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Proteins differentially expressed in human beta-cells-enriched pancreatic islet cultures and human insulinomas ☆

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ABSTRACT

In view of the great demand for human beta-cells for physiological and medical studies, we generated cell lines derived from human insulinomas which secrete insulin, C-peptide and express neuroendocrine and islet markers. In this study, we set out to characterize their proteomes, comparing them to those of primary beta-cells using DIGE followed by MS. The results were validated by Western blotting. An average of 1800 spots was detected with less than 1% exhibiting differential abundance. Proteins more abundant in human islets, such as Caldesmon, are involved in the regulation of cell contractility, adhesion dependent signaling, and cytoskeletal organization. In contrast, almost all proteins more abundant in insulinoma cells, such as MAGE2, were first described here and could be related to cell survival and resistance to chemotherapy. Our proteomic data provides, for the first time, a molecular snapshot of the orchestrated changes in expression of proteins involved in key processes which could be correlated with the altered phenotype of human beta-cells. Collectively our observations prompt research towards the establishment of bioengineered human beta-cells providing a new and needed source of cultured human beta-cells for beta-cell research, along with the development of new therapeutic strategies for detection, characterization and treatment of insulinomas.

1. Introduction

The vertiginous increase in the number of diabetic patients in the world has rendered Diabetes mellitus (DM) to be considered as a severe Public Health problem. Type 1 DM (DM1) is a pathology characterized by auto-immune destruction of beta-cells, which causes an absolute deficiency in insulin production, leading to several neurological, renal, ocular and cardiovascular complications. This auto-immune destruction occurs when the beta-cells are infiltrated by macrophages and lymphocytes that secret a variety of cytokines to which the beta-cells are extremely sensitive, resulting in beta-cell apoptosis (Nielsen et al., 1999, 2004).

Because of the absolute deficiency in insulin production, DM1 patients require administration of daily doses of exogenous insulin to maintain normal glucose levels in their bloodstream. In order to adequately correct the blood glucose levels and minimize the complications which appear as a result of the frequent metabolic imbalance, insulin therapy requires severe adherence of the patient to the treatment (DC and CTR 1993). Hypoglycemic episodes

Abbreviations: CALD1, Caldesmon; MAGE-A2, Melanoma Associated Antigen-A2; NR6A1, Nuclear Receptor Subfamily 6 Group A Member 1; OTUD7A, OTU domain-containing protein 7A.

☆ *Significance*: The results obtained in the present study point at the potential value of the proteins uncovered here, since almost all proteins presenting increased levels in insulinoma cells, such as MAGE2, were first described in this study and could be related to cell survival as well as to resistance to chemotherapeutic agents. It is worth mentioning that the data obtained may also contribute to the still scarce knowledge on the molecular biology of human insulinomas. Finally, our study warrants further validation of some of these proteins in larger cohort samples as well as consideration for the development of insulinoma biomarkers and detection.

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and weight gain are also consequences of the continuous exogenous insulin application (Dong and Woo, 2001; Kido et al., 2000).

Therefore, it is crucial to understand the mechanisms involved in beta-cells proliferation and differentiation in order to develop protocols to augment the available number of these cells for transplantation and also, to allow induction of endogenous beta-cells regeneration in recently-diagnosed DM1 patients and in insulin-dependent type 2 DM (DM2) patients, where a significant reduction in beta-cell mass is also observed.

One of the main obstacles in human beta-cell research has been the lack of a proper human pancreatic beta-cell line not only because of difficulties in obtaining them, but also in culturing these cells for long periods of time (Adcock et al., 1975; Gragnoli, 2008). Therefore, rodent insulinoma cell lines have been widely used to study both physiological and pathophysiological mechanisms involved in glucose metabolism and to establish *in vitro* models for the beta-cell damage occurring in type 1 diabetes (Frodin et al., 1995; Gylfe and Hellman, 1986; Hill et al., 1987; Meglasson et al., 1986; Trautmann and Wollheim, 1987; Verspohl et al., 1995). Nevertheless, studies comparing sequences lying upstream of, or flanking the transcription start site of the insulin gene among different species, led to the conclusion that the rodent promoters are markedly different from the human one (Hay and Docherty, 2006). Moreover, nitric oxide formation, shown to be an important mediator of cytokine-induced beta-cell death in rodents, does not affect cytokine-mediated beta-cell death in human islets (Eizirik et al., 1994, 1996). The signal transduction pathways induced by the pro-inflammatory cytokines IL-1β and TNF-α differ (Ortis et al., 2010, 2008), and although both are present during islet inflammation, they seem to appear at different time windows (Eizirik et al., 2009). These important differences in the cytokine-induced response in rodent and human beta-cells urge caution when we directly translate the research results obtained in rodents to the human disease etiology and therapy.

Insulinomas are the most common pancreatic endocrine neoplasms, representing approximately 17% of all neuroendocrine tumors of the digestive tract (Buchanan et al., 1986), constituting rare tumors, with an incidence of four patients per million inhabitants a year (Boden, 1989; Oberg and Eriksson, 2005; Tucker et al., 2006). Most insulinomas originate in individuals with a mean age of 50 years, being usually small (less than 2 cm), solitary and almost always benign (more than 90% of cases) (Boden, 1989; Oberg and Eriksson, 2005; Proye, 1987). Malignant insulinomas are often associated with local invasion of adjacent tissues or with liver and lymph nodes metastasis (Oberg and Eriksson, 2005; Broder and Carter, 1973). The main clinical manifestation of insulinomas is hypoglycemia, due to uncontrolled insulin secretion by the tumor (Oberg and Eriksson, 2005).

For insulinoma treatment, the usual method of choice is tumor ablation (Oberg and Eriksson, 2005; Menegaux et al., 1993); however, a major issue faced by surgeons is the exact localization of the tumor in the pre- and, mainly, intraoperatory, since no imaging technique is sufficiently accurate (Tucker et al., 2006; Machado et al., 1998). Due to the fact that these are rare tumors, knowledge about genetic changes associated with initiation and progression of these tumors is very limited.

Only a few human beta-cell lines have been described, with long-term passage resulting in loss of insulin secretion (Gartner et al., 2006; MacFarlane et al., 1999). Therefore, we have established and characterized human insulin-releasing cell lines, which may not perfectly mimic the primary beta-cell physiology, but still represent valuable tools for the study of the molecular events underlying beta-cell function and dysfunction (Labriola et al., 2009).

In order to further characterize the differences between normal human beta-cells-enriched islets cultures and our developed beta-cell lines, we set out to study the molecular mechanisms involved in beta-cell proliferation and secretory function, by comparing the protein expression profiles of these primary cultures of human pancreatic islets and the human cell lines derived from isolated insulinomas through two-dimensional gel electrophoresis (2D-DIGE) and mass spectrometry. The results obtained in this study are relevant to increase knowledge on the molecular biology of insulinomas, aiming at selection and/or validation of new therapeutic targets and diagnostic biomarkers, since these differentially expressed proteins could be related to the malignancy displayed by these cells.

2. Materials and methods

2.1. Human pancreatic islet isolation and culturing

Human pancreases from adult brain-dead donors (mean age 42 ± 3 years, $n = 14$) were removed in accordance with Brazilian regulations and the local institutional ethics committee. For characterization of human islet donors, see Supplementary Table 1. Pancreatic islets were isolated after ductal distension of the pancreas and digestion of the tissue with Liberase HI (Roche Diagnostics, Indianapolis, IN, USA) or Collagenase (Serva Electrophoresis GmbH, Heidelberg, Germany), utilizing the Ricordi automated method (Ricordi et al., 1988), with modifications, as previously described (Adams and Cory, 1998; Shapiro and Liu, 1995).

Islets were maintained in CMRL 1066 medium (5.6 mM glucose) (Mediatech-Cellgro, Miami, FL, USA) supplemented with 10% fetal calf serum (FCS) (Cultilab, Campinas, SP, Brazil) and 100 units/mL streptomycin/ampicilin.

In all cases, the culture medium was changed every 2–3 days. At confluence or when cell clusters became overcrowded, the cells were detached with 0.025% trypsin, washed, and resuspended in fresh medium. All cell culture experiments were performed at 37 °C in a 5% CO_2 humidified atmosphere. These cultures display at least 85% of beta-cells, according to our previous results (Labriola et al., 2007; Maria-Engler et al., 2004).

2.2. Primary cultures of human insulinoma cells

Ex vivo primary culture of insulinomas (APM and CPR cells) and nesidioblastosis (VGA cells) were obtained from independent donors after surgical resection and tissue processing. Tumor tissue samples were removed upon informed consent from patients enrolled in a protocol of the Bile Ducts and Pancreas Service duly approved by the Institutional Board of the Hospital das Clínicas, FMUSP and then processed. After collagenase digestion of these samples, cells were plated in culture flasks in CMRL 1066 medium (Mediatech-Cellgro) supplemented with ITS (10 mg/L insulin, 5.5 mg/L transferring, 0.0067 mg/L sodium selenite, 2 mg/L ethanolamine, GIBCO Corporation, Carlsbald, CA, USA), 100 units/mL streptomycin/ampicilin, 10% FCS and glucose (final concentration 11 mM) and allowed to attach for 24–48 h. In all cases, the culture medium was replaced every 2–3 days. At confluence or when the cell clusters became overcrowded, the cells were detached with 0.025% trypsin, washed, and resuspended in fresh medium. All cultures were maintained at 37 °C in a humidified atmosphere (Labriola et al., 2009). Data pertinent to the human insulinoma-derived cell line preparations used in this work, including insulin response data, are listed in Table 1.

2.3. Samples preparation

Primary cultures of human islets (passages 1–3), highly enriched in beta-cells, and APM cells (Labriola et al., 2009) were