Abstract

Introduction: Graves' disease (GD) and toxic multinodular goiter (TMNG) are two common disorders which are known to have different etiologies and pathogenesis. We searched for the molecular pathways that may underline the differences between these two devastating conditions.

Methods: Gel Electrophoresis (DIGE) was performed with the pools of protein extracts. Thyroid tissue samples for DIGE multispectral grid and Graves disease patients (12 of each) were used for protein extraction. After 2D separation, gels were imaged to reveal differentially expressed proteins which were identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF/TOF) and MASCOIT search. The identified proteins were classified on the basis of their functions in metabolic pathways by using PANTHER. In addition, Ingenuity Pathways Analysis (IPA) was performed to reveal the interconnections between relevant metabolic and canonical pathways.

Results: A total of 330±20 proteins spots were revealed on the gels. Fifteen regulated spots were identified and classified based on their molecular function and biological process that they are involved. Conclusion: Although our findings are preliminary, they hold importance by providing the first comprehensive comparative proteomics data for GD and toxic MNG.

Objectives

This study aimed at comparing the protein profiles of the thyroid tissues of patients with GD and toxic MNG in order to reveal the differentially expressed proteins which may have importance in understanding the pathogenesis and clinical features of these two diseases.

Methods

Thyroid tissue samples were obtained during surgery from 12 patients with GD and 12 patients with toxic MNG. For the DIGE experiment, the protein extracts were separately prepared in DIGE lysis buffer and equal amounts of proteins were pooled for each group to label with Cy3, Cy2 and Cy5 according to the instructions provided by the supplier (Life Tech, USA) as shown in Figure 1. The labeled proteins were subjected to 2D gel electrophoresis by using immobilized pH gradient strips (IPG) (pH 5-8, 17cm) and all 1D SDS-PAGE gel.

Results

Among 330±20 protein spots determined, 23 displayed difference in their abundancies between GD and TMNG groups. Protein spots excised from the gels were digested with tripsin and subjected to MALDI-TOF/TOF analysis. Fifteen spots were selected for PANTHER and IPA analysis, because some proteins appeared in more than one spot (Table 1). IPA predicted two main metabolic pathway networks with the scores of 29 and 16 (Figure 4). These networks were associated with Cell Death/Survival, Free Radical Scavenging, Cellular Movement, Cellular Growth and Proliferation. Data revealed close relationship with Endocrine and Gastrointestinal System Disorders (Table 2).

Conclusions

Majority of the differentially expressed proteins can be connected to malign diseases reported previously in the literature (1-2). However, as proteomics are being used to explore other diseases like GD, hidden connections are being revealed. Our results implicate that thyroiditis triggers the mechanisms concerning the cell proliferation and various protein synthesis in GD which let us to consider GD as a benign disease. The results of IPA analysis revealed that some of the proteins we identified were correlated to DM Type 1, DM Type 2, insulin resistance, metabolic syndrome and MODY. However these protein-disease associations can only be considered preliminary since the number of patients used in this study were limited. Future studies can be performed with larger patient groups to verify our findings and draw stronger conclusions.