Autophagy in the placenta as part of the pathogenesis of preeclampsia


The work was performed with the guidance of Prof. Chaimov-Kochman, the clinic of artificial insemination, the center for the study of human reproduction, Department of Obstetrics and Gynecology, Hadassah Ein Kerem, Jerusalem.

kochman@hadassah.org.il

For the second author: D. Goldmann-Weinber, the clinic of artificial insemination, Department of Obstetrics and Gynecology, Hadassah Ein Kerem, Jerusalem.
dwohl@hadassah.org.il
Introduction:

Preeclampsia is a multi-system disorder characterized by hypertension and proteinuria in the last half of pregnancy. Although most affected pregnancies deliver at term or near term with good maternal and fetal outcomes, Preeclampsia is a potentially dangerous syndrome with increased risk for maternal and/or fetal mortality or serious morbidity. Preeclampsia occurs in up to 7.5 percent of pregnancies worldwide.

The pathophysiology of preeclampsia likely involves both maternal and fetal/placental factors. Abnormalities in the development of placental vasculature early in pregnancy may result in relative placental underperfusion or ischemia, which then leads to release of antiangiogenic factors into the maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations of the disease. However, the molecular basis for placental dysregulation of these pathogenic factors remains unknown. The pathogenesis of preeclampsia, is thought to originate in the placenta (1). Characteristics often associated with preeclampsia, including an excessive inflammatory response (2), premature placental aging (Tenney-Parker change), metabolic syndrome, hypoxia and placental insufficiency are reminiscent of features of impaired autophagy.

'Autophagy' (in Latin = "self eating") refers to any intracellular process that involves the degradation of cytosolic components by the lysosome. There are at least three distinct autophagic pathways: Macroautophagy, Microautophagy and Chaperone-mediated autophagy. Macroautophagy, here referred to as 'autophagy', a lysosomal pathway of cellular component degradation, is known to play an essential role in such diverse processes as cell survival under stress and nutrient deprivation, cell differentiation, development, immunity and clearance of defective macromolecules. In addition to its role in normal cellular homeostasis, defects in autophagy are implicated in pathologies such as neurodegeneration, cancer, inflammation and aging (3-5). On the one hand, autophagy is an essential cellular process through which defective or harmful proteins are eliminated. On the other hand, it is a process through which cells can survive under stress conditions by digesting cellular organelles and proteins for reuse. Although previously described as a death pathway, autophagy is now considered an important survival phenomenon in response to environmental stressors to which most organs are exposed.

Although possible roles for autophagy in placental function have recently been suggested (6, 7), Studies of autophagy in the placenta are still lagging. Two main players in the regulation of autophagy, Beclin-1 (which plays a pivotal role in the regulation of autophagy by...
combining with positive and negative co-factors) and LC3-II (which required for the formation of the autophagosomal membranes), have been studied in preeclampsia. LC3-II was found to be increased in placentas of preeclampsia, while Beclin-1 was not (8).

To elucidate a possible association between autophagy and the clinical syndrome of preeclampsia we undertook an expression profile analysis with a directed focus of autophagy associated genes from placentas of preeclamptic pregnancies as compared to controls. We used microarray data including publicly available data from the NCBI Gene Expression Omnibus (GEO) platform, to analyze over 40 genes known to be regulated in autophagy. We compared datasets from 106 normal and 78 preeclamptic placental samples to determine whether they displayed statistically significant differential expression of these autophagy-related genes.

**Materials and Methods:**

1. **Expression profile analysis:** Five datasets were examined for a total of 78 preeclamptic and 106 control placental samples (Table 1). Datasets were carefully chosen for validation that was performed through additional methods such as RT-PCR. Additionally, at least two of the genes known to be associated with preeclampsia, FLT1 (fms-like tyrosine kinase-one) and ENG (endoglin)(9), were found to be upregulated in all the third trimester datasets that we used. Analysis of whole genome expression transcriptional profiling with RMA (quantile based) normalization using the PARTEK GENOMIC SUITE 6.6. PCA (principal component analysis) was performed to remove outlier samples. Unpaired t-tests were performed to detect statistically significant differentially expressed genes. 43 genes known to have a role in the macroautophagy pathway (Table 2) found in the "Amigo – Gene Ontology" Database [http://amigo.geneontology.org/cgi-bin/amigo/term-assoc.cgi?gptype=all&speciesdb=all&taxid=9606&evcode=all&term_assocs=all&term=GO%3A0016236&session_id=9416amigo1353235318&action=filter](http://amigo.geneontology.org/cgi-bin/amigo/term-assoc.cgi?gptype=all&speciesdb=all&taxid=9606&evcode=all&term_assocs=all&term=GO%3A0016236&session_id=9416amigo1353235318&action=filter) were analyzed with a t test. p <0.05 was considered statistically significant.
2. **Immunohistochemistry:** Immunohistochemistry was performed on archival FFPE normal third trimester (4 sections) and preeclamptic (8 sections) placental sections as well as a 17 day secretory endometrium sample. The KIAA1324 antibody (Sigma prestige antibody produced in rabbit) was used (as being the most upregulated gene in Preeclamptic placenta, see results section) according to the manufacturer’s instructions with citrate buffer antigen retrieval (1:100 dilution). The secondary antibody was Zytomed (Germany) systems HRP one-step polymer, antimouse/rabbit/rat. Color detection was with AEC (Zymed, CA) and GVA (Zymed, CA) mounting was used.

**Results:**

We analyzed five different microarray datasets, from five different studies, (10-14) (Winn et al. 2009, Sitras et al. 2009, Herse et al. 2012, Tsai et. al 2011, Founds et al. 2009), comprising gene expression data of placental tissue. Each dataset included both preeclamptic and control samples. Detailed information on the datasets is shown in Table 1. We analyzed each dataset separately for several reasons, but mainly because each study defined preeclampsia differently as regards severe (10, 11) or mild (12-14) disease.

The five datasets were created using three discrete microarray platforms: Affymetrix Human Genome U133A and U133B Array (10, 14), Illumina human-6 v2.0 expression beadchip (12, 13), and ABI Human Genome Survey Microarray Version2 (11).

Table 1 shows that the five datasets differ in the number of samples collected. In one dataset (14) the tissue collected differs from the others, as it was obtained via chorionic villus sampling at first trimester and not placental sampling at delivery.

$t$-test did not reveal a statistical difference between autophagy associated gene expression of normal and preeclamptic placental samples. Of the five datasets, very few autophagy genes differed significantly between the two groups, and only three of them had a fold change higher than 1.5 (in preeclamptic samples vs. control). They were the genes KIAA1324, WDR45L (which are involved in the vacuole formation stage of macroautophagy) and NPC1 (which is one of the negative regulators of macroautophagy). The gene mTOR
(FRAP1) displayed a 2-fold change in one of the datasets but was not significant (p=0.0624) (Figure 1A-E).

The gene KIAA1324 displayed a 1.6 fold overexpression in preeclampsia as compared to control samples. The gene, also known as EIG121, is a transmembrane protein that is estrogen-induced and is overexpressed in endometrial carcinoma (15). Overexpression of tetracycline inducible EIG121 in stably transfected cells coupled with starvation was found to upregulate autophagy (16). To investigate KIAA1324 protein expression in placenta we performed immunohistochemistry on normal third trimester and first pregnancy severe preeclampsia placental sections. As a positive control for the antibody we used secretory phase endometrium. As expected, staining was observed in the glandular epithelium (Figure 2A). The serum only control was negative (Figure 2B). In three of four normal third trimester placenta staining was observed surrounding fetal vessels; in six of eight of the preeclampsia placentas we observed staining in the smooth muscle cells surrounding fetal vessels Figure (Figure 2C,D). Overall however, the preeclamptic placental staining appeared less intense than the staining intensity observed in the normal sections.
**Table 1.** Characteristics of the five datasets used in the analysis

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study</th>
<th>Number of Cases</th>
<th>Sampling Procedure</th>
<th>Platform</th>
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<tbody>
<tr>
<td>Winn, 2009</td>
<td>GSE14722</td>
<td>12 severe preterm PE, 11 control preterm delivery</td>
<td>Basal plate biopsies of preterm labor (24-36 weeks) and preterm severe preeclampsia (24-36 weeks).</td>
<td>Affymetrix Human Genome U133A and U133B Array.</td>
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<tr>
<td>Tsai, 2011</td>
<td>GSE25906</td>
<td>23 preeclampsia, 37 control</td>
<td>The placental sample, excluding fetal membranes, was obtained within 5 cm of the placental umbilical insertion site.</td>
<td>Illumina human-6 v2.0 expression beadchip</td>
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<td>Founds, 2009</td>
<td>GSE12767</td>
<td>4 CVS samples of PE, 8 matched controls</td>
<td>A nested case control study of CVS tissues collected at 10–12 weeks’ gestation in 160 patients with singleton fetuses.</td>
<td>Affymetrix Human Genome U133 Plus 2.0 Array</td>
</tr>
<tr>
<td>Sitras, 2009</td>
<td>GSE10588</td>
<td>17 preeclampsia, 26 control</td>
<td>Chorionic tissue was dissected from a standardized location – approximately 2 cm from the umbilical cord insertion, from the middle layer of placenta midway between maternal and fetal surfaces.</td>
<td>ABI Human Genome Survey Microarray Version 2</td>
</tr>
<tr>
<td>Herse, 2012</td>
<td>Biobank Collection Oslo University Hospital</td>
<td>22 preeclampsia, 24 control</td>
<td>Placental biopsies were excised from a macroscopically normal looking, centrally located cotyledon, omitting the decidual layer.</td>
<td>Affymetrix Human Genome U133 Plus 2.0 Array</td>
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Table 2. Autophagy related genes and function

<table>
<thead>
<tr>
<th>Induction</th>
<th>Nucleation</th>
<th>Expansion</th>
<th>Vacuole formation</th>
<th>Co-Regulators of Autophagy and Apoptosis:</th>
<th>Degradation of misfolded proteins</th>
<th>Positive regulation of Macroautophagy</th>
<th>Negative regulators of Macroautophagy</th>
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<tr>
<td>mTOR</td>
<td>RB1CC1</td>
<td>ATG3</td>
<td>ATG9A</td>
<td>CLN3</td>
<td>HDAC6</td>
<td>LARP1</td>
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<td>ATG4B</td>
<td>ATG9B</td>
<td>SQSTM1</td>
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<td>PAFAH1B2</td>
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<td>ATG13</td>
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<td>KIAA1324</td>
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Figure 1: Analysis of datasets: Each point in the graph represents one gene of the autophagy pathway which was analyzed. The X axis represents the expression fold-change values (preeclamptic samples vs. control samples). The Y axis represents the level of significance for gene expression. The horizontal line marks statistical significance (p=0.05).

Figure 2: Immunohistochemistry of KIAA1324

Expression of KIAA1324 in the glandular epithelium of secretory phase endometrium (A 200X) magnification and inset 400X magnification, negative control third trimester placenta (B 400X), third trimester placenta with faint positive staining surrounding fetal blood vessels (C 400X), preeclampsia placenta with positive staining of smooth muscle cells surrounding large fetal vessels (D 200X), preeclampsia placenta negative for KIAA1324 (E 400X).

Discussion

Although it is tantalizing to suggest that autophagy plays a role in normal placental function and that the preeclamptic placenta would have defects in the autophagic pathway, our initial analysis does not support this hypothesis. We investigated this notion through microarray data and publicly available data from the NCBI Gene Expression Omnibus (GEO) platform of preeclamptic as compared to normal placenta, as well as first trimester CVS samples in pregnancies that later did or did not develop preeclampsia. We did not observe major statistically significant differences between the RNAs expressed by the preeclamptic and normal placentas.
This was a surprising result as autophagy not only plays a role in normal cellular homeostasis but plays a role during nutrient starvation, which could help explain placental function during nutritional variability in maternal blood. Furthermore, considering the known contributions of autophagy to processes such as aging, immunity, inflammation, and cell survival, it seemed reasonable to suspect an association between defects in autophagy and preeclampsia.

However, our findings are not without caveats. Among the possible mechanisms that may diminish the need for autophagy in the placenta is placental reserve. Placental reserve is essential for fetal survival as fluctuations in nutrient availability are to be expected. Placental reserve is highly dependent on transport efficiency and placental size. Along with the placental capacity of adaptability of active transport, placental reserve could offer a way to meet fetal nutritional demands in times of limited nutrient availability (17).

Another possibility that may explain our negative results is that while not rising to statistical significance, the small differences observed for some of the genes (less than 1.5 fold) may be enough to trigger the autophagy pathway and perhaps to sustain it. These small RNA differences could reflect larger differences if the data could be analyzed in protein arrays or by western blot. In other words, the significance of defective autophagy in preeclampsia may be revealed on the level of protein expression but not on RNA (4). In addition, marked differences in gene expression may be observed according to the number of samples collected per placenta, whether they were pooled or not, or the quality of the RNA: all of these factors, as well as the anatomical site where the placental sample was taken, may impact the results (18).

The placental samples used in this current investigation were from samples (either third trimester or chorionic villous sampling) where the number of extravillous trophoblasts would constitute only a minor fraction of the cell types. Therefore, if the defect in autophagy in preeclampsia was to be found in extravillous trophoblasts then it would not be observed by this methodology. Furthermore, since the basis for development of preeclampsia is thought to be in the first trimester of pregnancy, with defective remodeling by extravillous trophoblast of uteroplacental spiral arteries then perhaps a defect in autophagy expression would not be detectable in the third trimester samples evaluated from four of five sources used here for the array data (19).
Summary:

Autophagy, a mechanism of cell survival during times of stress, may be involved in normal placental maintenance, and in addition to its role in normal cellular homeostasis, defects in autophagy are implicated in pathologies such as neurodegeneration, cancer, inflammation and aging (3-5). Characteristics often associated with preeclampsia, an hypertensive disorder of pregnancy, including an excessive inflammatory response (2), premature placental aging (Tenney-Parker change), metabolic syndrome, hypoxia and placental insufficiency are reminiscent of features of impaired autophagy. Thus, the disruption of autophagy might contribute to the pathophysiology of preeclampsia. However, studies of autophagy in the placenta are still lagging.

To elucidate a possible association between autophagy and the clinical syndrome of preeclampsia we undertook an expression profile analysis with a directed focus of autophagy associated genes from placentas of preeclamptic pregnancies as compared to controls. We used microarray data including publicly available data through the NCBI Gene Expression Omnibus (GEO) platform, to analyze over 40 genes known to be regulated in autophagy. We compared datasets from 106 normal and 78 preeclamptic placental samples to determine whether they display statistically significant differential expression of these genes.

Of the five datasets, very few autophagy genes differed significantly between the two groups, and only three of these (KIAA1324, WDR45L and NPC1) had a fold change higher than 1.5 (in preeclamptic samples vs. control).

To investigate KIAA1324 protein expression in placenta we performed immunohistochemistry on normal third trimester and first pregnancy severe preeclampsia placental sections. Overall however, the preeclamptic placental staining appeared less intense than the staining intensity observed in the normal sections.

Although preeclampsia displays many of the features suggestive of altered autophagy, including premature placental aging and placental insufficiency, defective placental autophagy as a cause of preeclampsia is not supported by whole placental tissue microarray differential expression profiling. We suggest that further investigations and alternate approaches be taken to unveil the role that autophagy may play in normal placental function and possible dysfunction in preeclampsia.
Autophagy in the placenta as part of the pathogenesis of preeclampsia

 Tattoos are made of cell death and survival, and it is possible that it is a part of the placenta's ability to nourish the fetus in utero. The autophagy process is abnormal and has been shown to be involved in pathologies such as cancer, infections, aging and neurodegenerative processes (3-5). Additionally, many features of a malformed pregnancy, including an increased risk of complications, premature aging of vessels, and hypoxia are situations where there are indications that the autophagy process is weak (6, 7). Therefore, it was hypothesized that the autophagy process plays a role in the pathogenesis of pregnancy-related disorders.

In order to investigate this hypothesis, and to find a relationship between the autophagy process and the condition of pregnancy, we conducted a study using data from the NCBI Gene Expression Omnibus (GEO) platform, in order to analyze the expression of more than 41 genes known to be involved in the autophagy process. After conducting the analysis, we found that only a few genes were significantly different between the two groups tested, namely KIAA1324, WDR45L and NPC1 (fold change > 0.5 in the affected group compared to the control group). The mTOR (FRAP1) gene showed high expression (3-fold), but was not statistically significant. In order to investigate the expression of the KIAA1324 protein in the placenta, we conducted immunohistochemical staining of sections of placenta from third trimester and of women who suffered from a severe form of preeclampsia. The staining in the affected group was weaker than that observed in placenta taken from normal cases.

Thus, although pregnancy expresses many features that are suitable for an autophagic process, the hypothesis that a flaw in the autophagy process in the placenta is the cause of preeclampsia is not supported by our study.
References: