Three mechanisms in the pathogenesis of pre-eclampsia suggested by over-represented transcription factor-binding sites detected with comparative promoter analysis

B. Vásárhelyi¹,⁵, Á. Cseh¹, I. Kocsis¹,², A. Treszl¹,², B. Györffy³ and J. Rigó Jr⁴

¹Research Laboratory of Pediatrics and Nephrology, Hungarian Academy of Sciences, ²First Department of Pediatrics, ³Szentágothai János Knowledge Centre and ⁴First Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary
⁵To whom correspondence should be addressed at: First Department of Pediatrics, Budapest, Bókay u. 53, H-1083 Hungary. E-mail: vasbar@gyerl1.sote.hu

Microarray studies generating lists of genes with altered expression in placentas from pregnancies complicated with pre-eclampsia (PE) have so far been published in several different studies. Working under the assumption that altered gene expression in PE may be the result of altered expression of regulatory transcription factors (TFs), we looked for over-represented TF-binding sites (TFBSs)—which indicate the involvement of TFs in regulatory networks—in lists of genes (n = 143) compiled in these studies. We compared the prevalence of TFBSs in the promoter regions of 68 genes with the background prevalence of TFBSs in promoters of the human genome. The prevalence of the E47, sterol regulatory element binding protein (SREBP) and NFKB-p50 TFBSs was higher (P < 0.005) in the promoter sequences of the PE gene lists than in the background model. Each of these TFBSs could be implicated in the development of PE. The E47 protein is an E-protein or basic helix-loop-helix (bHLH) TF. Data support the role of bHLHs in the differentiation of placental tissue. SREBP-1, a lipid-sensing sterol regulatory element-binding protein, is a critical regulator of fatty acid homeostasis in the placenta. The target genes of NFKB-p50 determine inflammatory response, and aberrant cytokine homeostasis is a further sign of PE. These TFs may provide an insight into the pathogenesis of the disease.

Key words: microarray/pre-eclampsia/transcription factor/transcription factor-binding site

Introduction

Pre-eclampsia (PE) is a major cause of maternal mortality, affecting approximately 5% of all pregnancies (Anonymous, 2000). PE is the cause of 150,000 deaths worldwide each year. PE also affects fetal development, causing intrauterine growth retardation. Growth retardation is even more pronounced when the disease starts before 32nd week of pregnancy (Ventura et al., 2001). Most cases of PE present with increased blood pressure, proteinuria and edema, but some cases may progress into a complex disorder that involves the central nervous system, liver and kidneys and results in multiple organ failure (Gillham and Hayman, 2004). The etiology of PE remains unclear; it is aptly termed as ‘disease of theories’. Blood pressure usually normalizes and proteinuria subsides after termination of pregnancy, lending support to the assumption that the placenta plays an important role in the pathogenesis and pathophysiology of PE (Redman and Sargent, 2003). Placental dysfunction may be caused by uncoordinated expression of genes in the placenta. We used data from four recent microarray studies comparing gene expression in placental tissue of women with PE to gene expression in placental tissue of women without PE (Reimer et al., 2002; Tsoi et al., 2003; Pang and Xing, 2004a; Soleymanlou et al., 2005). From these data, we generated a list of genes showing altered expression in the placentas of women with PE. Altered gene expression in PE may be linked to altered expression of regulatory transcription factors (TFs). TFs may up-regulate or down-regulate gene expression. Transcriptional regulatory regions—so called promoter sequences—located before the start codon of each gene contain binding sites (TFBS) for TFs. The TFBS is short—usually 4–10 bases long—and one TF may bind to several TFBSs (Aerts et al., 2003). Therefore, one TF may bind to the promoters of several genes or the promoter sequence of one gene may contain several TFBSs. TFs with over-represented TFBSs in the promoter region may play a role in the regulation of expression of co-regulated genes. If they do, certain TFBSs should be over-represented in the promoter regions of genes with altered expression.

To test our hypothesis, we looked for over-representation of some TFBSs in the promoter regions of gene sets with altered expression in PE.

Methods

We searched the PubMed archive (http://www.ncbi.nlm.nih.gov/entrez/) for papers presenting lists of genes with altered expression in PE using the keywords PE, placenta, DNA-chip or microarray. We found four articles in which the altered expression of 143 genes in placental tissue was described (Reimer et al., 2002; Tsoi et al., 2003; Pang and Xing, 2004a; Soleymanlou et al., 2005).

For TFBS analysis, we applied the same method as described previously (Mayer et al., 2004). We used the Java program TOUCAN for comparative promoter analysis of the selected genes (Aerts et al., 2003). Proximal promoter sequences (1 kb upstream and 0.05 kb downstream of the transcriptional start sites) were extracted from the genomic databases using the EZ-Retrieve (Zhang et al., 2002). The TOUCAN tool MotifScanner, which searches the
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TRANSFAC database (Wingender et al., 2001), was used to detect TF binding sites (TFBSs) in the sets of sequences. The prior (stringency level) was set to a value of 0.1, and the human promoter set of the Eukaryotic Promoter Database (EPD) was chosen as a third-order background model. We applied TOUCAN’s statistics tool to the data produced by MotifScanner to detect over-representation (showing positive significance values) in the sets of the selected genes. The level of significance was set at $P = 0.005$.

**Results**

The promoter sequences were available for 68 genes and we identified 226 different TFBSs. The prevalence of three TFBSs was significantly higher in the analyzed promoter sequences of PE gene lists than in the background model: E47 protein (TF accession number: T00207) (prevalence: 0.147 versus 0.0000229, $P = 0.0011$), sterol regulatory element binding protein (SREBP-1) (TF accession number: T01556) (prevalence: 0.221 versus 0.0000278, $P = 0.0002$) and nuclear factor-kappa-beta-p50 (NFkB-p50) (TF accession number: T00593) (prevalence: 0.176 versus 0.0000513, $P = 0.0005$).

These TFBSs were present on 26 genes. Table I lists the genes with over-represented TFBS in the analyzed promoter sequences. Most of the 26 genes contain TFBS for one of the three identified TFBS while both over-represented TFBSs in the analyzed promoter sequences. The results of our analysis suggest that these TFBSs could be implicated in the development of PE.

**E47 protein**

An association between altered E47 function and PE could be implied by two different mechanisms. E47 protein belongs to E-proteins or basic helix-loop-helix (bHLH) TFs. Although the exact role of E47 in pregnancy has not been investigated, other structurally related E-proteins have been found to play a role in placental development (Hemberger and Cross, 2001; Liu et al., 2004). Data obtained in knockout mice models show that trophoblast differentiation and placental morphogenesis are regulated at multiple checkpoints by bHLH factors and their regulators. In the placentas of women with PE, abnormalities of trophoblast differentiation could be caused by bHLH TFs, including E47. In normal pregnancy, cytotrophoblast invasion of the uterine spiral arteries is accompanied by the loss of musculo-elastic tissue in these vessels (Wang and Alexander, 2000). An important structural abnormality of the placenta in PE is the incomplete cytotrophoblast invasion of the uterine spiral arteries. As a result, spiral arteries fail to lose their elasticity and instead of becoming low resistance vascular channels as in normal pregnancy, the resistance of arterioles in PE pregnancies remains high. E47 could contribute to altered musculo-genesis in the spiral arteries. Although the importance of heterodimers of E47 proteins with muscle-specific receptor (MSR) proteins has been tested only in the development of skeletal muscles (Carlsen and Gundersen, 2000), other bHLH/MSR heterodimers have been shown to play a role in smooth muscle differentiation (Dhulipala et al., 2001). Altered E47 protein expression could contribute to inadequate loss of musculo-elastic tissue in the vascular wall in PE.

<table>
<thead>
<tr>
<th>UniGeneID:gene</th>
<th>Name</th>
<th>TFBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>494457:NINJ1</td>
<td>Ninjurin 1 (NINJ1)</td>
<td>NFkB50</td>
</tr>
<tr>
<td>117938:COL17A1</td>
<td>Collagen, type XVII, alpha 1</td>
<td>SREBP1</td>
</tr>
<tr>
<td>512152:HLA-G</td>
<td>HLA-G histocompatibility antigen, class I, G</td>
<td>NFkB50</td>
</tr>
<tr>
<td>789:CXCL1</td>
<td>Chemokine (C-X-C motif) ligand 1</td>
<td>NFkB50</td>
</tr>
<tr>
<td>2164:PPBP</td>
<td>Pro-platelet basic protein [chemokine (C-X-C motif ligand 7)]</td>
<td>E47</td>
</tr>
<tr>
<td>151413:GMFB</td>
<td>Glia maturation factor, beta</td>
<td>SREBP1</td>
</tr>
<tr>
<td>248100:CHRM4</td>
<td>Cholinergic receptor, masculenic 4</td>
<td>E47</td>
</tr>
<tr>
<td>368794:AP1B1</td>
<td>Adaptor-related protein complex 1, beta 1 subunit</td>
<td>SREBP1</td>
</tr>
<tr>
<td>1360:CYP2B6</td>
<td>Cytochrome P450, family 2, subfamily B, polypeptide 6</td>
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<td>Glucosidase, beta</td>
<td>SREBP1, E47</td>
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<td>293970:ALDH6A1</td>
<td>Aldehyde dehydrogenase 6</td>
<td>SREBP1, E47</td>
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<tr>
<td>502914:DPP3</td>
<td>Dipeptidylpeptidase 3</td>
<td>NFkB50</td>
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<td>524368:VDR</td>
<td>Vitamin D receptor</td>
<td>E47</td>
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<tr>
<td>119689:CGA</td>
<td>Glycoprotein hormones, alpha polypeptide</td>
<td>SREBP1</td>
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<td>247917:CHRM1</td>
<td>Cholinergic receptor, masculenic 1</td>
<td>E47</td>
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<tr>
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<td>Insulin-like growth factor binding protein 6</td>
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<td>Cholecystokinin</td>
<td>E47</td>
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<td>Bone morphogenetic protein 2</td>
<td>NFkB50</td>
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<td>2430:TCLF1</td>
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<td>184907:GPR1</td>
<td>G protein-coupled receptor 1</td>
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<td>194148:YES1</td>
<td>V-yes-1 Yamaguchi sarcoma viral oncogene homologue 1</td>
<td>SREBP1, E47</td>
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<td>422662:VRK1</td>
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<tr>
<td>496487:ATF4</td>
<td>Activating transcription factor 4</td>
<td>SREBP1</td>
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</table>
**SREBP**

Epidemiological data show that PE and cardiovascular disorders share several risk factors, including insulin resistance, obesity and diabetes (Wolf *et al.*, 2004). PE also presents an increased maternal risk of cardiovascular disease later in life. SREBP has recently emerged as a possible link between these disorders. SREBPs are essential for the control of intracellular lipid accumulation. Intracellular lipid accumulation might be a link between insulin resistance, visceral obesity and increased lipid deposition in non-adipose tissue, including the arterial wall, therefore, SREBPs are being investigated as a possible target for atherosclerosis (Muller-Wieland and Kotzka, 2002). The role of SREBPs in lipid homeostasis of the human placenta has also been suggested. Recent studies have demonstrated that SREBPs regulate the transcription of ACC and FAS, important enzymes for the synthesis of fatty acids (Duttaroy, 2004). Insulin is known to act through SREBP-1c by augmenting the nuclear content of SREBP-1c while LCPUFA suppresses the nuclear content. SREBP may also play a role in insulin resistance syndrome. Studies have proposed that, along with other TFs for fatty acid regulation, such as peroxisome proliferator activating receptor gamma (PPAR-g), alterations in the amount and activity of SREBPs could offer a key to understanding the association of insulin resistance with cardiovascular risk factors at the cellular or gene regulatory level. In PE, several mechanisms may contribute to the possible alteration of SREBP expression. Consumption of large amounts of polyunsaturated fatty acids could increase risk of PE (Clausen *et al.*, 2001). As expression of SREBP has been shown to be regulated by cholesterol and polyunsaturated fatty acids, altered SREBP expression after ingestion of fatty acids could be an explanation for the link between nutrition and PE (Kim *et al.*, 2002; Jump, 2002).

Another possible explanation for the alteration of the hypothesized alteration of SREBP expression is a change in HCG production (Richardson *et al.*, 2005). In PE, HCG levels are abnormally high (Kharfi *et al.*, 2005). Because HCG has been shown to cause a shift between SREBP isoforms as well as increase the expression of SREBP-1c, therefore, the link between HCG and SREBP could be a mechanism leading to altered expression of SREBP-regulated genes.

**NFKB-p50**

NFKB-p50, the third identified TF, belongs to the NFKB/Rel family, which plays an important role in the intracellular regulation of immune response, inflammation and cell cycle regulation (Liou and Hsia, 2003). The most prevalent activated form of NFKB is the heterodimer of subunit NFKB-p65, associated with either subunit NFKB-p50 or -p52. p50 homodimers lack the transcription activation domain but still bind to B-consensus sites and therefore function as transcription repressors. A growing number of NFKB/Rel target genes have been identified in the past several years. These include cytokines, chemokines, cytokine/chemokine receptors, adhesion molecules, survival factors, cell cycle regulators and inducible effector enzymes. Inadequate functioning of the cytokine network has been implicated in the disturbance of trophoblast remodelling in PE pregnancies by inducing enhanced apoptosis (Allaire *et al.*, 2000; Kharfi *et al.*, 2003) as well as in the triggering and maintenance of the maternal systemic inflammatory response. One study has postulated that NFKB-p50, as the conductor of cytokine regulation, plays a role in PE. This hypothesis was supported recently by an immunohistochemical study which showed increased expression of NFKB-p50 in PE (Aban *et al.*, 2004). This finding was confirmed by the results of our TFBS analysis, which also indicate an over-representation of binding sites for NFKB-p50.

Although the results of our analysis provide attractive hypotheses for the pathogenesis of PE, one should take into account the limitations of our analysis.

Promoter analysis of gene lists in pre-eclampsia

In this study we analyzed promoter sequences of genes with altered expression published in four papers. These papers listed more than 350 genes that are up- or down-regulated in PE; however, only a minority of these genes were listed in the papers (n = 143) and only a fraction of them (n = 68) possessed well-defined promoter sequences in EZ—Retrieve (Zang *et al.*, 2002). The length of promoter sequences used for the analysis was also arbitrary (1000 bases). (We postulated that TFs with TFBSs on these proximal promoter regions would have the greatest effect on gene transcription.) If longer promoter sequences and/or more genes had been involved in the analysis, it may have affected our results.

Furthermore, our computer-assisted analysis does not provide information about the functional impact of the identified TFs on gene activation. Our results are putative and the resulting hypothesis should be tested by experimental methods. One way to investigate the complex interaction of TFs and DNA is to use functional assays such as an electrophoretic mobility shift assay (EMSA). (Fried and Coothers, 1984). (EMSA) make use of the observation that protein : DNA complexes migrate more slowly than free DNA molecules when subjected to non-denaturing polyacrylamide or agarose gel electrophoresis used to investigate sequence-specific interactions.)

Interestingly, the TFs highlighted in our analysis are different from those identified by Pang and Xing (2004a,b) and Reimer *et al.* (2002). A probable explanation for this apparent contradiction is the methods used. These authors drew their conclusions after the comparison of gene expression data while we used a computer-simulated statistical analysis. Our analysis, however, does not exclude the implication of TFs other than E47, SREBP-1 and NFKB-p50 in the pathogenesis of PE (Indeed, 4 of the genes with TFBSs for these TFs are TFs themselves or regulators of cell signalling). There may be a mutual interaction between E47, SREBP-1 and NFKB-p50 and the products of these genes, but the analysis of these connections is beyond the scope of our present study.

In summary, altered gene expression of the placenta may contribute to the development of PE. Based on the results of our computer-simulated analysis of the promoter regions of genes with altered gene expression, we hypothesize that the altered function of three TFs in the placenta may contribute to the disease. These TFs play a role in the regulation of tissue differentiation, lipid homeostasis, inflammation and apoptosis.

Acknowledgements

This study was supported by grants OTKA T046086 and NKFP 1A/002/2004.

References


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Submitted on September 27, 2005; accepted on December 7, 2005