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Abstract
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INS-1 Cells Undergoing Caspase-Dependent Apoptosis Enhance the Regenerative Capacity of Neighboring Cells

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OBJECTIVE—In diabetes, β-cell mass is not static but in a constant process of cell death and renewal. Inactivating mutations in transcription factor 1 (tcf-1)/hepatocyte nuclear factor 1α (hnf1α) result in decreased β-cell mass and HNF1A-maturity onset diabetes of the young (HNF1A-MODY). Here, we investigated the effect of a dominant-negative HNF1A mutant (DN-HNF1A) induced apoptosis on the regenerative capacity of INS-1 cells.

RESEARCH DESIGN AND METHODS—DN-HNF1A was expressed in INS-1 cells using a reverse tetracycline-dependent transactivator system. Gene(s)/protein(s) involved in β-cell regeneration were investigated by real-time quantitative RT-PCR, Western blotting, and immunohistochemistry. Pancreatic stone protein/regenerating protein (PSP/reg) serum levels in human subjects were detected by enzyme-linked immunosorbent assay.

RESULTS—We detected a prominent induction of PSP/reg at the gene and protein level during DN-HNF1A–induced apoptosis. Elevated PSP/reg levels were also detected in islets of transgenic HNF1A-MODY mice and in the serum of HNF1A-MODY patients. The induction of PSP/reg was glucose dependent and mediated by caspase activation during apoptosis. Interestingly, the supernatant from DN-HNF1A–expressing cells, but not DN-HNF1A–expressing cells treated with zVAD.fmk, was sufficient to induce PSP/reg gene expression and increase cell proliferation in naïve, untreated INS-1 cells. Further experiments demonstrated that annexin-V–positive microparticles originating from apoptosing INS-1 cells mediated the induction of PSP/reg. Treatment with recombinant PSP/reg reversed the phenotype of DN-HNF1A–induced cells by stimulating cell proliferation and increasing insulin gene expression.

CONCLUSIONS—Our results suggest that apoptosing INS-1 cells shed microparticles that may stimulate PSP/reg induction in neighboring cells, a mechanism that may facilitate the recovery of β-cell mass in HNF1A-MODY. Diabetes 59:2799–2808, 2010

Maturity-onset diabetes of the young (MODY) is a familial form of non–insulin-dependent diabetes characterized by early onset of disease, autosomal dominant inheritance, and insulin secretory defects (1). MODY type 3 results from mutations in the gene encoding transcription factor-1/hepatocyte nuclear factor 1α (tcf-1/hnf1α) (2), recently denoted as HNF1A-MODY. HNF1A belongs to a network of transcription factors controlling organ-specific gene expression during embryonic development and in adult tissues (3). Previous studies demonstrated that HNF1A is expressed in adult β-cells and regulates genes involved in glucose and lipid homeostasis as well as β-cell–specific genes (4–10).

Transgenic mice carrying a deletion of the hnf1α gene have defective glucose-stimulated insulin secretion without insulin resistance in target tissues (3), similar to individuals with HNF1A-MODY (11). Findings from animal and cellular models of HNF1A-MODY suggest a decline in functional β-cell mass as the primary mechanism of this defect (8,12,13). Previous studies from our and other laboratories have shown that gene knockout of HNF1A or expression of dominant-negative mutants of HNF1A (DN-HNF1A) inhibits AKT signaling, decreases cell proliferation, and renders cells susceptible to apoptosis (8,14,15). Evidence is growing that β-cell mass is not static but in a constant process of cell death and renewal (16). However, it remains unclear in these models how increased β-cell apoptosis is linked to a compensatory stimulation of regenerative processes.

The Regenerating (Reg) gene family belongs to the calcium-dependent lectin gene superfamily (17). Pancreatic stone protein (PSP) was identified from extracts of calcified pancreatic concrumps (18). Regenerating protein (reg) was independently identified in a screen of a regenerating islet-derived cDNA library taken from 90% depancreatized rats (19) and was found to be identical to PSP (20). Subsequent studies highlighted the potential role for the Reg gene family in pancreatic regeneration (21). We investigated the expression of the PSP/reg gene during DN-HNF1A–induced apoptosis and found PSP/reg to be prominently upregulated. We also provide biochemical evidence that apoptotic cells shed microparticles that may stimulate PSP/reg induction in neighboring cells, thereby linking β-cell apoptosis with β-cell regeneration.

RESEARCH DESIGN AND METHODS

INS-1 cells overexpressing HNF1A in an inducible system. Rat insulinoma INS-1 cells overexpressing wild-type HNF1A (WT-HNF1A) or DN-HNF1A under control of a doxycycline-dependent transcriptional activator have been described previously (4,8). Cells were cultured in RPMI-1640 at 6 mmol/l glucose supplemented with 10% FBS (PAA, Co¨ lbe, Germany), 2 mmol/l

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l-glutamine, 1 mmol/l pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mmol/l HEPES (pH 7.4), and 50 μmol/l 2-mercaptoethanol (Sigma, Dublin, Ireland). For experiments investigating glucose dependence of PSp/reg induction, expression of DN-HNF1A was induced for 24 h and continued for a further 2 h in medium supplemented with glucose at 3, 6, 12, and 18 mmol/l. For caspase inhibition, cells were preincubated with 100 μmol/l NEM (Nikon, broad-spectrum caspase inhibitor zVAD.fmk (Bachem, St. Helen's, U.K.) for 45 min prior to induction.

**Real-time quantitative RT-PCR.** CDNA synthesis was performed using 1.5 μg total RNA as the template and Superscript II reverse transcriptase (Invitrogen, Paisley, U.K.) primed with 50 pmol random hexamers (New England Biolabs, Ipswich, MA). Real-time PCR was performed using the LightCycler 2.0 (Roche Diagnostics, Indianapolis, IN) and the Quantifast SYBR Green PCR kit (Qiagen). Specific PCR primers were designed using Primer3 software (sequences available on request). For absolute quantification of Hnf1a copy number, a gene-specific PCR amplon of known concentration was prepared as a standard. Melting curve analysis and gel electrophoresis was utilized to verify specificity of all PCR products. The data were analyzed using LightCycler software, version 4.0, with all samples normalized to β-actin.

**Western blotting.** For Western blotting analysis, 25 μg protein lysates were obtained from INS-1 cells overexpressing WT-HNF1A and DN-HNF1A as described (9). The rabbit polyclonal anti-PSp/reg antibody (22) was diluted 1:20,000 in Tris-buffered saline containing 1% bovine serum albumin. The primary mouse monoclonal anti-β-actin antibody was utilized at a 1:1,000 dilution (Sigma, Germany). FITC was excited at 488 nm with an argon laser, using a 488/543-nm multichroic beam splitter, and the emission was collected at a 490- to 550-nm spectral band. Rhodamine was excited at 543 nm with a helium/neon laser, using the same beam splitter and the emission was collected at a 450-700 nm spectral band. DAPI fluorescence was detected in the 355 nm and a 0.1-logarithmic gain. The gate for microparticles was defined as events with a 0.1-μm diameter and expressing externalized phosphatidylserine. Phosphatidylserine was labeled using FITC-labeled annexin V (Roche Diagnostics, Meylan, France) containing the signal sequence of the yeast Hnf1a copy number, and a 0.1-logarithmic gain. The gate for microparticles was defined as events with a 0.1-μm diameter and expressing externalized phosphatidylserine. Phosphatidylserine was labeled using FITC-conjugated annexin V (Roche Diagnostics, Meylan, France) in the presence or absence (negative control) of CaCl2 (5 mmol/l) as reported earlier (25).

**Determination of caspase-3–like protease activity.** For Western blotting experiments using recombinant human PSp/reg protein as a control. Slides were incubated for 1 h at room temperature in rhodamine-labeled anti-rabbit secondary antibody (1:100; Jackson Immunoresearch, Suffolk, U.K.). Slides were incubated again overnight at 4°C and a 405-nm dichroic beam splitter. DAPI fluorescence was detected in the 490- to 550-nm spectral band. Rhodamine was excited at 543 nm with a helium/neon laser, using the same beam splitter, and the emission was collected at a 450- to 600-nm spectral band. DAPI was excited with the 405 nm DPSS laser and a 405-nm dichroic beam splitter. DAPI fluorescence was detected in the 420- to 480-nm spectral band. Images were processed using Zeiss LSM 4.2 software (Carl Zeiss). Rhodamine-positive cells in the periphery and interior of the islet were quantified as a percentage of total islet cells using Image J software.

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>HNF1A-MODY</th>
<th>HNF1A-MODY negative</th>
<th>Type I diabetes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>female:male</td>
<td>10:6</td>
<td>4:3</td>
<td>6:4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.8 ± 20</td>
<td>27.9 ± 11</td>
<td>31.8 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 4</td>
<td>26.6 ± 7.9</td>
<td>25.5 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>7.6 ± 0.3*</td>
<td>5.4 ± 3.5</td>
<td>7.8 ± 0.3*</td>
<td>P &lt; 0.05*</td>
</tr>
<tr>
<td>PSp (ng/ml)</td>
<td>18.8 ± 2.7*</td>
<td>8.5 ± 3.8</td>
<td>17.3 ± 1.7*</td>
<td>P &lt; 0.05*</td>
</tr>
</tbody>
</table>

Data are means ± SE. Patients were age, sex, and BMI matched. *P < 0.05 vs. HNF1A-MODY-negative group.
PSP/reg levels were compared with standard amounts of protein of recombinant human PSP/reg.

**Statistical analysis.** Results were expressed as means ± SE. Statistical analysis was conducted using the SPSS version 15.0 software package for Windows (SPSS). Differences between treatments were analyzed by Student t test, as well as one-way ANOVA and a subsequent Tukey tests. Nonparametric data were analyzed by Mann-Whitney U test. Differences were considered to be significant at P < 0.05.

**RESULTS**

The inducible expression of DN-HNF1A leads to a potent induction of PSP/reg mRNA and protein. Inducible, dominant-negative suppression of HNF1A function in INS-1 cells reduces the expression of HNF1A target genes involved in glucose and lipid homeostasis (4) and induces a caspase-dependent apoptosis (8). To investigate the relationship between DN-HNF1A expression, activation of apoptosis, and induction of the regenerative PSP/reg gene in particular, INS-1 cells stably transfected with respective rtTA systems were induced to express either DN-HNF1A or WT-HNF1A. We observed a time-dependent induction of DN-HNF1A or WT-HNF1A mRNA in the respective INS-1 cells (Fig. 1A). This correlated with a significant reduction in mRNA levels of pdk1, a known HNF1A target gene (7), in response to DN-HNF1A expression but not WT-HNF1A (Fig. 1B). Examination of the expression of the Reg family of genes in INS-1 cells demonstrated that DN-HNF1A led to a potent induction of the PSP/reg gene at 24 h (~15-fold) and 48 h (~30-fold). Induction of WT-HNF1A for up to 48 h did not significantly regulate PSP/reg expression (Fig. 1C).

We next investigated PSP/reg protein levels after induction of DN-HNF1A. Cleavage of PSP/reg converts the 16-kDa proteins into a 14-kDa insoluble fibrillar protein (28). Western blot analysis demonstrated a significant upregulation of the PSP/reg protein (16 kDa) following DN-HNF1A induction and a time-dependent increase in the 14 kDa form of PSP/reg (Fig. 1D).

**High extracellular glucose levels potentiates PSP/reg mRNA induction.** We next determined whether the induction of PSP/reg mRNA was glucose dependent. Interestingly, we found that the DN-HNF1A induction of PSP/reg mRNA expression was modulated by varying extracellular glucose concentrations for 2 h following DN-HNF1A induction. The increase in PSP/reg mRNA expression was potentiated in a concentration-dependent manner, with the highest induction evident at 18 mmol/l (Fig. 2A).

**Elevated PSP/reg levels in an HNF1A-MODY animal model and in the serum of HNF1A-MODY patients.** We analyzed paraffin-embedded pancreatic sections from 5-month-old diabetic (Fig. 2B) mice expressing DN-HNF1A in β-cells and compared them with wild-type C57BL/6JBonTac mice. Sections were costained for PSP/reg and insulin. PSP/reg was expressed at low levels in wild-type islets (30 ± 1% PSP/reg-positive cells per islet; n = 8 islets from n = 2 animals) and was found almost exclusively in peripheral islet cells. In contrast, DN-HNF1A islets showed significantly elevated PSP/reg-positive islet cells (82 ± 2% PSP/reg-positive cells per islet; n = 8 islets from n = 2 animals). Moreover, PSP/reg-positive cells were found to be widespread throughout the transgenic islets and were also detectable in insulin-negative islet cells. Strong expression was also observed in the surrounding acinar cells (Fig. 2B). Interestingly, high magnification analysis suggested that many islet cells with elevated PSP/reg immunoactivity were positioned in the vicinity of cells displaying apoptotic nuclear morphology (Fig. 2C).

We next sought to determine whether increased PSP/Reg1a levels could also be detected in HNF1A-MODY diabetic subjects. We analyzed serum PSP/Reg1a levels in a group of 16 diabetic subjects with HNF1A-MODY using ELISA. Results were compared with serum levels in seven HNF1A-MODY–negative, nondiabetic family members. PSP/Reg1a levels were significantly elevated in HNF1A-MODY subjects (Fig. 2D). Interestingly, we also detected elevated PSP/Reg1a serum levels in the serum of patients with type 1 diabetes (n = 10 patients) (Fig. 2D).

**Induction of PSP/reg mRNA and protein by DN-HNF1A function involves the activation of caspases.** Previously we demonstrated that DN-HNF1A–induced apoptosis of INS-1 cells required caspases and involved the activation of the mitochondrial apoptosis pathway (8). To assess whether the activation of executioner caspases influenced DN-HNF1A–induced PSP/reg mRNA induction, we first determined the time course of DEVDase activation (indicative of executioner caspase 3/7 activity) by monitoring the cleavage of a fluorogenic caspase substrate. In agreement with our earlier report (8), there was a significant caspase activity in the time frame of 24–48 h post induction. Expression of WT-HNF1A or doxycycline treatment of parental INS-1 cells did not activate caspases (Fig. 3A). Furthermore, DN-HNF1A induction mediated nuclear morphological changes indicative of apoptosis with condensation and fragmented nuclei. Pretreatment with the pancaspase inhibitor zVAD.fmk inhibited the occurrence of this apoptotic morphology confirming the caspase dependence (Fig. 3B).

Given the similar kinetics of PSP/reg mRNA induction and caspase activation, we explored the possibility that caspase activation regulated PSP/reg expression. Indeed, DN-HNF1A induction of PSP/reg expression was completely inhibited by pretreatment with zVAD.fmk (Fig. 3C and D). Next we addressed whether DN-HNF1A induction of PSP/reg mRNA could be sufficiently explained by its ability to activate apoptosis. Indeed, induction of apoptosis in INS-1 cells with the topoisomerase inhibitor etoposide, was sufficient to increase PSP/reg mRNA levels in a caspase-dependent manner (Fig. 3E).

**Conditioned medium from DN-HNF1A–induced INS1 cells results in a prominent increase in PSP/reg mRNA expression in naïve INS1 cells.** Executioner caspases such as caspase 3 inactivate transcription and translation processes (29), suggesting that apoptosing cells were unlikely to be the source of increased PSP/reg mRNA during DN-HNF1A–induced apoptosis. We investigated whether this increased expression was due to apoptosing cells sending a paracrine signal to neighboring cells. After 48 h of DN-HNF1A induction in the presence and absence of zVAD.fmk, the conditioned medium was added directly to naïve INS-1 cells resulting in a 16-fold increase in PSP/reg mRNA levels in the naïve INS1-cells (Fig. 4A). This induction was absent in cultures incubated with the conditioned medium of zVAD.fmk pretreated DN-HNF1A–induced cells (Fig. 4A) or heat-inactivated conditioned medium (Fig. 4B), supporting the hypothesis that apoptotic cells secrete heat-sensitive factor(s) that stimulate(s) PSP/reg gene induction in neighboring cells.
Removal of microparticles inhibits \( \text{PSP/reg} \) gene induction. Microparticles are small (100 nm) membrane vesicles originating from blebbing membranes of apoptotic cells and can be identified by annexin V staining. These microparticles may contain nuclear proteins as well as nucleic acids (30,31). We examined if microparticles were present in our conditioned medium and, if so, whether these could modulate \( \text{PSP/reg} \) expression. After 48 h of DN-HNF1A induction in the presence and absence of zVAD.fmk, we enriched for microparticles in the superna-

**FIG. 1.** The inducible expression of DN-HNF1A leads to a potent induction of \( \text{PSP/reg} \) mRNA and protein. A: The time course of the \( \text{hnf1a} \) gene induction in DN-HNF1A and WT-HNF1A induced INS-1 cells, represented by absolute quantitative PCR (qPCR). Data are presented as cDNA copy number per microliter. Data are represented as means ± SE from \( n = 3 \) cultures. The experiment was repeated three times with similar results. \( *P < 0.05 \) indicates the difference from noninduced controls. B: Quantification of the pyruvate dehydrogenase kinase (Pdk1) gene expression. DN-HNF1A and WT-HNF1A INS-1 cells were induced with doxycycline from 0 to 48 h. Pdk1 mRNA expression was examined using real-time qPCR relative to \( \beta\)-actin. Data shown are the means ± SEM of \( n = 3 \) cultures. The experiment was repeated four times with similar results. \( *P < 0.05 \), difference from noninduced controls. C: Quantification of \( \text{PSP/reg} \) gene expression following DN-HNF1A and WT-HNF1A induction in INS-1 cells. INS-1 cells were treated with 500 ng/ml doxycycline from 0 to 48 h. mRNA expression of \( \text{PSP/reg} \) was examined using real-time qPCR relative to \( \beta\)-actin. Expression levels were normalized to control cells and data represent means ± SEM from \( n = 3 \) cultures. \( *P < 0.05 \), difference from noninduced controls. Experiments were repeated six times with similar results (D) whole cell lysates (25 µg) were analyzed by Western blotting on 15% SDS-PAGE. Membranes were probed with a polyclonal antibody recognizing \( \text{PSP/reg} \). The 14-kDa fragment represents a \( \text{PSP/reg} \) cleavage product. \( \beta\)-Actin served as a loading control. Approximate molecular weights are provided on the right side of the figure. Similar results were obtained in two separate experiments.
High extracellular glucose potentiates PSP/reg mRNA induction in DN-HNF1A expressing INS-1 cells. A: INS-1 cells were induced to express DN-HNF1A for 24 h. Cultures were continued for 2 h at either 3, 6, 12, and 18 mM glucose concentrations. After this period, mRNA expression of PSP/reg was analyzed by real-time qPCR. Expression levels were normalized to noninduced control cells and data are represented as means ± SEM from n = 3 cultures. *P < 0.05, difference from noninduced controls; #P < 0.05, difference from induced cultures at 3 mM glucose. Experiment was repeated three times with similar results. B: PSP/reg immunoreactivity in islet cells in an in vivo mouse model of DN-HNF1A-induced apoptosis. Paraffin-embedded pancreatic sections on slides from 5-month-old wild-type C57BL/6J BomTac control mice and RIP-DN-HNF1A transgenic mice were double stained using antibodies against PSP/reg and insulin. Primary antibodies were recognized by secondary antibodies coupled to Rhodamine (red) for PSP/reg and to FITC (green) for insulin, respectively. Cell nuclei were recognized by secondary antibodies coupled to Rhodamine (red) for insulin. Primary antibodies were stained with DAPI (blue). Bar = 10 μm. C: PSP/reg immunoreactivity in islets of DN-HNF1A mice. Arrows indicate cells exhibiting apoptotic nuclear morphology. Bar = 10 μm. D: PSP/reg protein is detectable in the serum of HNF1A-MODY subjects and type 1 diabetic subjects. Serum from HNF1A-MODY patients, their MODY-negative family members, and type 1 diabetic patients were analyzed by a specific ELISA detecting human PSP/reg. The square indicates the normal range (R.G., historic data). Individual values, the average as well as the SD are plotted. PSP/reg levels in HNF1A-MODY and type 1 diabetic subjects were significantly different from MODY-negative, control family members (*P < 0.05). (A high-quality digital representation of this figure is available in the online issue.)

**DISCUSSION**

The present study provides evidence that β-cell apoptosis may stimulate β-cell regeneration. We demonstrate 1) that dominant-negative suppression of HNF1A leads to apoptosis and a potent induction of PSP/reg; 2) this induction was potentiated by increased extracellular glucose concentrations; 3) the induction of PSP/reg was inhibited by the broad-spectrum caspase inhibitor zVAD.fmk; 4) conditioned medium from DN-HNF1A–induced cells was sufficient to induce PSP/reg expression in naïve INS-1 cells, suggesting that apoptosing INS-1 cells secrete factors that stimulate PSP/reg induction in neighboring cells; 5) removal of...
FIG. 3. Activation of caspases during DN-HNF1A–induced apoptosis triggers PSP/reg induction. A: Time course of caspase-3–like protease activity in whole-cell extracts. INS-1 cells were induced to overexpress WT-HNF1A or DN-HNF1A for 0, 16, 24, and 48 h. As a control, parental INS-1 cells were treated with doxycycline for 0, 16, 24, and 48 h. Caspase protease activity was measured by cleavage of the fluorogenic substrate Ac-DEVD-AMC (10 μmol/l). Activities are represented as increase in AMC fluorescence (in AU) per 1 h per microgram of protein. Data represent means ± SE from n = 6 cultures. Experiments were repeated two times with similar results. *P < 0.05, difference from noninduced controls. B: Treatment with the broad spectrum caspase inhibitor zVAD.fmK (100 μmol/l) inhibits apoptosis after induction of DN-HNF1A. Cultures were simultaneously pretreated with doxycycline and zVAD.fmK or vehicle (DMSO; zVAD.fmK) for 48 h. Apoptotic cell morphology was assessed by DAPI staining of nuclear chromatin. C: zVAD.fmK inhibits PSP/reg mRNA induction. The mRNA expression of PSP/reg was examined using real-time qPCR. Expression levels were normalized to noninduced plus noninduced zVAD.fmK–treated control cells. Data are represented as means ± SE from n = 3 cultures. *P < 0.05, difference from noninduced controls. #P < 0.05, difference compared with doxycycline alone–treated cultures. The experiment was repeated three times with similar results. D: PSP/reg protein expression was detected by Western blotting. A duplicate experiment showed similar results. Membrane was stripped and reprobed with anti–β-actin as a loading control. E: PSP/reg gene induction in parental INS-1 cells treated with etoposide is caspase dependent. INS-1 cells were simultaneously treated with etoposide (20 μmol/l) or vehicle (0.1% DMSO) for 24 h in the presence and absence of zVAD.fmK (100 μmol/l). Following treatment, mRNA expression of PSP/reg was examined using real-time qPCR. Expression levels were normalized to control cells. Data are represented as means ± SE from n = 3 separate cultures. *P < 0.05, difference compared with etoposide alone–treated cultures. Data shown are the mean of n = 3 separate experiments.

microparticles from this conditioned medium inhibited PSP/reg induction, suggesting that microparticles released from blebbing membranes mediated this effect; 6) treatment of INS-1 cells with rPSP/reg protein reversed the DN-HNF1A–induced decrease in cell proliferation and insulin mRNA; and 7) moreover, PSP/reg serum levels were significantly elevated in HNF1A-MODY patients and type 1 diabetes compared with HNF1A-MODY–negative, nondiabetic family members.

**Regulation of β-cell mass in HNF1A-MODY.** Evidence is growing that functional β-cell mass is decreased in animal models of MODY and in type 2 diabetes, with an increased apoptosis rate largely contributing to this decrease (12,13,33). However, a decrease in β-cell mass does not necessarily implicate that new β-cells are not generated during disease progression. Indeed, β-cell mass is a result of a homeostatic balance between the generation of new β-cells and the rate of their apoptosis (34). As HNF1A-MODY and type 2 diabetes evolve over several years or decades, the destruction or apoptosis rate of β-cells may eventually exceed the rate of β-cell generation during disease progression.

Although β-cell regeneration may occur during disease progression, it is not clear whether this happens through self-replication of mature β-cells, through neogenesis of progenitor cells residing in the pancreas or recruited from...
Conditioned culture medium was heat inactivated at 95°C for 15 min.

Results.

Noninduced controls; concentration levels were normalized to noninduced controls. Data are represented as means ± SE from n = 3 cultures. *P < 0.05, difference from noninduced controls. Experiments were repeated four times with similar results. C: The conditioned medium from cells treated as described in A above was centrifuged and filtered through 0.20 μm filters and added to naïve INS-1 cells for 24 h. Unfiltered conditioned medium served as a control. PSP/reg gene expression was analyzed by real-time qPCR. Data are represented as means ± SE from n = 3 cultures. *P < 0.05, difference from noninduced controls. Experiments were repeated three times with similar results. D: INS-1 cells were induced with doxycycline in the presence and absence of zVAD.fmk. The conditioned culture medium was added to naïve cells for a further 24 h. Supernatants were collected and detached cells were precipitated at (300g), followed by additional 800g centrifugation. Microparticles were quantified as described in Research Design and Methods.
detected increased expression of PSP/reg in insulin-positive islet cells in 5-month-old transgenic HNF1A-MODY mice, confirming previous findings that PSP/reg is expressed in islets during injury and regeneration processes (17,19). Moreover, PSP/reg was also found in insulin-negative islet cells (Fig. 2B). In this context, a recent study has suggested α-cells to be the major source of β-cell regeneration/conversion in mice in vivo (41). We also detected increased PSP/reg levels in the serum of HNF1A-MODY patients. We can only speculate whether the source of PSP/reg in the serum of HNF1A-MODY patients was the endocrine or exocrine pancreas (i.e., islet or acinar cells). We can likewise not exclude that these levels came from other organs potentially affected in HNF1A-MODY, such as the liver, where PSP/reg is also expressed (42). However, we also detected elevated levels of PSP/reg in subjects with type 1 diabetes, arguing for a role for pancreatic injury and β-cell apoptosis as a source for elevated PSP/reg serum levels.

**Microparticles as signal transducers or enablers.** In our study, we detected that apoptotic INS-1 cells released annexin V–positive microparticles into the medium in a caspase-dependent manner. Microparticles are being increasingly described as efficient vehicles for the release of signaling molecules. Microparticles enable communication between different cell types (43) and also deliver proteins to cells that do not normally express them (44). Microparticles deriving from apoptotic cells have been shown to carry nuclear proteins as well as nucleic acids (31,45). Filtration experiments suggested that the absence of microparticles abolished the effect of the conditioned medium to induce *PSP/reg* mRNA in naïve INS-1 cells. This suggested that cytokines, peptide hormones, or other soluble messengers present in the conditioned medium did not mediate this effect. Interestingly, heat inactivation of the conditioned medium was sufficient to inhibit *PSP/reg* mRNA expression in naïve INS-1 cells. It is therefore tempting to speculate that microparticles may contain signaling molecules or nuclear proteins which, upon uptake by neighboring cells, enhanced *PSP/reg* gene expression in these cells.

In conclusion, our study demonstrates that the execution of caspase-dependent apoptosis in INS-1 cells stimulates the induction of regenerative genes in neighboring cells, suggesting a role for MPs and *PSP/reg* in this process. These findings provide new mechanistic insights into a fundamental aspect of β-cell mass regulation.

*FIG. 5. Treatment of INS-1 cells with recombinant PSP/reg protein reverses the phenotype of HNF1A-MODY cells. A: INS-1 cells were induced to express DN-HNF1A for 24 h before being cultured for a further 24 h in the presence and absence of 10 ng/ml of rPSP/reg for 24 h. Proliferation was assessed by BrdU incorporation into the cells. Data are a means ± SE from *n* = 6 cultures. Experiments were repeated three times with similar results. *P* < 0.05, difference from noninduced controls. #P < 0.05, difference compared with doxycycline alone–treated cultures. B and C: DN-HNF1A INS-1 cells were treated as described in A above. Insulin (B) and *p27*kip1 (C) gene expression was analyzed by real-time qPCR. Data are represented as means ± SE from *n* = 3 cultures. *P* < 0.05, difference from noninduced controls. #P < 0.05, difference compared with doxycycline alone–treated cultures. Experiments were repeated three times with similar results. D: The supernatant of apoptosing INS-1 cells induced by DN-HNF1A overexpression is sufficient to stimulate cell proliferation in naïve INS-1 cells. Cells were induced with 500 ng/ml of doxycycline in the presence and absence of zVAD.fmk (100 μmol/l) in 0.05% serum at 6 mmol/l glucose for 48 h. The conditioned culture medium was added to the noninduced, naïve cells for a further 24 h. After treatment, cell proliferation was assessed by BrdU incorporation. Data are a means ± SE from *n* = 6 cultures. Experiments were repeated three times with similar results. *P* < 0.05, difference from noninduced controls. #P < 0.05, difference compared with doxycycline alone–treated cultures.*
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