A potential role for islet-1 in post-natal angiogenesis and vasculogenesis

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Summary
The LIM-homeobox transcription factor islet-1 (Isl1) marks a cell population which gives rise to myocardial, pacemaker, endothelial and smooth muscle cells, which are derived from the secondary heart field during heart embryogenesis. Isl1+ precursors have the potential of self-renewal and differentiation into endothelial, cardiomyocyte and smooth muscle lineages. The primary objective of this study was to determine whether retroviral gene delivery of Isl1 to endothelial cells and mesenchymal stem cells (MSCs) could promote angiogenic and vasculogenic properties. To this end, endothelial cells and rat MSCs were retrovirally transduced to express Isl1. Isl1 expression in endothelial cells resulted in enhanced proliferation and adhesion to fibronectin. In addition, increased IL-1β and VEGF secretion was evident in Isl1 transduced endothelial cells, concomitant with increased migratory and tube formation properties of the endothelial cells. Isl1 expression in MSCs promoted their vasculogenic properties and resulted in enhanced in vitro tube formation. Finally, Isl1 expressing endothelial cells induced enhanced in vivo vascularisation in C57BL/6J mice. These data suggest, for the first time, that Isl1 promotes postnatal angiogenesis and vasculogenesis by improving the angiogenic properties of endothelial cells and MSCs.

Keywords
Bone marrow stromal cells, cell therapy, angiogenesis, gene therapy, Isl1

Introduction
The cardiogenic mesoderm consists of two populations or fields of cardiac precursor cells that contribute to different parts of the heart (1–3). Cells from the primary heart field give rise to parts of the atrial chambers and the left ventricular region (4, 5). Cells from the second heart field are added to the developing heart tube and give rise to the outflow tract, the right ventricular region and the main parts of the atrial tissue (2, 6–8). The transcription factor islet-1 (Isl1) defines and is required for secondary heart field development (2). Moreover, lineage tracing studies have shown that Isl1 progenitors differentiate not only to myocardial cells of the secondary heart field progeny, but also to pacemaker, smooth muscle and endothelial cells. It has also been demonstrated that when isolated and cultured in vitro, Isl1+ progenitors have the potential of differentiation into endothelial cells, cardiomyocytes and smooth muscle cells (9). Interestingly, after birth, the mammalian heart harbors a rare subset of Isl1+ progenitors, which can also be self-renewed, and triggered into fully differentiated myocytes (8).

Angiogenesis and vasculogenesis are promising therapeutic strategies to provide new venues for blood flow in patients with severe ischaemic heart and peripheral vascular diseases. Considering that Isl1 is a fundamental transcription factor in heart embryogenesis, and that embryonic progenitors that are positive to Isl1 have the ability to differentiate to endothelial cells, the aim of this study was to investigate whether gene delivery of Isl1 to endothelial cells (ECs) and mesenchymal stem cells (MSCs), could promote angiogenesis and vasculogenesis. By retroviral infection, we transduced the Isl1 gene to ECs and MSCs. We examined the functional properties of ECs that are activated during angiogenesis: proliferation, migration, adhesive capacity and paracrine activity (10, 11). Additionally, we examined the functional properties that are used in vasculogenesis of bone marrow stem cells: paracrine activity, tube incorporation capacity and differentiation markers (12, 13).

We found that Isl1 expression in ECs resulted in their enhanced proliferative, paracrine, migratory and adhesive properties as well as enhanced in vivo vascularisation in mice. Similar to its effects in ECs, Isl1 expression in MSCs promoted their vasculogenic properties as well.

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**Materials and methods**

**Ethics statement**

All the animal protocols were approved by Tel Aviv Sourasky Medical center Animal use and care Committee.

**Animals**

Twenty male eight-week-old C57BL/6J mice were obtained from Harlan Laboratories, Jerusalem, Israel.

**Endothelial cells**

H5V cell line that was produced from all of the embryonic murine heart (31) was grown in 37°C and 8% CO2 in Dulbecco's modified Eagle medium (DMEM/F12 (Biological Industries, Beit Haemek, Israel), supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum (FCS).

**Mesenchymal stem cells**

BM-mononuclear cells were isolated from Wistar inbred rats' bone marrow. Cells were separated using density gradient centrifugation (Axis-Shield) and grown on fibronectin in Dulbecco’s modified Eagle medium (DMEM) medium (Biological Industries, Beit Haemek, Israel) 15% FCS, in 37°C and 8% CO2. After 72 hours (h), non-adherent cells were washed and medium was changed every 72 h for 16 days.

**Retroviral transduction of ECs and MSCs**

Cmmp-Isl1-ires-gfp retroviral vector (a generous gift of Prof. Cecilia Lundberg, Dept Experimental Medical Science, Lund University), or cmmp-ires-gfp serving as its retroviral control vector, were transfected into Phoenix packaging cells (ATCC, Manassas, VA, USA) using standard Ca3PO4 protocol. Medium was changed after 24 h. Cells were grown for additional 24 h, and virus was collected and centrifuged for 5 minutes (min)/1,500 x rpm/4°C. Viral titer was determined as >6 x 104 IU/ml using NIH 3T3 cells. At 40% confluence, MSCs and ECs were incubated with viral supernatant for 8 h in the presence of 5 μg/ml polybrene (Sigma-Aldrich, Rehovot, Israel). At 72 hours post-infection, infection efficiency was determined by GFP flow cytometry analysis. Infected ECs was sorted using Fluorescence Activated Cell Sorting (FACS) for GFP positive population, to reach purity of 70–90% of ECs that are infected to express Isl1 or mock vector. MSCs were not sorted by FACS since infection rates routinely reached 60–70%.

**Experimental groups**

Unless mentioned otherwise all experiments were conducted on three groups: ECs and MSCs that were transduced to express Isl1-ires-gfp (group 1) were compared to a control group that consisted of ECs or MSCs that were transduced to express gfp alone (group 2), and to non-transduced (NT) ECs or MSCs that were not infected with any virus (group 3).

**Antibodies**

The following antibodies were used: monoclonal anti-Isl1 39.4D5 (Developmental Studies Hybridoma Bank, IA, USA). Polyclonal rabbit anti-Tie-2, polyclonal goat anti-CD31 and monoclonal mouse anti-Flk-1 (Santa Cruz, Santa Cruz, CA, USA), PE anti rabbit, PE anti-goat, PE anti-mouse secondary antibodies (Jackson Laboratories, Bar Harbor, ME,USA), polyclonal goat anti-CD31 (Jackson Laboratories). Biotin anti mouse CD105, biotin anti mouse CD105, biotin anti mouse CD54 (BioLegend, San Diego, CA, USA).

**Western blot analysis**

A total of 50 μg of whole-cell protein extract per sample, prepared from Phoenix packaging cells, was separated on 8% polyacrylamide gel and electroblotted on nitrocellulose membranes. Isl1 was detected using the specific antibodies described above. Corresponding HRP-conjugated secondary antibodies (Santa Cruz), in combination with enhanced chemiluminescence reagent (Amersham, Louisville, CO, USA) were used for signal detection.

**Thymidine incorporation assay**

Thymidine incorporation assay was performed as described before (32–34). Briefly, the proliferation index was determined by first synchronising the cells at the G1-S boundary by culturing cells for 24 h in a serum-free medium. Then, 4 x 10^4 H5V cells were seeded in a 96-well plate and treated with serum-rich medium. At 24 h after seeding, 3H-thymidine (1 μCi/well) was added for 16 h, and then proliferation was assayed by scintillation counting (β counter).
**Cell cycle and apoptosis analysis**

The proliferation index was determined by first synchronising the cells at the G1-S boundary by culturing cells for 24 h in a serum-free medium, and then cells were cultured for another 24 h with serum-enriched medium. Cell cycle analysis was determined by fluorescence-activated cell sorting (FACS) following staining with propidium iodide. Cells were collected by centrifugation, washed with phosphate-buffered saline (PBS), and permeabilised in 90% ethanol, 10% PBS prior to DNA staining. The permeabilised cells were incubated with 50 μg/ml propidium iodide, 0.1 mg/ml RNase A (Sigma), 0.1% Nonidet P-40, and 50 μg/ml trisodium citrate for 30 min prior to analysis using a Becton Dickinson FACSort analyser. The cell cycle profile was analysed using the Cell Quest software.

Assessment of apoptotic cells was carried out by annexin V staining as recommended by the manufacturer (R & D Systems Europe Ltd., Oxon, UK). Briefly, centrifuged cells were resuspended in binding buffer (100 mM HEPES, pH 7.4, 1.5 mM NaCl, 50 mM KCl, 10 mM MgCl₂, and 18 mM CaCl₂) and incubated with 0.5 μg/ml fluorescein-conjugated annexin V and 20 μg/ml propidium iodide for 30 min at room temperature prior to FACS analysis.

**Adhesion assays**

For adhesion to fibronectin, 10^4 H5V cells/per well were seeded in 96-well plates coated with fibronectin for 30 min. Non-adherent cells were washed away and adherent cells stained using XTT-based colorimetry (Biological Industries, Beit Haemek, Israel). Optical density at 450 nm, proportional to viable cells number, was measured using ELISA reader. Fibronectin-coated wells (standardised for each group) served as background.

**Cell-based ELISA of ICAM-1 and VCAM-1**

A total of 2x10^4 H5V cells/well were seeded in 96-well plates coated with fibronectin for 30 min. Non-adherent cells were washed away and adherent cells stained using XTT-based colorimetry. Optical density at 450 nm, proportional to viable cells number, was measured using ELISA reader. Fibronectin-coated wells (standardised for each group) served as background.

**Migration assay**

Isl1 expressing H5V cells were grown 48 h post-infection in serum-free conditioned media. Media were then collected and loaded on the lower chamber of a modified Boyden chamber (Neuroprobe, Gaithersburg, MD, USA) housing 8 μm polycarbonate filter. A total of 5x10^4 ECs in 50 μl serum-free medium were loaded into the top chamber. Migrating cells were fixed and stained with Diff-Quick Stain (Dade Behring AG, Newark, NJ, USA), and counted in four high-power fields (x20).

**Matrigel tube formation assay**

Conditioned Medium from Isl1 expressing ECs/MSCs was collected after 72 h in culture. Non-transduced ECs were cultured with the conditioned medium for 24 h. Then, 2x10^5 cells/well were seeded on 24-well plates coated with 200 μl Matrigel (BD, Franklin Lakes, NJ, USA) for 4 h.

A total of 2x10^5 Isl1 expressing MSCs or, alternatively, 10^5 MSCs mixed with 10^5 non-transduced H5V were seeded on 24-well plates coated with 200 μl Matrigel (BD). Tube formation was evaluated blinded to studied groups, after 4 h by phase-contrast microscopy (x40) and scored as described elsewhere (35):

0, individual cells, well separated;
1, cells begin to migrate and align themselves;
2, capillary tubes visible, no sprouting;
3, sprouting of new capillary tubes visible;
4, closed polygons begin to form;
5, complex mesh-like structures develop.

**Antibody array**

RayBio® Mouse Cytokine Array C series 1000 assay (RayBiotech Inc., Norcross, GA, USA) was performed according to manufacturer’s instructions. Briefly, 500 μg of whole H5V cell extract proteins were incubated with arrayed antibody membranes, which were exposed to specific biotin-antibody cocktail. Signals were detected using labelled-streptavidin and exposure on X-OMAT AR films. Signals quantification and data analysis were performed using TINA 2.0 software. Significance threshold was set as 1.5-fold and proteins showing such an induction/repression in at least one experimental group are presented ± standard deviation (SD) (p<0.05).

**In vivo Matrigel angiogenesis assay**

Transduced H5V (2x10^6) cells in 50 μl PBS were mixed with 400 μl liquid Matrigel and were subcutaneously injected into two groups of mice (n=10). After eight days, mice were sacrificed, and 5 μm thick frozen sections were prepared from the excised plugs. Immunohistochemical assessment of vascular density was performed by CD31 and Hematoxylin co-staining. Quantitative analysis was performed under a light microscope (x20) in high-power fields (n=10/per mouse).
Flow cytometry analysis

After 16 days in culture, MSCs were stained for typical mesenchymal, haematopoietic and mononuclear markers: CD29, CD90, CD11b, and CD45, and analysed by flow cytometry. Seven days after retroviral infection of MSCs, cells were gently removed from culture dishes using cell scraper, washed, and diluted in PBS containing 1% BSA. Cells were stained against CD31, Flk1, Tie2, and isotype control and analysed by flow cytometry (FACScan, BD).

Statistical analysis

Comparison between all of the studied groups in all experiments was performed employing the One-way ANOVA test. Level of significance was set at p<0.05(*). Results are expressed as mean ± SD unless otherwise specified. Results of all experiments represent data collected from three independent repeats, unless mentioned otherwise.

Results

Retroviral transduction of Isl1 to ECs

At 40% confluence, H5V EC line was infected with the retroviral vector cmmp-Isl1-ires-gfp or with control vector cmmp -ires-gfp. Infected cells were sorted to GFP positive cells by fluorescence-activated cell sorting (FACS) in order to reach purity of 70–90% Isl1 expressing cells. Is1 protein expression in transfected ECs was confirmed by Western blot and was compared to controls of ECs transfected with control vector, and non-transfected endothelial cells. Abbreviations: NT, non-transduced.

Efficacy of Isl1 expression was confirmed by Western blot (Fig. 1C).

Control groups in all experiments were ECs infected with control vector or non-transduced ECs, unless mentioned otherwise.

Is1 forced expression in endothelial cells ameliorates the functional properties that are required for angiogenesis

In order to form new blood vessel, endothelial cells are activated to proliferate, to migrate into the forming vessel, to adhere to the extracellular matrix and to adjacent cells, while the whole process is conducted through paracrine activity by the secretion of angiogenic cytokines.

We examined the proliferative capacity of Isl1 expressing endothelial cells by both propidium iodide stain and thymidine incorporation assay. Cell proliferation was increased (2.79 ± 0.54 folds of control, p<0.05) as measured by "H-thymidine incorporation (Fig. 2B). Cell cycle analysis by propidium iodide staining confirmed the effect of increased proliferation (Fig. 2A). Combined staining for Annexin V and PI demonstrated that there was no significant difference in apoptotic cells between groups (data not shown).

Increase in adhesion capacity of ECs to extracellular matrix (ECM) and attainment of an appropriate cellular shape is crucial for EC growth, differentiation, and survival (14). Herein, we assessed the ability of Isl1 expressing H5V endothelial cell line, to adhere to fibronectin. Indeed, an increased number of adherent cells was observed in the Isl1 expressing group as compared to controls (1.45 ± 0.045 fold, p<0.05) (Fig. 2C).
It had been previously shown that the adhesion molecule ICAM-1 regulates endothelial cell motility, and that the adhesion molecule VCAM-1 mediates adhesion between ECs and mural cells. Both properties are essential for angiogenesis (15, 16). Isl1 expression in ECs resulted in a significant increase of ICAM-1 and VCAM-1 (1.49 ± 0.09 fold, p<0.05 and 1.35 ± 0.1 fold, p<0.05, respectively), compared to controls, as demonstrated by cell-based ELISA (Fig. 2D).

Next, we examined whether Isl1 expression in ECs will affect the cells cytokine secretion. Using a cytokine array, cell extract of Isl1–expressing ECs was examined by comparative analysis to an extract of ECs expressing control vector. Twenty-four different cytokines were examined. Significance threshold was set as 1.5-fold, and proteins showing such an induction/repression in at least one experimental group are presented ± SD (p<0.05). According to the threshold definition, a significantly increased expression of VEGF (1.55 ± 0.47 folds of control, p<0.05) and IL-1β (1.58 ± 0.11 folds of control, p<0.05), both pro-angiogenic cytokines (17–20) were found. The data apply to the paracrine activity induced by Isl1.

**Figure 2: Functional properties of Isl1 expressing ECs.**

A) Cell cycle analysis and comparison of Isl1-expressing ECs to controls, by PI stain and fluorescence-activated cell sorting (FACS) analysis. Prior to PI staining, cells were synchronised at G1-S boundary by culturing with serum-free medium. Then serum was added for another 24 hours and PI staining was conducted. B) Thymidine incorporation assay of Isl1-expressing ECs versus controls. Cells were seeded in a 96-well plate. Twenty-four hours after seeding, 3H-thymidine was added for 16 hours, and then proliferation was assayed by scintillation counting (β counter). C) 10^4 H5V cells/per well were seeded in 96-well plates coated with fibronectin for 30 minutes, non-adherent cells were washed away. Remaining cells were stained with XTT-based colorimetry. D) Cell-based ELISA for VCAM1 and ICAM1 adhesion molecules. Abbreviations: NT, non-transduced; S, synthesis.
In the following experiment we aimed to assess Isl1 influence on paracrine activity. Isl1-expressing ECs were grown for 72 h, and their conditioned media was collected. The migratory capacity of non-transduced ECs towards the Isl1-enriched medium was tested using a Boyden chamber apparatus, and showed an increase of 2.1 ± 0.33 folds (p<0.05) (Fig. 3A), shedding more light on the paracrine affects attributed to Isl1.

We next sought to assess whether the paracrine effect of Isl1-enriched medium could enhance tube formation of non-transduced ECs; to this end, non-transduced ECs were treated for 72 h with conditioned medium that was collected from Isl1-expressing ECs. Then, treated cells were seeded on Matrigel-coated plates for 4 h. In accordance with the migration results, Isl1-enriched medium ameliorated non-transduced cells’ tube formation capacity as early as 4 h after seeding on matrigel (4.53 ± 0.71 fold, p<0.05) (Fig. 3B).

**In vivo Matrigel plug assay**

In view of the encouraging *in vitro* data we then sought to examine the consequences of Isl1 expression on angiogenesis *in vivo*, in a mouse Matrigel plug assay. Anti-CD31 staining of the excised plugs revealed increased vascular density in plugs which contained Isl1-expressing ECs as compared to controls of Matrigel plugs that were mixed with ECs expressing control vector (Fig. 4A). In concordance with the histology findings of enhancement in vascular density, plugs composed of ECs expressing Isl1 were larger in size compared to their control cell counterparts (representative picture, Fig. 4B).
**Figure 4:** Isl1 expression in endothelial cells enhances in vivo vascularisation.

A) Isl1-expressing endothelial cells (ECs) or ECs expressing control vector were mixed with Matrigel and injected subcutaneously into C57BL/6J mice. Eight days post-injection plugs were removed and subjected to immunohistochemical assessment of vascular density by CD31 and Hematoxylin co-staining. Quantitative analysis was performed under a light microscope (x20) in high-power fields (n=10). Data are presented as an average of the number of vessels that was counted per microscopic field. B) Representative plug of Isl1/ECs (1) compared to plug of control vector/ECs (2).

**Figure 5:** Mesenchymal stem cells (MSC's) phenotype and angiogenic markers.

A) Primary culture of rat MSCs: morphology was analysed by microscopy (X20) at days 4, 7 and 14. Lower right: MSCs transduced with cmmp-Isl1-ires-gfp. B) Flow cytometry analysis for MSCs phenotype. C) Seven days post-transduction of MSCs with Isl1-ires-gfp; we performed immunostaining for Phycoerythrin (PE) conjugated endothelial markers: flk-1, tie2 and CD31. A significant increase in CD31 expression was detected in MSCs that express Isl1. Data are presented as summary of CD31-positive cells of Isl1-expressing ECs as folds of CD31-positive cells of control ECs.
Cell culture, phenotypic characterisation of MSCs and Isl1 transduction

Next, we sought to assess the effects of Isl1 expression in MSCs. MSCs were isolated according to standard techniques for the isolation of mononuclear cells from bone marrow (BM) using density gradient centrifugation. In vitro culture-expanded adherent MSCs were uniformly fibroblast-like in appearance (Fig. 5A). Analysis by flow cytometry performed at passage 3 revealed that the cells expressed CD90 and CD29 but were negative for CD45, and only a small minority of the cells was positive for CD11b, consistent with the previously demonstrated MSC phenotype (21) (Fig. 5B).

Figure 6: In vitro tube formation capacity of Isl1-expressing mesenchymal stem cells (MSCs). A) 2x10⁵ Isl1-positive MSCs or MSCs expressing control vector, were seeded on Matrigel. Wells were examined after 6 hours by phase-contrast and fluorescent microscopy (x40) and tube formation index was scored 1–5 (1– separated cells, 5– closed polygons). B) 10⁵ MSCs expressing Isl1 or MSCs expressing control vector + 10⁵ ECs were seeded on Matrigel and tube formation index was evaluated as described above. C) Non-transduced ECs were cultured for 72 hours with medium collected from Isl1-expressing MSCs or from MSCs expressing control vector. Then, ECs were seeded on Matrigel and tube formation index was assessed as described above. Abbreviations: NT, non-transduced; CM, conditioned medium.
Isl1 expression in MSCs improves their vasculogenic properties

Seven days post-transduction with Isl1, phenotypic differentiation was assessed by flow cytometry analysis for the PE-conjugated endothelial markers, Flk1, Tie2, and CD31. Whereas no significant change was detected in Flk1 and Tie2 levels, there was a significant increase in the expression of CD31 in MSCs expressing Isl1 compared to MSCs expressing the control vector (2.1 ± 0.7 fold, Fig. 5C).

We next examined whether Isl1 expressing MSCs will form better tubes on Matrigel, and whether Isl1 expression will result in a paracrine effect of MSCs on ECs, when MSCs expressing Isl1 are seeded together with ECs on Matrigel. When seeded alone on Matrigel, MSCs that expressed Isl1 were shown to form more organised tube structures compared to control MSCs expressing the control vector (1.7 ± 0.7 fold, ▶ Fig. 6A). In order to examine the ability of MSCs to incorporate into growing vessels and their paracrine effects on the vasculogenic capacity of ECs, we performed two additional Matrigel tube formation assays. First, we seeded a mixture of MSCs with ECs and found a more developed capillary-like organisation as well as an increase in incorporation of the Isl1-transduced MSCs into the tubes, compared to MSCs expressing control vector (3.2 ± 0.5 fold, ▶ Fig. 6B). Second, we demonstrated that conditioned medium of MSCs expressing Isl1 induced a better capillary network of non-transduced ECs on Matrigel (2.1 ± 0.5 fold, ▶ Fig. 6C) compared with medium obtained from MSCs which expresses the control vector.

Discussion

Several studies have confirmed the pivotal role of Isl1 progenitors in heart embryogenesis (8, 9, 22). Additionally, the in vitro differentiation potential of Isl1 progenitors to all heart lineages was also demonstrated (9).

Recent studies have focused on embryonic pathways that are re-established postnatally, and provide improved angiogenic and vasculogenic effects in in vivo ischaemic models (23–25). In the current study, we induced expression of Isl1 in adult ECs and MSCs aiming to examine the effect of this master transcription factor on postnatal angiogenesis and vasculogenesis. We hypothesised that forced expression of this transcription factor has a cardinal role in specification of embryonic myocardial lineages, may lead to reprogramming of transduced cells and improve their ability to promote angiogenesis and vasculogenesis. However, the question of whether Isl1 expression is encountered in pathophysiological conditions in ECs and / or MSCs, is of great interest and may be examined in future studies.

Gene therapy and cell therapy for cardiovascular diseases are emerging approaches that that receive considerable interest in recent years (26). We have previously demonstrated an improved differentiation potential to the endothelial lineage of bone marrow cells after overexpression of HIFα or transcription factors (27). These data support the notion that there is a potential therapeutic advantage in virus based gene delivery of transcription factors, since angiogenic and vasculogenic processes are controlled by several programs and not by single genes. In the current study, we have demonstrated that retroviral gene delivery of Isl1 to ECs and MSCs promotes their angiogenic and vasculogenic properties.

Proliferation, migration, sprouting and pruning of EC are basic elements in the angiogenic process (10, 11). Isl1 expression in ECs promoted their proangiogenic properties in a direct and paracrine manner. This effect was demonstrated by studying the proliferation, migration, vessel incorporation and adhesive properties of endothelial cells. Accordingly, we have found that the adhesion molecules ICAM-1 and VCAM-1 were upregulated. Both these molecules were recently shown to be essential during angiogenesis (15, 16). The protein array data supported the proangiogenic effects of Isl1 transduction by demonstrating an increase in the expression of the pro-angiogenic cytokines VEGF and IL-1β (17, 20). The increase in VEGF and IL-1β expression may point to a potential advantage of using a transcription factor in gene therapy, since more then one angiogenic signaling pathway was apparently activated.

What is known about this topic?
- The LIM-homeobox transcription factor islet-1 (isl1) marks a cell population which gives rise to myocardial, pacemaker, endothelial and smooth muscle cells, which are derived from the secondary heart field during heart embryogenesis.
- Isl1+ precursors have the potential of self-renewal and differentiation into endothelial, cardiomyocyte and smooth muscle lineages.
- Interestingly, after birth, the mammalian heart harbors a rare subset of Isl1+ precursors, which can also be self renewed, and triggered into fully differentiated myocytes.

What does this paper add?
- Isl1 retroviral over expression in adult endothelial cells (ECs) resulted in their enhanced proliferative, paracrine, migratory and adhesive properties.
- Isl1 retroviral over expression in adult ECs resulted in enhanced in vivo vascularisation in mice.
- Isl1 retroviral over expression in MSCs promoted their vasculogenic properties.
- These data suggest a potential role for isl1 in post natal angiogenesis and vasculogenesis.
As the paracrine-induced properties of Isl1 expressing ECs were clearly evident, medium from these cells was collected and was shown to improve migratory capacity and tube formation on Matrigel of non-transduced ECs. Moreover, in an in vivo Matrigel plug assay in mice, enhanced vascularisation into plugs mixed with Isl1+ ECs was evident. This observation supports the collective effects of Isl1 expression on postnatal angiogenesis.

We next addressed the question of whether Isl1 expression in MSCs could influence their vasculogenic properties. Indeed, seven days after Isl1 was retrovirally transduced to MSCs, we observed upregulation of CD31, suggesting differentiation towards endothelial lineage. Incorporation of bone marrow cells into growing vessels has been shown to be part of the vasculogenic process (11, 28). In this study we show that Isl1 expression in MSCs improved their in vitro capillary formation ability on Matrigel, as well as their incorporation into forming capillaries when seeded together with non-transduced ECs.

An additional mechanism for the contribution of BM cells to postnatal vessel growth is proarteriogenic paracrine effects (28–30). Accordingly, we have shown that Isl1 expression in MSCs induces a paracrine effect on tube formation of non-transduced ECs, after the latter were cultured with conditioned medium that was collected from Isl1 positive MSCs population. In summary, Isl1-forced expression may influence proangiogenic and provasculogenic programs in adult EC and MSC. These findings suggest the potential Isl1 have in promoting angiogenesis and vasculogenisis in ECs and MSCs by triggering the intrinsic proangiogenic functional properties of these cells, as well as by enabling paracrine amplification on angiogenesis.

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References