGAATTCT-3′	5′-GATGGAGCCAGTCTCGTAATACCT-3′	81	NM_001604	40
Nkx2.2	5′-TCTACGACAGCAGCGACAAC-3′	5′-TTGTCATTGTCCGGTGACTC-3′	154	O95096	40
Nkx6.1	5′-TCTTCTGGCCCGGAGTGA-3′	5′-CCAACAAAATGGATCCTTGATGA-3′	84	NM_006168	40
Albumin	5′-ACTTTTATGCCCCGGAACTC-3′	5′-AGCAGCAGCACGACAGAGTA-3′	598	NM_000477	40
PPARγ2	5′-GCGATTCCTTCACTGATAC-3′	5′-GCATTATGAGACATCCCCAC-3′	580	NM_015869	40
Leptin	5′-TGCCCATCCAAAAAGTCCA-3′	5′-GAAGTCCAAACCGGTGACTTTCT-3′	122	NM_000230	40
Adiponectin	5′-TGGGCCATCTCCTCCTCA-3′	5′-AATAGCAGTAGAACAGCTCCCAGC-3′	103	NM_004797	40
APRT	5′-GCGTGGTATTCAGGGACATC-3′	5′-CAGGGCGTCTTTCTGAATCT-3′	284	NM_000485	28

All primers were run at 60 °C.