Protein Profiling of Gestational Diabetes Mellitus

by Jessalyn L. Chuang

Abstract—The purpose of this study was to utilize the Protein Pathway Array (PPA) method, a multiplex immunoblot assay combined with computational analysis, to analyze the dysregulation of placental protein signals between normal and Gestational Diabetes Mellitus cases (GDM). This aided in finding novel biomarker candidates for the early detection and treatment of GDM. Results showed that there was significant dysregulation of 21 proteins involved in GDM placentas as compared to those in normal placentas, including 15 upregulated and 6 downregulated proteins. These 21 proteins can be used as diagnostic biomarkers for GDM earlier in gestation and as potential targets for intervention.

I. INTRODUCTION

Gestational Diabetes Mellitus (GDM), characterized by hyperglycemia, occurs in 3-5% of pregnancies. This number is projected to rise by up to 20% due to increased obesity [1]. In order to improve detection, a heightened understanding of the pathogenesis and biological features of GDM is critical.

The placenta is representative of the interactions between maternal and fetal bloodstreams and as a result, can serve as a valuable tool for studying micro-anatomical perturbations due to GDM [2]. Examining proteins involved in placental regulation is helpful as the mechanisms that trigger and sustain the disease need to be fully elucidated. By using PPA, hundreds of proteins in tissue samples can be analyzed for biomarkers whose dysregulation may play a role in GDM pathogenesis.

It was hypothesized that there will be significant dysregulation of the following proteins involved in GDM as compared to those in normal placentas: cell cycle regulators, angiogeneic growth factors, cytokines and other proteins involved in inflammatory processes, proteins required for cell formation, proteins involved in glucose conversion, oxygen moderators, transmembrane proteins, and proteins involved in cell communication.

II. METHODOLOGY

PPA was performed using 40 protein specific or phosphorylation site-specific antibodies from macroscopically normal tissue of the maternal side of the placenta around the umbilical cord. This tissue is derived from the decidua basalis, a section of the endometrium that develops into the placental maternal side. 108 trials were conducted for each protein. The signals of each protein were analyzed with densitometric scanning (Quantity One software package, Bio-Rad) [2].

Class comparison analysis was completed in Microsoft Excel by using an unpaired Student's t test with $p < 0.05$ to identify proteins differentially expressed between GDM and normal cases. This was followed by use of the Significant Analysis of Microarray (SAM) tool, which aided in finding significant genes in a set of microarray experiments.

III. RESULTS

21 proteins showed significant differences between normal and GDM cases, including 15 upregulated proteins and 6 downregulated proteins. (Table 1)

The results demonstrated significant alterations in proteins involved in multiple signaling pathways. Notably, the upregulation of cell cycle regulators CDK6 and cyclin B1 indicate reactions to counteract hematopoietic cell impairment. An upregulation of VEGF suggests an increase of angiogenesis, which causes the plethoric anatomy common to the GDM placenta. Stress responses may be lower due to cPKCα and Hsp90 downregulation, proteins that increase in amount when tissue is damaged and when heat stress occurs, respectively. Other components affected include proteins involved in glucose conversion, oxygen level regulation, immune response, cell communication, and transmembrane proteins in GDM placental tissue. [1]

Table 1
Summary of Proteins detected in GDM placentas

Upregulated		*No Changes*		*Downregulated*
CDK6	HIF-3α	Akt	CREB	cPKCα
Cyclin B1	N-cadherin	β-actin	E-cadherin	Cyclin D1
Cyclin E	NFkB52	β-catenin	H-Ras	Hsp90
p34	NFkBp50	EGFR	Mesothelin	Calretinin
p38B	PTEN	GAPDH	NFkBp65	KRAS
p42	VEGF	MDM2	WT1	OPN
PCNA	Vimentin	Notch4	β-actin	
GAPDH		p44		

IV. DISCUSSION

Regulator subunits, including cyclin B1, controls CDK6, which in turn induces VEGF expression [1]. If cyclin B1 is manipulated, the other two proteins can be downregulated, leading to GDM prevention. One possible protein that can target cyclin B1 is genestein, an enzyme inhibitor involved in gene replication that can cause anticancer proliferation effects through G2/M arrest in cell cycle progression [3].

In addition, another study found that although the use of rapamycin subunits dramatically decreased cPKCα expression, Hsp proteins, including Hsp90, were stabilized, allowing for greater responses to stress [4]. As a result, the use of rapamycin may also serve as another prevention method.

The dysregulated proteins in this study may serve as diagnostic biomarkers and therapeutic targets. Altered signaling pathways may be responsible for clinical presentation of GDM and fetal growth hindrance as the expression levels of some of the proteins are strongly correlated with clinical symptoms.

Practically, SB-FI-95 may not be effective enough to serve as an FABP5 inhibitor in its current form; however, further in vitro testing should be performed to verify its activity against FABP7, another therapeutic target. The data supports the conclusion that a greater focus should be placed on mono-amide α-truxillic acid derivatives, especially those containing a fused ring moiety, due to these specific compounds' demonstrated affinity for FABP5 in silico and in vitro. The continued use of computational methods for lead optimization can help narrow the search for more potent, yet selective, FABP inhibitors and thereby a new class of next-generation analgesic agents.

V. ACKNOWLEDGEMENTS

Research was conducted at the Icahn School of Medicine at Mount Sinai under the guidance of Dr. Fei Ye. I would like to thank Ms. Alison Huenger for her support for this study.

VI. REFERENCES

[1] Gauster, M., G. Desoye, M. Tötsch, and U. Hiden. Curr Diab Rep Current Diabetes Reports 12.1 (2011): 16-23.
[2] Ye, Fei, and David Y. Zhang J Mol Biomark Diagn Journal of Molecular Biomarkers & Diagnosis 02.02 (2012): n. pag.
[3] Choi, Yung H., W. Ho Lee, K. Y. Park, and L. Zhang. "P53-independent Induction of P21 (WAF1/CIP1), Reduction of Cyclin B1 and G2/M Arrest by the Isoflavone Genistein in Human Prostate Carcinoma Cells." Japanese Journal of Cancer Research 91.2 (2000): 164-73.
[4] Hall, M. N., and F. Tamanoi, "Structure, Function and Regulation of TOR Complexes from Yeasts to Mammals." Amsterdam: Elsevier/Academic, pp. 110-111, 2010.

**Research supported by the Icahn School of Medicine at Mount Sinai.*
J. L. Chuang is a student at Manhasset High School, Manhasset, NY 11030 USA (corresponding author to e-mail: jchuang181@gmail.com)
Dr. F. Ye is a professor of pathology at the Icahn School of Medicine at Mount Sinai, New York, NY 10029 USA (corresponding mentor to e-mail: fei.ye@mssm.edu