PROTEOMIC ANALYSIS OF THYROID TISSUE IN GRAVES' DISEASE AND TOXIC MULTINODULAR GOITER

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Abstract

Introduction
Graves' disease (GD) and toxic multinodular goiter (MNG) are two common disorders which are known to have different etiologies and pathogenesis. We searhed for the molecular pathways that may underline the differences between these two devastating conditions.
Methods / Design
Difference Gel Electrophoresis (DIGE) was performed with the pools of protein extracts. Thyroid tissue samples from toxic multinodular guatr patiens and Graves' disease patients (12 of each) were used for protein extraction. After 2D separation, gels were imaged to reveal differantially expressed proteins which were identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF/TOF) and MASCOT 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 conanical 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 comparing the protein profiles of the thyroid tissues of patients with GD and toxic MNG in order to reveal the differantially 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 Cy2, Cy3 and Cy5 according to the instructions provided by the supplier (Life Tech, USA) as shown in Figure 1. The labelled proteins were subjected to 2D gel electrophorersis by using immobilized pH gradient strips (IPG) (pH 5-8, 17cm) and a12% SDS-PAGE gel.

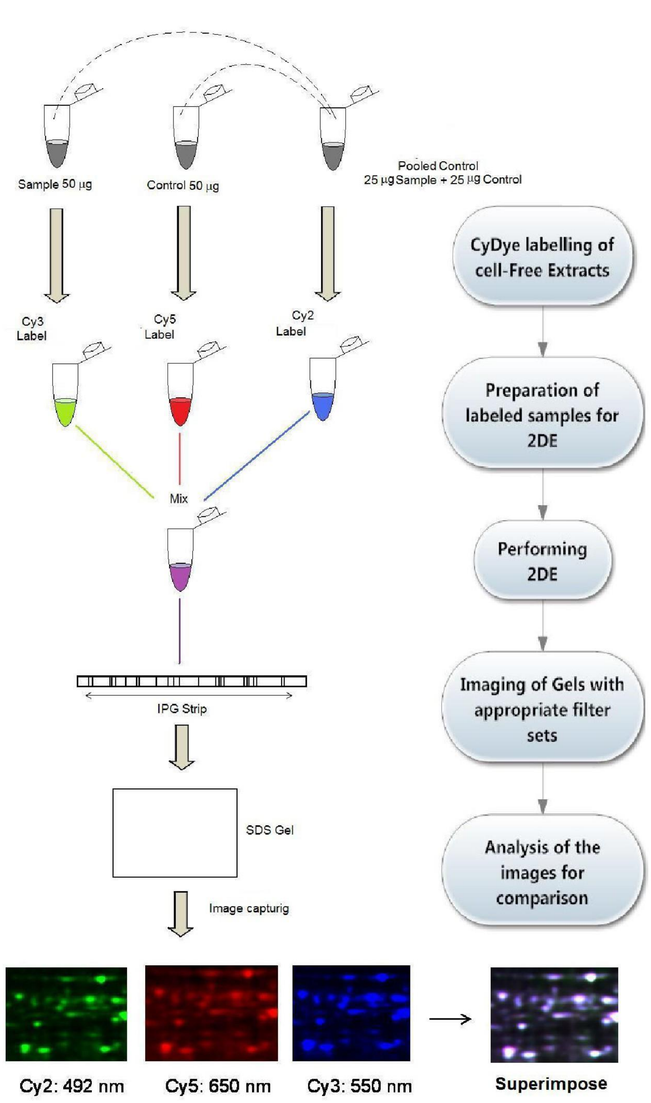


Figure 1. Outline of DIGE experiment

Gels were imaged with VersaDoc4000 MP (BioRad, USA) by using Quantity One software (BioRad, USA) (Figure 2). PDQuest Advance 2D-analysis software (BioRad, USA) was used for comparison. (Figure3). Regulated protein spots were cut from a preperative gel by using EXQuest Spot Cutter (BioRad, US) (Figure 3). After in gel tryptic digestion, peptides were desalted and concentrated by using ZipTip. MS/MS analysis were performed using MALDI TOF/TOF (AbSciex 5800) instrument. PANTHER and IPA were used to classify and idetify relevant metabolic functions and pathways, respectively.

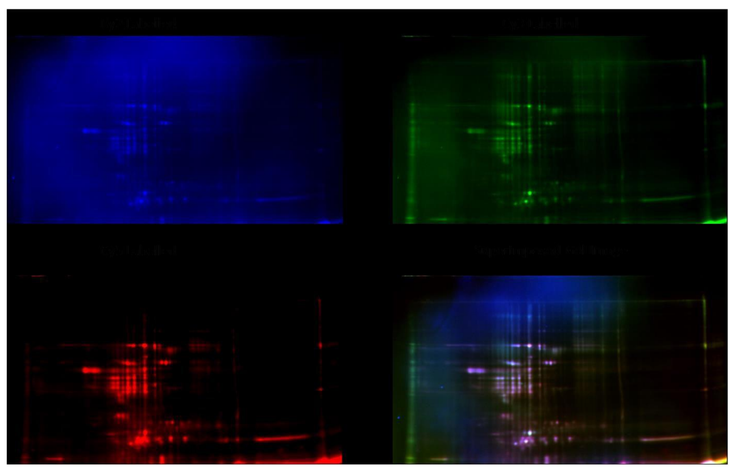


Figure 2. DIGE images for comparison of regulated spots.

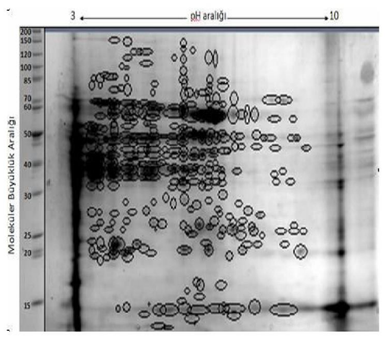


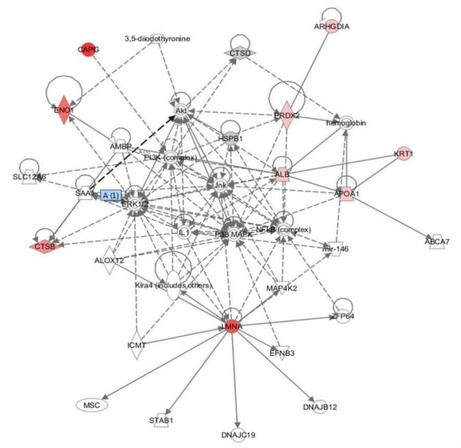
Figure 3. Regulated spots detected by PDQuest analysis was cut from this preperative gel.

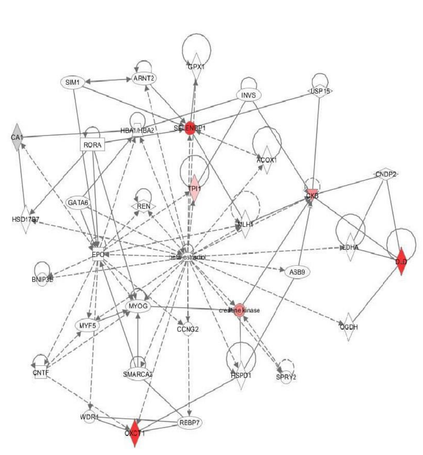
Results

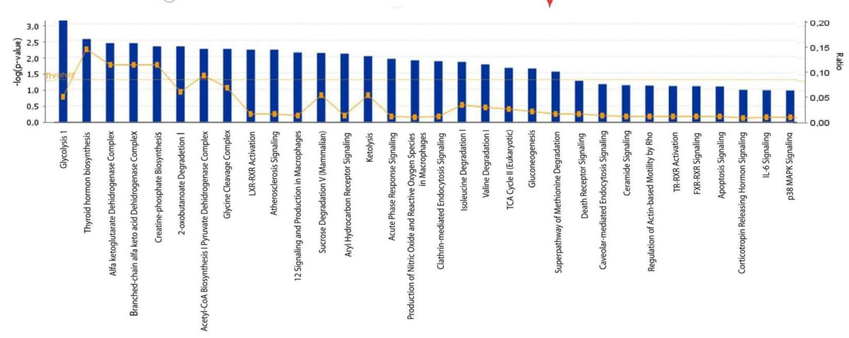
Among 330±20 protein spots determined, 23 displayed difference in their abundancies between GD and TMNG groups. Protein spots excised from the gels were digested with tripsin and subjected to MALDI-TOF/TOF analysis. Fifteen spots were selected for PATNHER and IPA analysis, because some proteins appeared in more than one spot (Table1).
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).

System	Disorder	p-value	Protein	Number of proteins
Endocrin/GİS	Pankreatit s	1.86x10^{9}	ALB, APOA1, CAPG, CTSB, CTSD, PRDX2	6
Endocrin	Dilate KMP+hypergonadism	9,91x10^{4}	LMNA	1
Endocrin/GİS	Type 1DM	1.44x10^{3}	ALB, APOA1, CA1, CTSB, HSPB1	5
Endocrin	Insulin resistance	1.54x10^{3}	ALB, APOA1, CA1, LMNA	4
Endocrin/GİS	MODY	2,97x10^{3}	APOA1	1
Endocrin/GİS	Type 2 DM	3,93x10^{3}	ALB, APOA1, CA1	3
Endocrin	Pancreatic tumor	6,00x10^{3}	CTSB, CTSD, ENO1	3
Endocrin	Metabolic syndrom	7,02x10^{3}	APOA1, CA1	2
Endocrin	Testis hipoplasia	1,87x10^{2}	ARHGDIA	1
GİS	Digestive system tumors	8,07x10^{4}	ALB, ARHGDIA, CAPG, CTSB, CTSD, ENO1, SELENBP1, TPI1	8
GİS	Mandibuloacral disostosis	9,91x10^{4}	LMNA	1
GİS	Liver amiloidosis	2,97x10^{3}	APOA1	1
GİS	Islet cell tumors	2,97x10^{3}	CTSB	1
GİS	Hepatocelluler Carcinoma	1,09x10^{3}	ALB, CTSB, TPI1	3
GİS	Colorectal Carcinoma	1,24x10^{3}	ALB, ARHGDIA, CAPG, CTSB, SELENBP1	5

Table2. Proteins related to Endocrine and Gastrointestinal System Disorders .







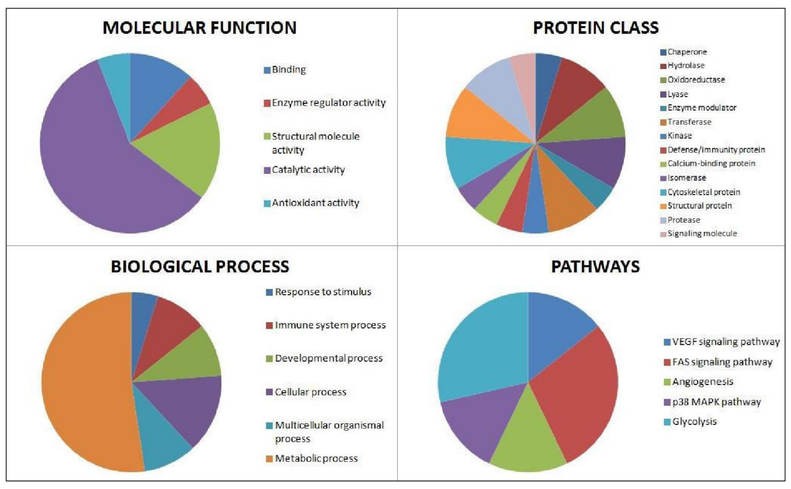


Figure 4. Main networks revealed by IPA and PANTHER analysis.

Conclusions

Majority of the differentially expressed proteins can be connected to malign diseases reported previously in the literature (1-2-3). However, as proteomics are being used to explore other diseases like GD, hidden connections are being revealed. Our results implicate that thyrotoxicosis 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.

Swiss Prot No	Protein	Celluler location	MA (kDA)	Gen symbol	Function
Q13228	Selenium-binding protein 1	N/C/M	52	SELENBP1	Protein transport
P02545	Prelamin-A/C	N	74,139	LMNA	The arrengement of chromatin , nuclear membrane and telomer
P40121	Macrophage-capping protein	C/N	38,499	CAPG	Makrophage function
P12277	Creatine kinase B-type	C	42,644	CKB	Generating energy for skeletal muscle, heart, brain and ve spermatozoa
P55809	Succinyl-CoA:3-ketoacid coenzyme A transferase	M	56,158	OXCT1	Ceton body catabolism
P09622	Dihydrolipoyl dehydrogenase, mitochondrial	M	54,177	DLD	Branched chain amino acids catabolism and capasitation of spermatozoa
P06733	Alpha-enolase	C/M	47,169	ENO1	Glycolysis, plaminogen activation, transcription; growth control, tolerans to hypoxia, immunoglobulin production
P52565	Rho GDP-dissociation inhibitor 1	C	C23,207	ARHGDIA	Proliferation, apoptosis, gen expression
P32119	Peroxiredoxin-2	C	21,892	PRDX2	Elimination of peroxides; signaling cascades of the growth factors and TNF α
P07858	Cathepsin B	C (Lis)	37,822	CTSB	Protein turnover, tumor invasion ve metastasis
P02647	Apolipoprotein A-I	C	30,778	APOA1	Cholesterol transport from tissues to the liver
P07339	Cathepsin D	Lis	44,552	CTSD	Protein turnover (especially in breast cancer and Alzheimer's disease pathogenesis)
P04792	Heat shock protein beta-1	C/N	22,783	HSPB1	Stress resistance and actin integrity
P00915	Carbonic anhydrase 1	C	28,87	CA1	Hydration of carbon dioxide
P60174	Triosephosphate isomerase	C	30,791	TPI1	Gluconeogenesis and glycolysis

Table1. MALDI TOF/TOF (MS/MS) analysis of the regulated spots

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*This study was performed at Kocaeli University DEKART Proteomics L aborotory.