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Differential expression of cell-cycle regulators in human beta-cells derived from insulinoma tissue

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ABSTRACT

Introduction. The low frequency of beta-cell replication in the adult human pancreas limits beta-cell regeneration. A better understanding of the regulation of human beta-cell proliferation is crucial to develop therapeutic strategies aiming to enhance beta-cell mass.

Methods. To identify factors that control beta-cell proliferation, cell-cycle regulation was examined in human insulinomas as a model of increased beta-cell proliferation (n = 11) and healthy pancreatic tissue from patients with benign pancreatic tumors (n = 9). Tissue sections were co-stained for insulin and cell-cycle proteins. Transcript levels of selected cell-cycle factors in beta-cells were determined by qRT-PCR after performing laser-capture microdissection.

Results. The frequency of beta-cell replication was 3.74 ± 0.92% in the insulinomas and 0.11 ± 0.04% in controls ($p = 0.0016$). p21 expression was higher in insulinomas ($p = 0.0058$), and Rb expression was higher by trend ($p = 0.085$), whereas p16 ($p < 0.0001$), Cyclin C ($p < 0.0001$), and p57 ($p = 0.018$) expression levels were lower. The abundance of Cyclin D3 ($p = 0.62$) and p27 ($p = 0.68$) was not different between the groups. The reduced expression of p16 ($p < 0.0001$) and p57 ($p = 0.012$) in insulinomas and the unchanged expression of Cyclin D3 ($p = 0.77$) and p27 ($p = 0.55$) were confirmed using qRT-PCR.

Conclusions. The expression of certain cell-cycle factors in beta-cells derived from insulinomas and healthy adults differs markedly. Targeting such differentially regulated cell-cycle proteins may evolve as a future strategy to enhance beta-cell regeneration.

1. Introduction

Pancreatic beta-cell mass reduction in patients with either type 1 or type 2 diabetes has led to the idea that beta-cell mass regeneration is a potential diabetes treatment [1–6]. However, the frequency of beta-cell replication in adult humans is very low [7]. This is in contrast to prenatal development and childhood, during which time replicating beta-cells can be

Abbreviations: BMI, body mass index; HbA_{1c}, glycated hemoglobin; fwd, forward; rev, reverse; bp, base pairs.

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frequently detected [8,9]. The reasons underlying the decline in beta-cell proliferation with aging are unclear, but most likely involve changes in the expression of cell-cycle regulators [10,11]. Therefore, a deeper understanding of the cell-cycle factors that control human beta-cell replication is needed.

In rodent models, cell-cycle control of beta-cell replication has been studied in great detail. In particular, the generation of specific knockout mice has provided insights into the roles of various cell-cycle proteins. Using such models, pivotal roles for Cyclin D2 [12], p27 [13,14], and p53 [15] in the regulation of cell-cycle entry and progression have been proposed. Furthermore, age-dependent p16 accumulation has been associated with the loss of proliferative capacity in beta-cells [16]. In isolated human islets, beta-cell proliferation has been induced by over-expression of Cyclin D3, Cyclin C and cdk6 [17,18]. Moreover, induction of beta-cell replication by suppression of $p57^{KIP2}$ in isolated human islets has been recently described [19].

However, for obvious reasons, similar gene knockout, over-expression, or knockdown experiments cannot be performed in humans in vivo. Therefore, our current knowledge pertaining to cell-cycle regulation in beta-cells is largely based on rodent models or in vitro data. The mechanisms controlling beta-cell proliferation in human tissue have been examined in few studies [10,20].

One approach to identify factors that may be involved in the molecular control of cell-cycle progression in humans is to study the expression of relevant cell-cycle regulator proteins under conditions of high and low rates of beta-cell replication. Therefore, we previously studied the abundance of such factors in the prenatal human pancreas, as a condition of high proliferative activity, in comparison with the adult human pancreas, in which beta-cell replication is typically very infrequent [10]. These experiments showed that p16 and p27 expression is reduced in the prenatal pancreas, consistent with these factors playing inhibitory roles the in the control of human beta-cell proliferation [10]. In contrast, Cyclin D3 was increased during the prenatal development period.

Insulinomas are an experimental model that can be used to study human beta-cells under conditions of active proliferation. The average frequency of replication in insulinoma far exceeds that of healthy adult beta-cells [21]. Therefore, in the present study, the expression of cell-cycle factors in human insulinoma and normal adult human pancreatic tissues was compared.

2. Study Design and Methods

2.1. Study design

Pancreatic tissue from insulinoma patients and normal tissue from patients undergoing pancreatic surgery for the removal of adenomas (control group) were examined for differences in beta-cell replication as well as in the expression of various factors involved in cell-cycle regulation. Immunohistochemistry and quantitative PCR were performed to determine differential expression patterns in neoplastic and non-neoplastic beta-cells. The study protocol was approved by the ethics committee of the Ruhr-University Bochum (registration number 4505-12).

2.1.1. Pancreatic tissue samples

Pancreatic tissue samples from eleven (five female and six male) insulinoma patients and nine control (seven female and two male) patients with benign pancreatic adenomas were analyzed. Insulinoma patients and controls were not different with respect to age (60.3 ± 5.6 vs. 59.0 ± 5.6 years, respectively; $p = 0.88$) or BMI (27.9 ± 1.6 vs. 24.8 ± 0.88, respectively; $p = 0.12$). There were also no significant differences in either fasting glucose (81.0 ± 13.0 mg/dl vs. 118.6 ± 10.6, respectively; $p = 0.06$) or HbA_{1c} levels (4.8 ± 0.35% vs. 5.6 ± 0.17, respectively, $p = 0.31$). Human pancreatic tissue was obtained from patients during surgeries for the removal of pancreatic tumors between 2004 and 2012. The diagnoses of the insulinomas were made based on clinical symptoms (i.e., repeated fasting hypoglycemia) and prolonged fasting tests. The clinical diagnoses were confirmed by a trained pathologist in all cases. Pancreatic tissue was fixed in formalin and embedded in paraffin. Sections (5 μm) were cut from the paraffin blocks and processed for subsequent analyses. Because of limited availability of tissue sections, some analyses could not be performed on all cases. The number of cases available for analysis is given in the results section.

2.2. Immunohistochemical analyses

Pancreatic tissue sections were co-stained for insulin and one of the following cell-cycle proteins: KI67, p57, p16, Cyclin D3, p27, p21, or Cyclin C. All primary and secondary antibodies were diluted in Dako Antibody Diluent with Background Reducing Components (Dako, Golstup, Denmark; #S3022). In brief, immunohistochemistry was performed as follows:

2.2.1. KI67, insulin, and DNA

Sections were incubated with monoclonal mouse anti-human KI67 antibody (diluted 1:100; Dako; #M7240) overnight at 4 °C. KI67 was detected by using Dako REAL™ EnVision Detection System Peroxidase/DAB (Dako; #K5007) according to the manufacturer's protocol. To co-stain for insulin, tissue sections were incubated with guinea pig anti-insulin antibody (diluted 1:400; Dako; #A0564) for 30 min at 37 °C. Insulin was detected using Dako REAL™ Detection System Alkaline Phosphatase/RED (Dako; #K5005) according to the manufacturer's protocol.

2.2.2. p16, insulin, and DNA

Detection of the p16 antigen was performed using the CINtec® Histology Kit (mtm laboratories, Heidelberg, Germany; #9511). Co-staining for insulin and the nuclei was performed as described above.

2.2.3. p57, insulin, and DNA

Sections were incubated with mouse monoclonal anti-p57 (diluted 1:350; Gene Tex, CA, USA; #GTX23223) overnight at 4 °C. p57 was detected using Dako REAL™ EnVision Detection System Peroxidase/DAB according to the manufacturer's protocol. Co-staining for insulin and DNA was performed as described above.

2.2.4. Cyclin D3, insulin, and DNA

Sections were incubated with mouse anti-human Cyclin D3 antibody (diluted 1:600; Abcam, Cambridge, UK; #ab28283) overnight at 4 °C. Cy3-conjugated goat anti-mouse secondary