Research Article

Upregulation of FOXO3 in New-Onset Type 1 Diabetes Mellitus

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Forkhead box O (FOXO) transcription factors have been implicated in the development and differentiation of the immune cells. FOXO3 plays a crucial role in physiologic and pathologic immune response. FOXO3, cooperatively with FOXO1, control the development and function of Foxp3⁺ regulatory T cells (Treg). Since the lack of Treg-mediated control has fundamental impact on type 1 diabetes mellitus (T1DM) development, we investigated FOXO3 expression in patients with T1DM. FOXO3 expression was estimated in peripheral blood mononuclear cells (PBMCs) from newly diagnosed T1DM pediatric patients (n = 28) and age-matched healthy donors (n = 27) by real-time PCR and TaqMan gene expression assays. Expression analysis revealed significant upregulation of FOXO3 in T1DM (P = 0.0005). Stratification of the T1DM group according to the presence of initial diabetic ketoacidosis (DKA) did not indicate differences in FOXO3 expression in patients with DKA compared to a mild T1DM onset (P > 0.05). In conclusion, overexpression of FOXO3 is correlated with the ongoing islet autoimmune destruction and might suggest a potential role for this gene in the pathogenesis of type 1 diabetes mellitus.

1. Introduction

FOXO3 (forkhead box O3) protein belongs to the family of transcription factors included withal FOXO1, FOXO4, and FOXO6. FOXO3 is regulated via the phosphoinositide 3-kinase (PI3K)/serine/threonine-specific kinase (Akt) signaling pathway [1]. The active, nonphosphorylated FOXO3 form is localized in the nucleus and regulates gene transcription. Phosphorylation of FOXO3 in the PI3K/Akt pathway results in its exclusion from the nucleus and termination of transcriptional activity [2]. FOXO3 has been implicated in the regulation of diverse biological processes, including cell survival, proliferation, and apoptosis [3]. FOXO3 is expressed in immune cells, and recently, there has been a surge in interest to investigate the importance of FOXO3 in lymphoid homeostasis [4–6]. Upregulation of FOXO3 was observed in polymorphonuclear cells and peripheral blood mononuclear cells from patients with rheumatoid arthritis [7]. Overexpression of FOXO3 is mediated by T cell receptor stimulation [8]. In consequence, FOXO3 promotes polarization of CD4⁺ T cells towards the pathogenic T helper cells producing interferon γ and granulocyte monocyte colony-stimulating factor. FOXO3⁻/- mice exhibit reduced susceptibility to experimental autoimmune encephalomyelitis [8].

In this study, we investigate the expression level of FOXO3 in PBMCs from newly diagnosed type 1 diabetes mellitus pediatric patients. Upregulation of FOXO3 was observed in the T1DM group compared to the age-matched healthy controls—a finding that might suggest a potential role of this gene in autoimmunity.

2. Study Groups

The qRT-PCR of FOXO3 gene was conducted in 28 newly diagnosed T1DM subjects (mean age ± SD 11.2 ± 3.3 years, 4 females (14%), 24 males (86%)) and 27 age-matched
expression levels were calculated using the 2−ΔΔCt formula. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Statistical significance of the differences between relative expression levels was determined with an unpaired t-test. P values < 0.05 were considered statistically significant.

### 4. Results and Discussion

Type 1 diabetes mellitus is an autoimmune disorder that results from the lack of endogenous insulin secretion from the pancreatic beta cells. Although T-mediated destruction of beta cells is observed, the precise etiology and pathological mechanisms are still poorly understood. Genetic predisposition and environmental factors contribute to the development of type 1 diabetes mellitus [9]. HLA locus, specifically the haplotypes DRB1∗03-DQA1∗05-DQB1∗02 (DR3-DQ2) and DRB1∗04-DQA1∗03-DQB1∗03:02 (DR4-DQ8) are major genetic risk factors [10]. To date, around 60 non-HLA T1DM susceptibility loci have been identified, mostly related to immune response, for instance, genes encoding lymphocyte protein tyrosine phosphatase (PTPN22), cyto-toxic T-lymphocyte protein 4 (CTLA4), subunit alpha of the interleukin-2 receptor (IL2RA), and interferon-induced helicase C domain-containing protein 1 (IFIH1) [11–13].

Our previous study indicated FOXO3 as a potential target for miR-487a-3p, which is upregulated in T1DM [14]. These results prompted us to investigate the FOXO3 expression in type 1 diabetes mellitus patients. In order to reduce the interference of the initial metabolic status, PBMCs were collected from patients with normalized ketonaemia and glycemia and fully rehydrated. In addition, T1DM patients and control subjects included in the study did not present infection symptoms, confirmed by negative inflammatory tests (complete blood count, C-reactive protein tests). The type 1 diabetes mellitus group was further stratified according to the presence or absence of diabetic ketoacidosis (DKA) at initial presentation, which reflects severe and moderate disease onsets, respectively. Expression analysis revealed significant upregulation of FOXO3 in the new-onset T1DM group compared to the age-matched healthy controls (Figure 1).

#### Table 1: Clinical characteristics of type 1 diabetes patients.

| Clinical features | T1D | Severe T1D onset | Mild T1D onset |
|-------------------|-----|-----------------|---------------|
| Gender, F/M       | 4/24|                 |               |
| Age (y)           | 11.21 ± 3.33 | 11.33 ± 3.63 | 11.63 ± 3.18 |
| BMI (kg/m²)       | 17.33 ± 3.05 | 17.31 ± 3.18 | 17.62 ± 3.09 |
| HbA1c (%)         | 10.66 ± 1.67 | 10.59 ± 1.15 | 11.16 ± 1.86 |
| C peptide (%)     | 0.30 ± 0.08  | 0.27 ± 0.9    | 0.32 ± 0.08  |
| 25-OH-D³ (ng/ml)  | 18.19 ± 7.68 | 13.98 ± 3.27** | 21.73 ± 8.76 |
| DKA²              | 12 (43)       | 12 (100)       | 16 (0)       |
| IAA³              | 7 (25)        | 3 (25)         | 4 (25)       |
| GADA⁴             | 21 (75)       | 9 (75)         | 12 (75)      |
| IA2A⁵             | 23 (82)       | 11 (92)        | 12 (75)      |

BMI: body mass index; HbA1c: glycated haemoglobin A1c; 25-OH-D: 25-hydroxyvitamin D; DKA: diabetic ketoacidosis; IAA: antibodies to insulin; GADA: antibodies to glutamic acid decarboxylase; IA2A: antibodies to islet antigen-2; clinical features presented as mean ± standard deviation; ² number of subjects (%); ** P < 0.01, P values estimated by unpaired t-test, severe T1D onset vs. mild T1D onset subgroup.
However, we did not observe statistically significant differences in FOXO3 expression in patients with DKA compared to healthy controls (**P = 0.0005, mean fold change 1.54). (b) Expression level of FOXO3 gene in the T1D group stratified according to the presence of initial diabetic ketoacidosis (DKA). The patients with diabetic ketoacidosis did not present statistically significant differences in FOXO3 expression compared to the patients without DKA (P > 0.05). Horizontal lines indicate median with range; asterisks indicate significance, with P values estimated by unpaired t-test; T1D: type 1 diabetes patients; C: controls; DKA(+): cohort of patients with initial diabetic ketoacidosis; DKA(-): cohort of patients without initial diabetic ketoacidosis.

Yang et al. have demonstrated the mechanisms protecting FOXO3 from being targeted by certain miRNAs [16]. The Foxo3 pseudogene (Foxo3P) and the Foxo3 circular RNA (circ-Foxo3) act as a sponge and bind several miRNAs, including miR-22, miR-136, miR-138, miR-433, miR-762, miR-3614-5p, and miR-3622b-5p. Subsequently, Foxo3P and circ-Foxo3 ensure FOXO3 gene expression and protein activity.

5. Conclusion

Overexpression of FOXO3 in type 1 diabetes mellitus might suggest a potential role of this gene in the development of autoimmune disease. Further in vitro and ex vivo functional studies will address the issue of FOXO3 contribution to immune tolerance dysregulation.

Data Availability

Data presented in the manuscript are available upon request from corresponding author Magdalena Zurawek, magdalena.zurawek@igcz.poznan.pl.

Ethical Approval

The study was approved by the local Ethics Committee at the Poznan University of Medical Sciences (decision No. 656/15), and all procedures were in accordance with the Declaration of Helsinki.

Consent

Informed consent was obtained from the parents/legal representatives of the minor patients.

Conflicts of Interest

The authors declare that they have no conflict of interests.

Authors’ Contributions

MZ designed the study, performed experiments, analyzed data, and wrote the manuscript, MF and PF collected T1DM patients’ samples and clinical data, MC collected control subjects’ samples, and NR revised the manuscript.

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