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Author contributions

A.M. and J.E.B. hypothesized that Notch inhibition might aid reprogramming. J.K.I., J.T., A.M., A.U., L.L.R. and K.E. designed reprogramming and mechanistic experiments to test the hypothesis. J.K.I., J.T., A.C.C., L.A.W., Y.S., M.T.M., S.S., G.A. and H.A. performed reprogramming experiments and characterization of the iPSCs. C.B. and M.Z. performed bioinformatic analysis of transcriptional data characterizing the iPSCs. J.K.I., J.T., A.C.C. and Y.S. performed experiments to determine the mechanism of action of DAPT and Notch inhibition in reprogramming. K.E., J.K.I. and J.T. discovered and confirmed the mechanism of action of DAPT. K.E. and J.K.I. wrote the paper. All authors helped in paper revision.

Competing financial interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at http://www.nature.com/reprints/index.html. Correspondence and requests for materials should be addressed to H.A., A.M. or K.E.

ONLINE METHODS

iPSC reprogramming experiments. The IACUC committee of Harvard University approved the use of mice for all experiments included in this paper. *Oct4*:GFP neonatal mouse keratinocytes were isolated from P1-P2 pups using an overnight digestion in either 0.25% trypsin/EDTA or TrypLE (Life Technologies) at 4 °C. They were cultured in SFM medium (Life Technologies) on collagen IV–coated plates. Neonatal human epidermal keratinocytes (Lonza) were cultured in Epilife medium (Invitrogen) on collagen-coated plates. Keratinocytes were reprogrammed using retroviruses containing either mouse or human *OCT4*, *SOX2*, *KLF4* and *CMYC* produced in the pMXs backbone. Chemical treatment was initiated 1–2 d after viral transduction and readministered every other day until the end of the experiment unless otherwise specified. DAPT (EMD Millipore) was used at 10 μM for reprogramming experiments using *OCT4*, *SOX2*, *KLF4* and *CMYC* and 2.5 μM for *OCT4*, *SOX2* reprogramming experiments unless otherwise noted. DBZ was used at 2 μM. Irradiated mouse embryonic fibroblast feeders were added 6 d after transduction, and the medium was changed to mouse or human embryonic stem cell medium at that time. Colonies were scored as iPSC colonies if they were *Oct4*::GFP+ in mouse experiments or NANOG+/TRA-1-81+ in human experiments.

Gene expression analysis of iPSCs. Nanostring (Nanostring Technologies) and scorecard analysis was performed as described[34]. iPSCs were cultured in mTesr1 medium (Stem Cell Technologies) before RNA isolation. To measure their differentiation propensities, iPSCs were dissociated into embryoid bodies and cultured in human embryonic stem cell medium without bFGF for 16 d. Cells were then lysed, and total RNA was extracted using Trizol (Life Technologies) and purified using the RNeasy kit (QIAGEN). 300–500 ng of RNA was profiled on the Nano-String nCounter system (Nanostring Technologies) according to the manufacturer's instructions. A custom nCounter codeset covering 500 genes that monitor cell state, pluripotency and differentiation was used[34]. Data analysis was performed with the R statistics package as in ref. 34. Briefly, the lineage scorecard performs a parametric gene set enrichment analysis on *t* scores obtained from a pairwise comparison between the cell line of interest and the reference of ES cell–derived EBs.

Differentiation of iPSCs. For teratoma formation, 1–2 million human iPSCs were injected into the kidney capsule of nude mice and harvested 2 months later. Teratomas were sectioned and stained with hematoxylin and eosin for visualization. For the mouse iPSC chimera assay, 10 *Oct4*::GFP+ iPSCs were injected per ICR blastocyst, and 20 blastocysts were transplanted into each pseudopregnant female. Embryos were either allowed to develop to term or harvested at day E12.5 and dissected for genital ridge analysis using a stereomicroscope.

Gene expression analysis of reprogramming cultures. Illumina MouseRef-8 microarrays (Illumina) were used for genome-wide mRNA expression analysis of reprogramming mouse keratinocyte cultures treated with DMSO or 10 μM DAPT. For qPCR analysis, RNA was isolated using Trizol, cDNA synthesis was performed using the iScript cDNA synthesis kit (Bio-Rad), and the SYBR Green qPCR Supermix (Bio-Rad) was used for PCR product detection.

Western blots and immunofluorescence. Antibodies detecting mouse Notch (Santa Cruz Biotechnology, sc-6015, 1:1,000), human NOTCH (Abcam, ab27526, 1:1,000 and Santa Cruz Biotechnology, sc-23307, 1:1,000), cleaved human NOTCH (Cell Signaling Technology, 2421, 1:1,000), p53 (Santa Cruz Biotechnology, sc-56182, 1:1,000), Involucrin (Abcam, Ab53112) and p21 (Cell Signaling Technology 05-345, 1:1,000) were used for western blots. Blots were quantified using ImageJ software. Antibodies specific for NANOG (Abcam, AF1997, 1:400) and TRA-1-81 (Chemicon, MAB4381, 1:500) were used to identify human iPSCs. A γH2AX (Abcam, ab11175, 1:400) antibody was used to detect γH2AX foci. Cells in which γH2AX staining covered greater than half the nucleus were scored as positive for γH2AX foci.

UV irradiation assay. UV irradiation was performed at a dosage of 30 J. TUNEL staining was performed using a TUNEL kit (Pharmacia Biosciences).

shRNA and siRNA knockdown experiments. shRNAs and siRNAs were purchased from Sigma and added to reprogramming cultures within 1 d after addition of the reprogramming retroviruses. shRNAs (TRCN0000003753, p53 and TRCN0000287021, p21) were expressed in the pLKO.1 lentiviral backbone. siRNAs were used at 80 nM and were transfected into reprogramming cultures using RNAiMAX (Life Technologies).

Array CGH analysis of iPSC lines. Cell Line Genetics performed array CGH analysis of iPSC lines at passage 5 using 4x180K+SNP analysis.

Statistical analysis. For all experiments, error bars represent the s.d. between two or three biological replicates, and statistical significance was determined using a two-tailed homoscedastic Student's *t*-test.

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ERRATUM

Notch inhibition allows oncogene independent generation of iPS cells

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In the version of this article initially published, Julia TCW's name was misspelled as Julia T C W. In addition, her initials in the author contribution statement should have read J.T. instead of J.T.C.W. The error has been corrected in the HTML and PDF versions of the article.

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In the version of this article initially published, a black bar was erroneously placed in the scrambled shRNA column in Figure 3g. The error has been corrected for the PDF and HTML versions of the article.

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EDITORIAL

Stem Cells Bond Our Organs/Tissues and Engineering Products

Masashi Toyoda, PhD; Akihiro Umezawa, MD, PhD

The human body is made up of trillions of cells that work together. Cells make up tissues and organs, and a group of organs performs a certain function for homeostatic maintenance. Many diseases result from a homeostatic imbalance. Stem cells in organs and/or tissues play important roles in the regulation of homeostasis. Stem cell-based therapy has become a promising strategy for the treatment of many diseases. Therefore, regenerative medicine may one day restore the function of damaged organs or tissues. On the other hand, tissue engineering, such as cell-sheet and artificial organs that might supplement or completely replace the functions of impaired or damaged tissues, has developed remarkably.[1] An integrated strategy of tissue engineering including artificial organs and stem cell-based therapy medicine should give rise to a new regenerative medicine for organ failure.

Article p1762

Stents have been used for the treatment of coronary artery disease (CAD) for more than a decade. A coronary stent is placed in an artery to keep the vessel patent to maintain blood flow. Stent implantation is a major treatment option for CAD (eg, in bypass surgery) and has saved many patients' lives.

But no therapy is without risk. In fact, in clinical practice, persistent inflammation occurs around the stent, and can result in coronary restenosis and thrombosis. Until now, however, there has not been an appropriate experimental in vivo model to analyze the mechanisms of these side effects.

In this issue of the Journal, Sato et al[2] report on the established animal models of coronary stenting. In the past, many researchers used the endothelial injury model of CAD, in which there is transient injury to the endothelium, and then the mechanisms of restenosis of the artery are analyzed. But the stent implantation model has had difficulties because of inflammation around the stent. Sato et al used the stenting model of the rat abdominal aorta and showed that transplantation of adipose tissue-derived stem cells (ASCs) prevents this persistent inflammation.

Stem cells can self-renew and differentiate into multiple types of cells and have varying degrees of differentiation potential:

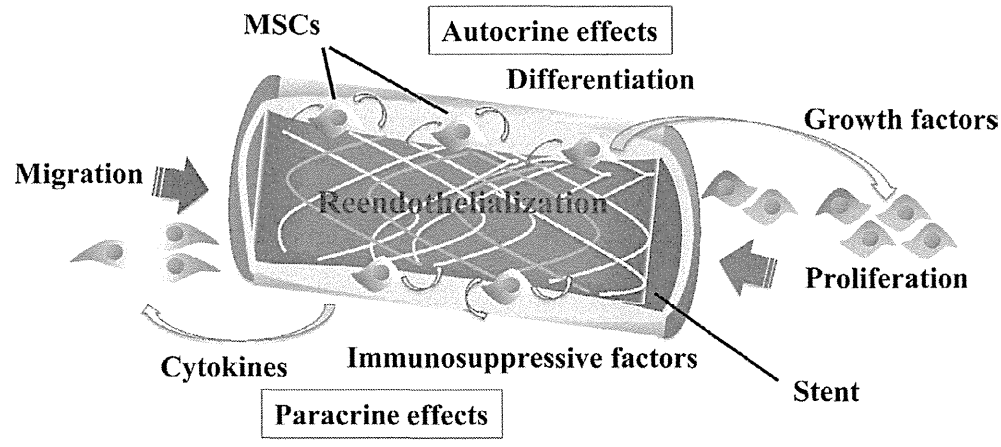

Figure. In stent implantation, administration of mesenchymal stem cells significantly stimulates reendothelialization through paracrine and autocrine effects on cell migration, proliferation, differentiation and cell fusion, leading to tissue/organ regeneration.

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pluripotency, ES cells and iPS cells; multipotency, somatic stem cells; and unipotency, or precursor cells. Mesenchymal stem cells (MSCs) are multipotent cells that can be derived from a variety of fetal and adult tissues such as bone marrow and adipose, and possess an immunosuppressive effect.[3] Therefore, MSCs are being used in clinical studies of a variety of diseases, and can be used in allogeneic settings without immunosuppressive therapy, and as cellular immunosuppressants that have the potential to control steroid-refractory acute graft vs. host disease.[4] In addition, the results of Sato et al suggest that the immunosuppressive effect of MSCs enables construction of a model to analyze the in vivo risk of stent therapy. The authors also show that transplantation of ASCs stimulates reendothelialization and inhibits neointimal formation after stent implantation in the animal model (**Figure**). They have previously reported that ASCs stimulate reendothelialization and inhibit neointimal formation in a wire injury model.[5] In the current experiment, they reproduced the effect of reendothelialization by ASC stimulation in the stent model and used 2 types of stents: a Driver coronary stent (bare metal) and a Cipher stent (sirolimus-eluting). Treatment with the Driver implant resulted in more effective reendothelialization by ASC stimulation than with the Cipher stent.

MSCs produce and secrete a broad variety of cytokines, chemokines, and growth factors, which influence the microenvironment through paracrine and autocrine effects on cell migration, proliferation, differentiation and cell fusion (**Figure**).[6,7] In fact, these factors are potentially involved in cardiac repair. The implantation of a cell-sheet over the damaged area of a failing heart has been shown to improve cardiac function through a paracrine effect.[8] Cell-sheets have recently been developed as a tissue engineering technology, and then put to practical use in several clinical studies. Paracrine effects of the cell-sheets by myoblasts, MSCs and cardiac progenitor cells improve cardiac function.[9] Major components of the regenerative mechanism in cell-sheet implantation would be both angiogenesis and the recovery of diastolic function in the heart. Most likely, MSC-released factors lead to tissue/organ remodeling, repair and regeneration in vivo. With stent implantation, MSC administration significantly stimulates reendothelialization and inhibits neointimal formation through paracrine factors.

Sato et al caution that the effect of reendothelialization might depend on the material type of the stent. In the future, factors affecting coronary restenosis after stent deployment in the coronary artery need to be identified. Peripheral technologies have indeed been developing along with the progress of regenerative medicine research, and development of stent design and efficiency and the safety of stent therapy should be expected in the form of combination products with cell-based products.

Cardiovascular disease is a growing problem in our aging society. It will be more important than ever to use stents as treatment for CAD. There are several animal disease models for hypertension, obesity and diabetes, and researchers will use these animals to study the mechanisms of heart disease and the development of stent therapy. The report by Sato et al therefore contributes to the growing number of clinical and preclinical studies of effective stent therapies for heart disease.

Disclosures

None declared.

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