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Proteomic identification of predictive biomarkers for malignant transformation in complete hydatidiform moles

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ABSTRACT

Introduction: Protein expression in cells are associated with oncogenesis. This study aims to explore proteomic profiles and discover potential biomarkers that can predict malignant transformation of hydatidiform mole.
Methods: Retrospective analysis was done in 14 cases of remission hydatidiform mole and 14 cases of hydatidiform mole who later developed malignancy (GTN group). Molar tissues were retrieved from −70 °C frozen tissue. Subsequently, a large-scale proteomic analysis was performed to identify proteins and compare their abundance levels in the preserved molar tissues from these two groups using a dimethyl-labeling technique coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: A total of 2,153 proteins were identified from all samples. 22 and 10 proteins were significantly up-regulated and down-regulated, respectively, in the GTN group compared with the mole group. These altered proteins were found in several biological groups such as cell-cell adhesion, secreted proteins, and ribonucleoproteins. Several hormone-related proteins were among the most up-regulated proteins in the GTN group including choriogonadotropin subunit beta (β-hCG) and alpha (α-hCG), growth/differentiation factor 15, as well as both pregnancy-specific beta-1-glycoproteins 2 and 3. In contrast, protein S100-A11 and L-lactate dehydrogenase A chain, were down-regulated in molar tissue from most patients in the GTN group.
Discussion: This study identified a set of differentially expressed proteins in molar tissues that could potentially be further examined as predictive biomarkers for the malignant transformation of CHMs. A molar proteome database was constructed and can be accessible online at http://sysbio.chula.ac.th/Database/GTD_DB/Supplementary_Data.xlsx.

1. Introduction

Molar pregnancy is a kind of gestational trophoblastic disease (GTD) presenting with hydatidiform mole (HM). Although it occurs with a low incidence, a significant number of these patients (16–20%), especially those with the complete form of the disease (a complete mole), eventually develop malignant transformation, a so-called gestational trophoblastic neoplasia (GTN) [1]. Many studies have tried to identify the etiology of malignant transformation so that early detection and diagnosis can become feasible [1,2]. Risk factors that contribute to the development of GTN have been suggested, including pre-evacuation uterine size larger than expected for gestational age, serum human chorionic gonadotropin (hCG) levels more than 100,000 mIU/ml, theca lutein cysts larger than 6 cm in diameter, and maternal age over 40 years [2]. However, precise decision making for post-evacuation management of hydatidiform mole patients is still challenging. Even though research studies have mentioned prophylaxis chemotherapy for patients with high-risk HMs, no sufficient evidence has been provided showing effective results [3]. Therefore, the identification of biomarkers that allow an accurate prediction of malignant transformation in patients with complete hydatidiform moles is required.

Currently, proteomic and bioinformatics technologies have been increasingly used. It is now known that altered protein expression profiles in functional cells are associated with a number of human

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diseases, especially cancer. Prior to the current decade, a combination of two-dimensional electrophoresis (2DE) and mass spectrometry (MS) was the conventional approach to proteomics. 2DE can separate proteins in tissues on the basis of differences in isoelectric point and molecular weight and MS can be performed to identify proteins of interest with the aid of genome sequence databases. Differential protein expression between normal and pathological tissue by using proteomic techniques can determine changes in relative amounts of the same protein and might detect a potential new biomarker [4,5]. There are few proteomic studies that have analyzed placental tissue [6–10]. The first use of proteomic analysis in placental trophoblast was in the comparison of the differential proteomic expression of cytotrophoblast and fibroblast cells [6]. More recent studies examined proteomic analysis in preeclampsia and eclampsia [7,9]. Ma, L et al. [8] reported 32 differentially expressed protein spots in malignant transformed moles using 2DE and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF–MS). Shi, Z. H et al. [10] compared protein profiles of benign moles with those of malignant-transformed HM using 2DE and MALDI-TOF–MS. They suggested that chloride intracellular channel protein 1 (CLIC1) could be a marker for malignant transformation; however, this data was limited due to a small sample size. Since there are few studies with proteomic analysis in gestational trophoblastic disease, further study of proteomic alterations in response to malignant-transformed moles is required.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has become increasingly accepted for protein identification and quantification due to its high-throughput nature [7,11]. However, investigation of proteins from molar tissues in patients with HM and GTN using an LC-MS/MS proteomic approach has not been reported to date. In this study, placental trophoblastic cells from molar tissue were collected for qualitative and quantitative analysis by LC–MS/MS. The primary objective of the current study is to explore prognostic biomarkers in order to predict HM that may undergo GTN. Using this approach, several potential biomarkers are indeed highlighted in this work.

2. Methods

2.1. Materials

We retrospectively reviewed medical records of women with hydatidiform mole (HM) initially diagnosed between January 2006 and June 2014 at King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand. The inclusion criteria of the study were HM patients who underwent either suction curettage or hysterectomy at our institution. Their tissues were preserved for both pathological (formalin fixation) and proteome characterization; tissues were washed with normal saline and immediately frozen at −70 °C. All patients were then divided into two groups; HM that remained benign (mole group) and HM that later progressed to GTN (GTN group). The mole group was defined by spontaneous regression of serum hCG level after evaculation and diagnosis of GTN diagnosis was done by FIGO criteria [12]. Patients who had incomplete medical records, insufficient tissues, or unavailable paraffin blocks were excluded. This study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Demographic and clinical data were collected including age, parity, gestational age, presenting signs and symptoms, initial diagnosis, history of abortion or molar pregnancy, previous medical history, pre-evacuation human choriogonadotropin (hCG) level, duration until hCG levels were undetectable, as well as stage and management after diagnosis of GTN. All tissue samples from paraffin blocks were obtained to confirm a diagnosis of complete hydatidiform mole (CHM) using p57 immunohistochemistry. After the review, samples in the mole group were paired with samples in the GTN group from the same year of tissue collection in order to match tissue quality after preservation. A protein analysis flowchart is shown in Fig. 1.

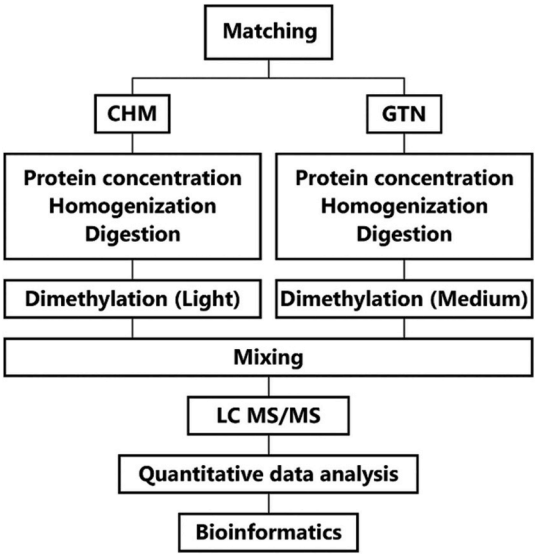

Fig. 1. Protein analysis flow chart.

2.2. Protein extraction and preparation

Frozen tissues were thawed and then washed with normal saline. For all cases, a part of thawed tissue was fixed in formalin and then reviewed by an independent gynecologic pathologist blinded from patient outcomes to confirm viability of the samples. The other part of the tissues was homogenized with a sonicator in lysis buffer containing 8 M urea. Prior to trypsin digestion, protein concentration was determined by micro BCA protein assay. Proteins were reduced with 10 mM DTT at 37 °C for 1 h and alkylated with 40 mM iodoacetamide (IAA) in the dark at room temperature for another 1 h. Excess unreacted IAA was quenched with 40 mM DTT in the dark at room temperature for 15 min. Lysate was digested overnight at 37 °C with trypsin. Finally, eluates were desalted with C18 reverse-phase column and then the samples were dried *in vacuo* prior to LC-MS/MS analysis.

2.3. LC-MS/MS protein identification

LC-MS/MS quantification was performed using a Q Exactive Plus mass spectrometer (Thermo Scientific, San Jose, CA). The matched tissues from the mole group and the GTN group were quantitatively compared using stable isotropic dimethyl labeling with light labeling (formaldehyde and cyanoborohydride) in the mole group and heavy labeling (deuterated and ^{13}C-labeled formaldehyde and cyanoborodeuteride) in the GTN group. The two samples were then mixed and analyzed by LC-MS/MS. The MS methods included a full MS scan at a resolution of 70,000 followed by 10 data-dependent MS2 scans at a resolution of 17,500. The normalized collision energy of HCD fragmentation was set at 32%. An MS scan range of 350–1400 m/z was selected and precursor ions with unassigned charge states, a charge state of +1, or a charge state of greater than +8 were excluded. A dynamic exclusion of 30 s was used. Using Proteome Discoverer™ Software 2.1 (Thermo), the MS raw data files were searched against a composite database containing the forward and reversed peptide sequences of the Human Swiss-Prot Database. The search parameters were set for the following fixed modifications: carbamidomethylation of cysteine (+57.02146 Da), as well as light and heavy dimethylation of N-termini and lysine (+28.031300 and + 36.075670 Da). For variable modification, oxidation of methionine (+15.99491 Da) was set. A maximum of four modifications and two missed cleavages per peptide were allowed. Parent and fragment monoisotopic mass errors were set at 10 ppm. A target–decoy approach was used to limit the false discovery rate of the identified peptides to less than 1%.

Bioinformatics was performed with the DAVID Functional Annotation Bioinformatics Microarray Analysis tool (http://david.abcc.niifcrf.gov/) [13] using the "functional annotation" feature to cluster protein functions and determine which functional clusters were over-represented in differentially altered proteins. Enrichment scores were reported as Benjamini-corrected p-values (modified Fisher's exact test).

Statistical analysis was performed with SPSS version 18 (SPSS Inc., Chicago). P value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic data and patient outcomes

Molar tissue samples were taken of twenty-eight HM patients. These patients were divided into 2 groups: those with HM that subsequently remained benign (mole group) and those with HM that later progressed to GTN (GTN group). Median age was 34 years (range 20–57 years) and most patients (57.1%) were multiparous. Median gestational age (GA) was 14 weeks (6–24 weeks) and abnormal uterine bleeding was the most common presenting symptom (60.0%). Initial baseline hCG levels were higher in the GTN group; median hCG level was 181,293 mIU/ml (40,478–1,235,126 mIU/ml) in the mole group and 525,020 mIU/ml (48,807–1,635,716 mIU/ml) in the GTN group, respectively ($p = 0.033$) (see Table1). 93.3% of patients in the GTN group had hCG level over 100,000 mIU/ml while only about half of the mole group reached similarly high levels of hCG. After subsequent tests of serum hCG level, hCG returned to normal in 14 patients (mole group) with a 90-day median remission time. In the other group (GTN), hCG levels reversed the initial downward trend at week 4 and the median time to diagnose GTN was 49 days (Fig. 2). Out of all patients in the GTN group, 58.3% were in stage I. Among them, all but one patient received chemotherapy for treatment and 7 patients (50%) needed second line treatment.

3.2. Proteomic data analysis

The data obtained with LC-MS/MS analysis was used to calculate protein abundance ratios for the GTN group compared with the mole group (GTN/mole) in each pair, expressed in log2 ratios. This database currently contains 2,153 proteins in placenta trophoblastic cells. Heterogeneity of placental tissue was responsible for inclusion of a variety of blood cells; we thus excluded plasma proteins [14]; a total of 1,951 proteins were subsequently identified. Seven hundred twenty proteins were present in at least 3 pairs of samples (Fig. 3 and Supplementary Table 1); 32 showed statistically significant differences (above the horizontal line in Fig. 3), including 22 up-regulated (shown in red on the right) and 10 down-regulated in the GTN group compared to the molar group (shown in green on the left).

All significantly different proteins between the GTN group and the mole group are shown in Table 2. Interestingly, significant up-regulated proteins included hormone-related proteins: choriogonadotropin subunit beta (β-hCG) (average log2-ratio of 3.34), choriogonadotropin subunit alpha chain (α-hCG) (2.00), growth/differentiation factor 15 (GDF15) (3.16), and pregnancy-specific beta-1-glycoproteins 3 and 2 (PSG3 and PSG2) (4.57 and 1.06, respectively). β-hCG levels were increased in 9 out of 13 applicable GTN/mole pairs. In the remaining pair, β-hCG was not found in either tissue. Of those 13 pairs, 11 pairs had higher pre-evacuation serum β-hCG levels in the GTN group than in the mole group. No correlation between β-hCG in cells and in serum was observed (Fig. 4). α-hCG were up-regulated in 5 out of 7 applicable pairs ($p = 0.008$). GDF15 increased in 8 out of 10 applicable pairs ($p = 0.016$). PSG3 and PSG2 had 5 out of 5 applicable pairs and 6 out of 7 applicable pairs, respectively, showing increases in the GTN group ($p = 0.038$ and 0.044). A number of non-hormone proteins were also significantly up-regulated in the GTN group. For example, 40S ribosomal proteins S3a, S8, and S21 also increased with average log2 ratios of 1.04 (8 out of 10 applicable pairs), 3.81 (7 out of 9 applicable pairs), and 3.77 (6 out of 8 applicable pairs), respectively. Though prolargin had the highest increase (an average log2 ratio of 7.70) in the GTN group, it was found only in 3 pairs. Among down-regulated proteins with significant decreases in the GTN group, 12 out of 14 applicable pairs (average log2 ratio of -0.91, $p = 0.01$) and 6 out of 8 applicable pairs (average log2 ratio of -1.01, $p = 0.05$) of protein S100-A11 and L-lactate dehydrogenase A chain were observed, respectively.

For the evaluation of relative protein abundance, the number of peptides detectable by LC-MS/MS (spectral counts) were normalized by the number of amino acid residues in the protein of interest. The top 30 most abundant proteins in mole tissue are shown in Fig. 5. Not surprisingly, housekeeping proteins such as actin and histone were observed at the highest abundance levels. Outside housekeeping proteins, β-hCG was among the most abundant proteins in the molar proteome. Other such proteins include decorin and lumican, both of which are extracellular matrix proteins.

Enrichment analysis was conducted with the online DAVID bioinformatic analysis tool [13]. We compared the differentially expressed protein list against all proteins identified in this study via LC-MS/MS. Functional clustering of proteins revealed that altered proteins in molar tissue were enriched in several biological groups such as cell-cell adhesion, secreted proteins, and ribonucleoproteins (see Table 3).

4. Discussion

Biomarkers that can predict which molar patients are likely to develop GTN have not been validated and implemented in a clinical setting [15]. Proteomic analysis can be used to identify prognostic biomarkers to distinguish malignant from benign disease. In this study, we compared the proteomic profile of benign mole versus GTN using a liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Together, these data offer new information about proteins expressed in molar tissues.

Table 1
Patients' demographic data and clinical characteristics between mole group and GTN group. (n = 28).

Clinical characteristics	Total (n = 28)	Mole group (n = 14)	GTN group (n = 14)
Age (years), mean (range, SD)	30.8 (13–53, 11.1)	30.5 (15–53, 8.9)	31.1 (13–50, 13.2)
Gestational age (weeks), median (range)	14 (6–24)	17 (11–24)	12 (6–21)
Parity, n (%)			
- Nulliparous	12 (42.9)	4	8
- Multiparous	16 (57.1)	10	6
Presenting symptoms, n (%)			
Vaginal bleeding	17 (60.7)	5	12
Enlarged uterus	1 (3.6)	0	1
Hyperemesis	4 (14.3)	4	0
Asymptomatic	6 (21.4)	5	1
Initial hCG (mIU/ml), median (range)	240,639 (40,478–1,635,716)	181,293 (40,478–1,235,126)	525,020 (48,807–1,635,716)
Cases with initial hCG > 100,000 mIU/ml	20	7	13

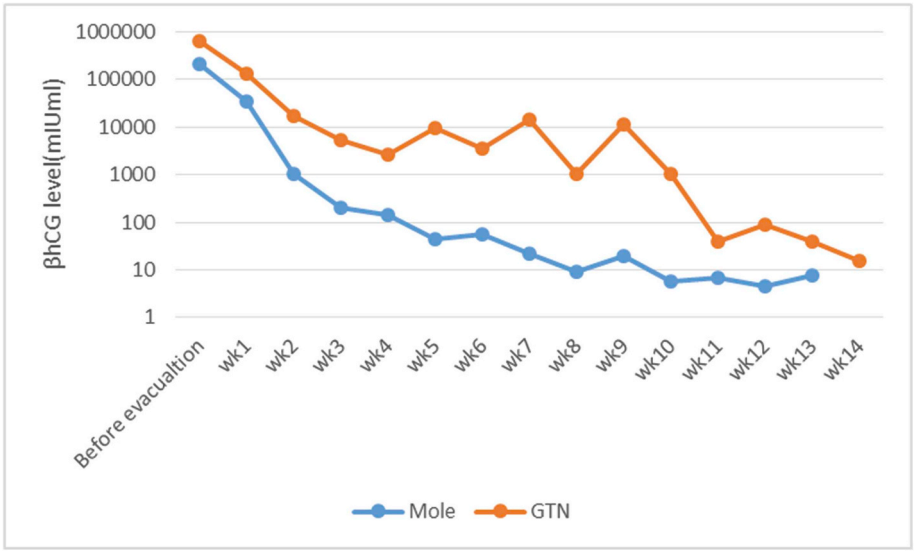

Fig. 2. Time-points of β-hCG levels between mole and GTN groups.

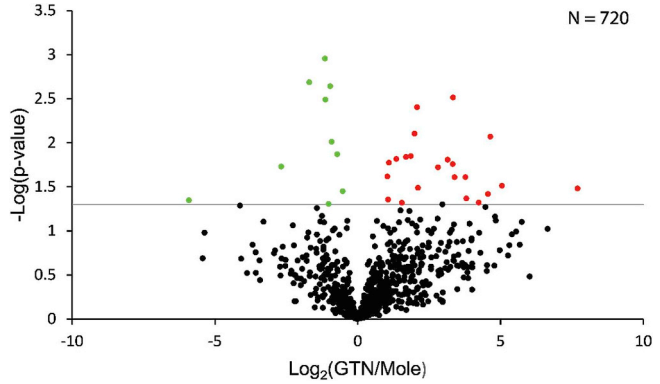

Fig. 3. Volcano plot of LC-MS/MS quantification between the GTN group and the mole group. The horizontal line represents the cutoff at p-value = 0.05 (-log10 (0.05) = 1.3). Significantly up-regulated and down-regulated proteins in the GTN group versus the mole group are indicated in red and green, respectively.

Through LC-MS/MS analysis and database searching, 1,951 proteins were identified, 32 of which showed a statistically significant difference. Most of these altered proteins were enriched in cell-cell adhesion, secreted protein, and ribonucleoprotein groups. In 2008, Ma et al. found down-regulated proteins in malignant-transformed HM such as chloride intracellular channel protein 3 (CLIC3), heat shock protein-27 (HSP27) and macrophage-capping protein (CAPG), in addition to several up-regulated proteins including septin-1 (SEPT1), peroxiredoxin-2 (PRDX2), voltage-dependent anion-selective channel protein 2 (VDAC2) [8]. All of these proteins were also detected in our study, but none of them showed significant difference between mole and GTN. Recently, Shi et al. suggested CLIC1 as a new biomarker for malignant transformed moles and proposed that its over-expression modulates cell division and antiapoptosis signaling resulting in cellular transformation [10]. Nevertheless, this data was limited due to a low sample size (3 samples). Until now, no sufficient evidence has been provided to confirm a useful marker for moles that undergo malignant transformation.

GTN, a malignancy comprised of syncytiotrophoblast and cytotrophoblast elements, is characterized by paraneoplastic disorders resulting from secretion of gestational hormones, especially human chorionic gonadotropin (hCG) proteins [1,16]. These patients are treated on the basis of an increase in serum hCG; however, hCG level is not a reliable marker for early identification of GTN after evacuation of moles. hCG, comprised of α and β subunits and produced by syncytiotrophoblasts, is a key marker for pregnancy and trophoblastic disease [16]. Previous studies proposed prediction of GTN by using several forms of serum hCG that have been found to be especially abundant in GTN, including hyperglycosylated hCG, hCG glycoforms and hCG β-core fragment [16–19]. Still, sufficient evidence has not been provided to reveal effective markers. Detection of hCG via immunohistochemistry (IHC) in GTD tissues was reported [20], but this method could not discern differences in expression between mole and GTN. Our study is the first to detect differential expression of hCG in trophoblastic tissues using LC-MS/MS analysis. β-hCG, as well as α-hCG, were significantly increased in GTN tissues compared with benign mole tissues. At present, the degree to which syncytiotrophoblastic cells secrete hCG peripherally is unknown, and there is no evidence of a correlation between hCG levels in tissue and in serum. Our study also showed no correlation between β-hCG in cells and in serum (Fig. 4). This lack of correlation could be attributed to any number of factors that alter serum hCG levels; hence, it might be more accurate to detect cellular levels of β- or α-hCG subunits from trophoblasts for prediction of GTN.

Pregnancy specific beta-1-glycoproteins (PSG), products of the trophoblast, are biochemical markers of differentiating trophoblast cells [21,22]. Thus, the high level of PSG seen in evacuated molar tissues could signal a state of differentiation that might be expected in GTN versus benign mole [23]. Earlier studies supported that PSG, whose structure is homologous to the carcinoembryonic antigen (CEA), may be acting as a molar trophoblastic adhesion molecule in a similar manner to CEA in colon cancer [24]. Additionally, PSG expression may be a marker for detection of colorectal carcinoma [22,25]. Based on our study, overexpression of PSG3 may represent a marker enabling early detection and monitoring of GTN.

Growth differentiation factor 15 (GDF15) of the transforming growth factor beta superfamily is highly expressed in human placenta compared with fetal or adult tissues [26]; however, there is no available data on GDF15 in molar tissue. In this study, GDF15 was significantly increased in GTN compared with HM. GDF15 has been reported to promote the proliferation of cervical cancer cells and metastasis in colorectal cancer [27,28].

Other interesting proteins that significantly increased in GTN were three human 40S ribosomal proteins. Dysregulation may contribute to cancer development. Several studies found that 40s ribosomal proteins are overexpressed in different cancers, including colorectal and esophageal carcinoma [29,30].

Table 2
Up-regulated and down-regulated proteins in the GTN group versus the mole group identified by LC-MS/MS. Note: UniProt ID, Protein identification based on the Protein knowledge base UniProtKB/Swiss-Prot ID (http://www.uniprot.org); SE, Standard error.

UniProt ID	Protein description	Average (Log2-ratios)	SE	Number of applicable pairs	p-value
Up-regulated proteins					
P01233	Choriogonadotropin subunit beta	3.34	1.01	13	0.003
P52597	Heterogeneous nuclear ribonucleoprotein F	2.08	0.46	4	0.004
P01215	Glycoprotein hormones alpha chain	2.00	0.63	7	0.008
Q6DRA6	Putative histone H2B type 2-D	4.64	1.21	4	0.009
P11940	Polyadenylate-binding protein 1	1.86	0.54	4	0.014
P12236	ADP/ATP translocase 3	1.69	0.55	5	0.015
P0DMV8	Heat shock 70 kDa protein 1A	1.36	0.52	12	0.015
Q99988	Growth/differentiation factor 15	3.16	1.18	10	0.016
O14980	Exportin-1	1.10	0.28	3	0.017
P09429	High mobility group protein B1	3.33	0.85	3	0.018
P62314	Small nuclear ribonucleoprotein Sm D1	2.81	0.74	3	0.019
P61247	40S ribosomal protein S3a	1.04	0.42	10	0.024
P02743	Serum amyloid P-component	3.39	1.14	4	0.025
P63220	40S ribosomal protein S21	3.77	1.50	8	0.025
P12532	Creatine kinase U-type, mitochondrial	5.06	2.07	7	0.031
Q9UBS4	DnaJ homolog subfamily B member 11	2.11	0.76	4	0.033
P51888	Prolargin	7.70	2.41	3	0.033
Q16557	Pregnancy-specific beta-1-glycoprotein 3	4.57	1.84	5	0.038
P62241	40S ribosomal protein S8	3.81	1.73	9	0.043
P11465	Pregnancy-specific beta-1-glycoprotein 2	1.06	0.46	6	0.044
P48307	Tissue factor pathway inhibitor 2	4.24	1.92	7	0.048
P68431	Histone H3.1	1.55	0.73	10	0.048
Down-regulated proteins					
P59998	Actin-related protein 2/3 complex subunit 4	−1.14	0.17	4	0.001
P01871	Ig mu chain C region	−1.69	0.63	7	0.002
O15511	Actin-related protein 2/3 complex subunit 5	−0.96	0.20	5	0.002
Q14847	LIM and SH3 domain protein 1	−1.13	0.25	5	0.003
P31949	Protein S100 -A11	−0.91	0.30	14	0.010
P31946	14-3-3 protein beta/alpha	−0.71	0.23	8	0.013
P10599	Thioredoxin	−2.67	0.97	9	0.019
P68402	Platelet-activating factor acetylhydrolase IB subunit beta	−0.52	0.15	3	0.036
Q06828	Fibromodulin	−5.90	1.77	3	0.045
P00338	L-lactate dehydrogenase A chain	−1.01	0.45	8	0.050

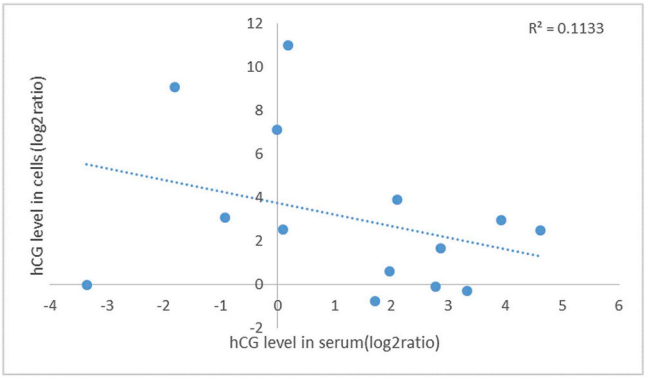

Fig. 4. Scatter plot between paired serum hCG log2 ratios and paired cell hCG MS log2 ratios (Correlation = 0.11).

The distinguishing downregulated protein in this study was S100-A11 (Calgizzarin), a calcium-binding protein of the S100 family, which has been reported as underexpressed in head and neck cancer [31]. Our findings showed S100-A11 was downregulated in 12 out of 14 paired specimens.

Song et al. reported that L-lactate dehydrogenase A chain (LDHA) or renal carcinoma antigen NY-REN-59 showed elevated expression in clear cell subtype of renal cell carcinoma [32]. In our own results, LDHA was decreased in 6 out of 8 paired specimens, in contrast with this report. The explanation was still not clear.

To our knowledge, this study is the first proteomic study of trophoblastic cells comparing benign and malignant moles using LC-MS/MS analysis. The strength of this study is the large sample size and the high number of differentially expressed proteins detected versus previous proteomic studies of molar samples [33]. The data demonstrated various proteins that could represent prognostic biomarkers for GTN. However, certain limitations exist. First, an attempt was made to optimally pair samples; however, a perfect match is not achievable. Secondly, the method of collecting tissue was not standardized and blood contamination may have obscured the proteomic data. Finally, in addition to the heterogeneous nature of the molar tissues, inconsistency of samples could be due to tissue collecting times varying from a few months to many years.

In conclusion, we performed large-scale quantitative proteomic analysis of molar tissues and identified several differentially expressed proteins that could serve as prognostic biomarkers predicting malignant transformation of moles. Among these proteins, hCG, PSG3, GDF15, and ribosomal proteins are particularly promising and worth further validation to test their sensitivity and specificity for early diagnosis followed by chemoprevention.

Conflicts of interest

This study was supported by Ratchadapisek Sompoch Endowment Fund of Chulalongkorn University (CU-58-001-HR), Thailand.

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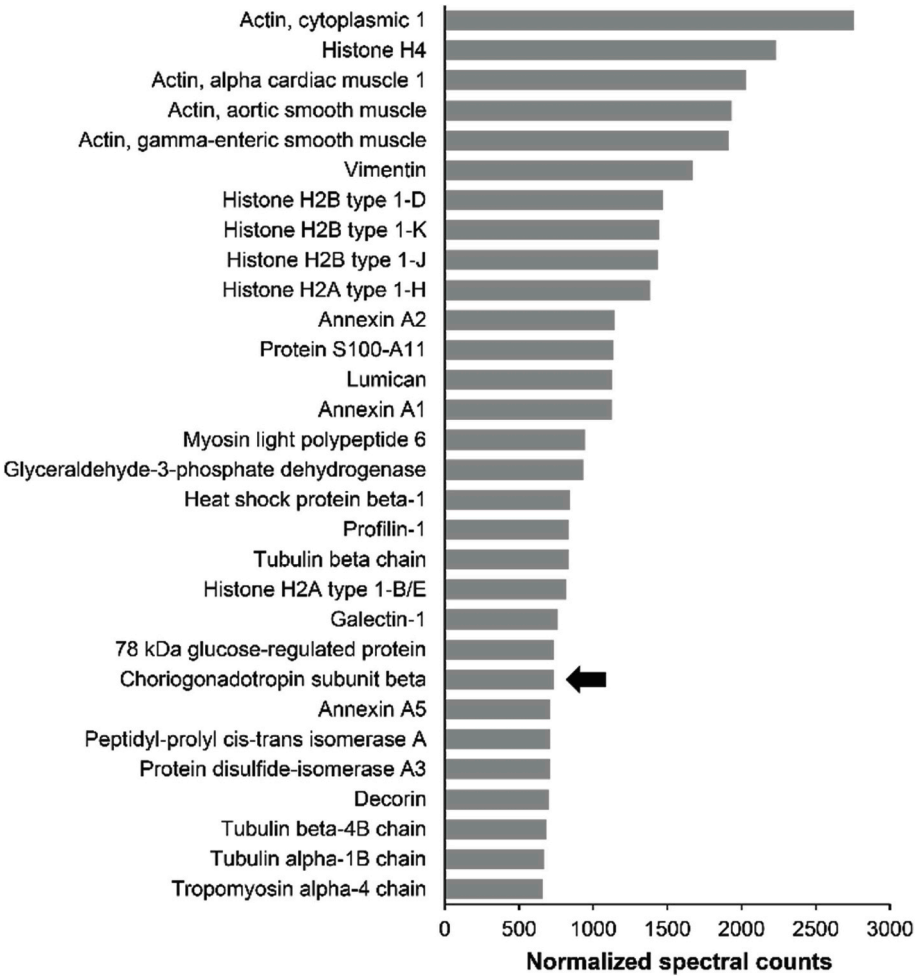

Fig. 5. Top 30 most abundant proteins identified in molar tissue. Normalized spectral counts = (number of spectra/total number of amino acid residues in a given protein) x 1,000. Arrow highlights the normalized spectral count for β-hCG.

Table 3
Three enriched biological groups of proteins that were differentially expressed in this study via DAVID. Red and green colors indicate up-regulated and down-regulated proteins (the GTN group versus the mole group), respectively.

Functional Annotation	UniProt ID	Protein Name	Benjamini-adjusted p-value
Cell-cell adhesion	P68431	Histone H3.1	0.030
	P31949	Protein S100-A11	
	P0DMV8	Heat shock 70 kDa protein 1A	
	P00338	L-lactate dehydrogenase A chain	
	P31946	14-3-3 protein beta/alpha	
	Q14847	LIM and SH3 domain protein 1	
Secreted	P51888	Prolargin	0.004
	Q16557	Pregnancy-specific beta-1-glycoprotein 3	
	P01871	Ig mu chain C region	
	P48307	Tissue factor pathway inhibitor 2	
	P01233	Choriogonadotropin subunit beta	
	P11465	Pregnancy-specific beta-1-glycoprotein 2	
	Q06828	Fibromodulin	
	P10599	Thioredoxin	
	Q99988	Growth/differentiation factor 15	
	P09429	High mobility group protein B1	
	P02743	Serum amyloid P-component	
	P01215	Glycoprotein hormones alpha chain	
Ribonucleoprotein	P52597	Heterogeneous nuclear ribonucleoprotein F	0.025
	P62314	Small nuclear ribonucleoprotein Sm D1	
	P61247	40S ribosomal protein S3a	
	P62241	40S ribosomal protein S8	
	P63220	40S ribosomal protein S21	

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.02.004.

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