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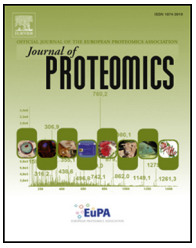

Quantitative proteomics reveals the link between minichromosome maintenance complex and glucose-induced proliferation of rat pancreatic INS-1E β-cells

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ABSTRACT

Proper functioning of pancreatic β-cells is a crucial for glucose homeostasis control, and therefore a main problem regarding type 2 diabetes onset and evolution. The ability of β-cells to proliferate upon certain stimuli, such as elevated glucose concentration, is an essential property to overpass a major problem of the pathology: the decrease of β-cell mass leading to a lack of insulin production. However, high glucose concentrations are also an inducer of β-cell dysfunction, when proliferation become unable to overcome insulin demand. The control of β-cell proliferation could represent an interesting target for the development of therapeutic molecules for type 2 diabetes treatment. To get new insights on β-cell replication, we investigated the modulation of nuclear proteins of INS-1E cells submitted to medium and high glucose concentrations for 24 h. Indeed, the nucleus should contain proteins responsible of proliferation-related events. The SILAC approach allowed us identifying 24 nuclear proteins whose expressions were modified by chronic high glucose. A wide Downstream Effects Analysis assigned the majority of the differentially expressed proteins to functions such as proliferation and cell cycle. Interestingly, our study linked for the first time the increase of expression of the 6 MCM components to glucose-induced stimulation in β-cells.

Biological significance
The current study represents a progress in the understanding of glucose-induced proliferation mechanisms in β-cells. We applied the SILAC strategy to INS-1E cells cultivated with medium or high glucose concentrations for 24 h, and we targeted nuclear proteins which have a central role in proliferation-related mechanisms. It allows

Abbreviations: MCM, mini chromosome maintenance; SILAC, stable isotope labeling with amino acids in cell culture; T2D, type 2 diabetes.

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quantifying 24 nuclear proteins, which are regulated by high glucose exposure. The vast majority of them are shown to be related to proliferation and cell cycle. We describe here for the first time than the 6 proteins of the MCM complex are involved in glucose-mediated proliferation in β-cells.

1. Introduction

Pancreatic β-cells are essential actors of glucose homeostasis. They are responsible for insulin secretion, this hormone inducing a decrease of blood glucose concentration in conditions of hyperglycemia. β-Cells arise from embryonic development, when cells of the primary pancreatic duct differentiate [1]. The pancreatic β-cell mass is dependent on the balance between proliferation/neogenesis and apoptosis. In adults, β-cells exhibit a low replication rate in normal conditions [2]. However, the pancreas possesses the ability to adapt its total β-cell mass to insulin demand, being able to increase their proliferation rate and neogenesis to counteract increased insulin requirements [3,4]. The tight control of β-cells replication is therefore a key element to maintain glucose homeostasis.

The main characteristic of type 2 diabetes (T2D) is chronic hyperglycemia that leads to glucotoxicity associated with β-cell dysfunctions, notably lack of insulin secretion. However, it was speculated that, at the onset of the disease, the pancreas may have the ability to compensate the lack of insulin secretion by increasing its total β-cell mass [5,6]. Nevertheless, a failure in this compensatory mechanism leads to chronic hyperglycemia preceding β-cell apoptosis. This phenomenon was observed in patients who exhibit a significant decrease of their β-cell mass when the disease is progressing [7,8]. Consequently, the identification of molecular mechanisms controlling β-cell proliferation now represents a central concern that could pave the way for the development of new therapeutic approaches aiming at counteracting the dysfunction of compensatory mechanisms occurring in diabetes. It was previously described that glucose influences β-cells proliferation through modulation of molecules being part of the insulin signaling pathway (mainly IGF1/IRS2 and downstream proteins such as PI3K, GSK3, or mTOR [9–11]), but also through the cAMP signaling pathway, or the overexpression of ChREBP and cyclins [12,13]. Additionally, glucose was shown to regulate β-cell proliferation through glycolysis pathways and Krebs cycle [14–16]. Glucose could also indirectly trigger β-cell proliferation through growth factors such as IGF-I [9].

Although it has been clearly described that glucose, as well as insulin or fatty acids, could trigger β-cell proliferation, all the pathways involved are still not unraveled [17–19]. In order to highlight the underlying molecular mechanisms linking glucose and β-cell proliferation, we decided to explore the effects of chronic high glucose exposition on β-cells using MS-based quantitative proteomics. More precisely, we centered this study on the nuclear proteome, the nucleus being a key player in the mechanisms associated to replication/proliferation and apoptosis. The nucleus is the control center of replication processes and contains all the cell cycle machinery (hosting cyclins, cyclin-dependent kinases and cyclin-dependent kinases inhibitors); therefore, it is of great interest to deeply study it [20–22]. For this, we applied the stable isotope labeling with amino acids in cell culture (SILAC) strategy to compare the nuclear proteomes of INS-1E cells submitted or not to short-term chronic high glucose concentration. This allowed to identify 24 proteins whose expression levels in the nucleus were impacted by high glucose exposure. Notably, this study highlighted the modulation of an entire complex of helicase proteins previously described as marker of proliferating cells: the MCM2-7 complex. This work therefore represents a comprehensive approach complementing the knowledge on β-cell molecular response to elevated glucose concentrations.

2. Materials and methods

2.1. Cell culture and stable isotopic amino acid incorporation

Rat insulinoma INS-1E cells were grown in a RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) at 37 °C and under 5% CO_2 and humidified atmosphere. SILAC experiments were conducted as described in Couté et al. [17]. Briefly, normal RPMI-1640 medium (Sigma-Aldrich) depleted in arginine, leucine, and lysine was supplemented with leucine (25 mg/L, Sigma), lysine (25 mg/L, Sigma), and arginine (100 mg/L, Sigma) for the "light" medium, and with $^{13}C_6$-leucine, $^{13}C_6$-$^{15}N_2$-lysine (Cambridge Isotope laboratories), and $^{13}C_6$-$^{15}N_4$-arginine in the same concentrations for "heavy" medium. Complete amino-acid incorporation was achieved after 4 weeks [23]. Glucose stimulation was performed for the last 24 h [23], using light and heavy RPMI media, respectively, supplemented with 2% FBS, and either 11 mM of glucose (D-(+)-Glucose, Sigma) for the medium concentration or 30 mM glucose for the high concentration. Labels were reversed for a second experiment using the same conditions.

2.2. Enrichment of nuclei

The cells were scraped in cold PBS, and the same number of cells from light and heavy conditions were pooled together and centrifuged at 800 rpm for 5 min at 4 °C. The pellet was resuspended into 15 volumes of cold RSB buffer containing 0.01 M Tris–HCl pH 7.4, 0.01 M NaCl, 7 mM MgCl2, anti-proteases (Roche) and anti-phosphatases (Roche) tablets according to manufacturer's instructions and incubated on ice for 30 minutes. NP-40 (0.3% final) was added, and the suspension was homogenized with a 0.4 mm Dounce. The remaining suspension was centrifuged 5 min at 1500 rpm at 4 °C, and the pellet was resuspended in 10 volumes of 0.25 M saccharose. Ten volumes of a 2 M saccharose solution was carefully loaded below the first layer, and the tube was centrifuged at 1800 rpm for 10 min at 4 °C. The pellet containing nuclei was finally resuspended in 0.34 M saccharose and frozen at −80 °C.