OBSTETRICS

Global "omics" evaluation of human placental responses to preeclamptic conditions

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BACKGROUND: Preeclampsia (PE) is a leading cause of maternal death. Its cause is still debated but there is general agreement that the placenta plays a central role. Perhaps the most commonly proposed contributors to PE include placental hypoxia, oxidative stress, and increased proinflammatory cytokines. How the placenta responds to these abnormalities has been considered but not as part of a comprehensive analysis of low-molecular-weight biomolecules and their responses to these accepted PE conditions.

OBJECTIVE: Using a peptidomic approach, we sought to identify a set of molecules exhibiting differential expression in consequence of provocative agents/chemical mediators of PE applied to healthy human placental tissue.

STUDY DESIGN: Known PE conditions were imposed on normal placental tissue from 13 uncomplicated pregnancies and changes in the low-molecular-weight peptidome were evaluated. A *t* test was used to identify potential markers for each imposed stress. These markers were then submitted to a least absolute shrinkage and selection operator multinomial logistic regression model to identify signatures specific to each stressor. Estimates of model performance on external data were obtained through internal validation.

RESULTS: A total of 146 markers were increased/decreased as a consequence of exposure to proposed mediators of PE. Of these 75 changed with hypoxia; 23 with hypoxia-reoxygenation/oxidative stress and 48 from exposure to tumor necrosis factor-α. These markers were chemically characterized using tandem mass spectrometry. Identification rates were: hypoxia, 34%; hypoxia-reoxygenation, 60%; and tumor necrosis factor-α, 50%. Least absolute shrinkage and selection operator modeling specified 16 markers that effectively distinguished all groups, ie, the 3 abnormal conditions and control. Bootstrap estimates of misclassification rates, multiclass area under the curve, and Brier score were 0.108, 0.944, and 0.160, respectively.

CONCLUSION: Using this approach we found previously unknown molecular changes in response to individual PE conditions that allowed development biomolecular signatures for exposure to each accepted pathogenic condition.

Key words: hypoxia, lipidomics, oxidative stress, peptidomics, placenta, preeclampsia, tissue culture, tumor necrosis factor-α

Introduction

Preeclampsia (PE) is a disorder of pregnancy characterized by hypertension and proteinuria. Its cause remains unknown. Despite increased understanding of its pathophysiology, PE incidence has increased in the United States over the past decade.[1] As many as 75,000 women die worldwide yearly from PE.[2] No established therapeutics exist and efforts to develop such have been hampered by the incomplete explanation of its cause. Currently, when PE cannot be temporized by clinical management, the pregnancy is ended. Typically, there is a rapid resolution of the hypertension, proteinuria, and other abnormalities. These findings and substantial other research suggest that the placenta is necessary for and a likely participant in PE.[3] Yet, the specific changes in the placenta that may lead to PE and their antecedent causes are still debated.[4]

Placentas from women with established PE demonstrate many alterations but it is unclear which are part of early PE pathogenesis. Among these, some appear to influence placentae very early to produce PE. These include hypoxia, oxidative stress, and increased exposure to proinflammatory cytokines.[5-7] These changes are thought to arise from inadequate remodeling of the maternal vasculature leading to poor placental perfusion.[8] Placentae experiencing hypoxia, oxidative stress, or inflammatory cytokines, eg, tumor necrosis factor (TNF)-α, initiate a cascade of events leading to maternal features of PE.[5-7] Knowing what effect these factors might have on normal placenta could be used to define specific placental changes that should be evident in PE.

Evaluating molecular changes in placenta has frequently involved proteomic analysis of tissue from women with and without established PE. Common methods primarily identify highly abundant, high-molecular-weight proteins, many being structural proteins and chaperones.[9,10]

The aim of this study was to document molecular changes in placenta with exposure to conditions thought to cause PE. To accomplish this, changes in the abundance of low-molecular-weight (LMW) biomolecular placental components were determined by a global, tissue "omics" approach for each condition thought to participate in PE, first to demonstrate that changes take place in response to that pathologic state and second to identify a set or signature of molecular responses characteristic of each. This should allow a PE placenta's composition to define its exposure to early and continued abnormalities.

Materials and Methods

Specimen collection

Institutional review board approval was obtained from Brigham Young

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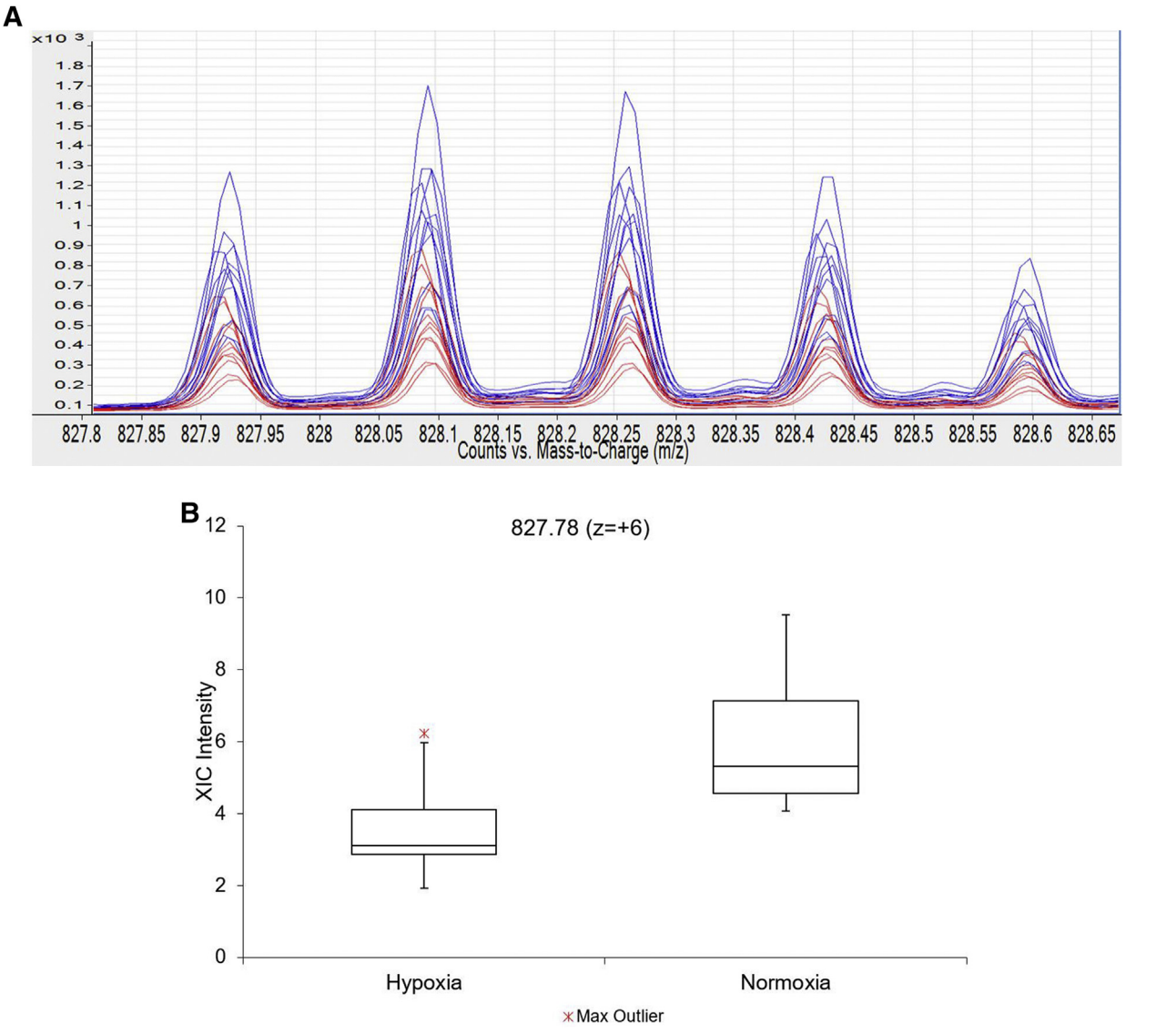
FIGURE 1
Changes in placental abundance of a representative peptide in response to hypoxia



Mass spectrometry overlay and box-and-whisker plot. **A**, Overlay of 26 mass spectra in region containing peptide mass-to-charge 827.78 ($z = +6$) with its isotope envelope. Placental explants under control (blue) and hypoxic (red) conditions. Species were less abundant in hypoxic placental explants. **B,** Box-and-whisker plot of peptide m/z 827.78 ($z = +6$).

XIC, extract ion count.

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University and Intermountain Health Care to collect human placentae. No patient personal or medical information was recorded in this process and no patient identifiers were provided. Thirteen placentas were collected immediately after elective cesarean delivery from uncomplicated term pregnancies. A full-thickness (~3 × 3 × 3 cm) block of placenta was dissected rapidly midway between the cord and placental edge, placed on ice, and processed within 30 minutes of delivery. No placenta came from a complicated pregnancy or from women with preexisting disease, eg, hypertension or diabetes.

Sample processing

Fetal membranes and decidua were removed. Explants were collected from the intervillous region representing a point midway between the chorionic and basal plates and ~1-cm thick. After initial washing with ice-cold, sterile, phosphate-buffered saline, the tissue was cut into thin sections (≤3 mm) and kept on ice to minimize proteolysis. Slices were washed another 8-10 times until nearly all blood was removed.