

IDENTIFICATION OF GENES AND GENE PRODUCTS INVOLVED IN THE PATHOGENESIS OF PRE-ECLAMPSIA

Pre-eclampsia (PE) is a very common pregnancy complication in humans. Approximately 3-10 percent, depending on the diagnosis criteria and the population of study, of pregnancies are affected by pre-eclampsia. Pre-eclampsia is a progressive multisystem disorder and a leading cause of maternal and fetal morbidity and mortality. It has been called a “disease of theories”, reflecting the confusion that surrounds the causes and pathophysiology of PE. Symptoms of PE are maternal hypertension and proteinuria and oedemas. PE is characterized by a state of generalized, maternal endothelial dysfunction.

An initial step in the pathogenesis of PE is inadequate placentation. Paternal or fetal genotypes may play a role in this phenomenon. This results in an insufficient placental perfusion and a reduced supply of nutrients and oxygen to the placenta, leading to a state of ischemia and oxidative stress. During gestation, there is a release of placental factors into the maternal circulation. Altered levels of some of these factors may contribute to the development of the generalised maternal endothelial dysfunction of PE. Maternal genotype may influence the susceptibility of the pregnant woman to the development of the symptoms associated with PE.

It is generally agreed that the placenta is the primary causative organ in the development of PE. The presence of a fetus and/or a uterus is not required, since PE can occur with hydatidiform mole and abdominal pregnancies. It is therefore speculated that aberrant expression of certain genes in the placenta may contribute to the development of pre-eclampsia. One of the factors released from the placenta during gestation are placental syncytiotrophoblast membrane fragments (STBM). STBM have been detected in the peripheral circulation in pregnant women and it has been shown that the level of circulating STBM is significantly increased in pre-eclamptic women compared to women with uncomplicated pregnancies. STBM could be a factor contributing to the endothelial cell dysfunction.

The objective of this Ph. D. thesis was to identify genes and gene products that may be involved in the pathogenesis of pre-eclampsia. Two different approaches were taken, one focusing on the placental contribution to the development of the syndrome, the other focusing on the maternal consequence, i.e. the endothelial dysfunction.

To study the placental gene expression, RNA was purified from tissue biopsies taken from pre-eclamptic placentas at term and matched normal controls. The mRNA expression profiles were analysed by Affymetrix microarrays. Verification of the expression of a selection of genes was performed using real-time PCR and immunohistochemistry. The results revealed [some](#) of the genes that have already been connected with pre-eclampsia as being differentially expressed between the pre-eclamptic group and the control group, e.g. inhibin beta A subunit and leptin. But also genes that have not previously been associated with the development of pre-eclampsia were found to have expression levels that were markedly different in the pre-eclamptic group compared to the control group. Among these were bradykinin B1 receptor and collagen XVII. Overall, genes associated with transcriptional regulation and vasoregulative pathways, the inhibin-activin system, eicosanoid and energy metabolism, were found to be regulated in pre-eclampsia, along with a number of hypothetical proteins and gene sequences with unknown functions. Comparisons with other studies revealed a great variability in the genes identified as potentially important to the pathogenesis of PE, depending on the gestational age of the placentas examined. It is speculated that the factors of importance to the pathogenesis of PE change throughout gestation. In order to identify the genes participating in the early events of PE, it will be necessary to examine placental samples from early gestation, such as for instance chorion villus biopsies.

To study the possible contribution of STBM to the maternal endothelial dysfunction, the effect of STBM treatment on endothelial cell gene expression was examined *in vitro*. Human umbilical vein endothelial cells

were cultured in the presence and absence of STBM. At specified time points, total RNA was purified from the cultures and analysed on Affymetrix microarrays. The expression levels of a number of genes were then verified by real-time PCR or ELISA. The results demonstrate an effect of the STBM on endothelial gene expression and support the theory of the vascular endothelial cell dysfunction following STBM treatment. They point to the involvement of the unfolded protein response and cell cycle arrest, but also to chemokines and cytokines, components of the extra cellular matrix, immunological and vasoactive factors. The aberrant expression of some or all of these factors may contribute to the development of PE. Furthermore, comparisons of the results of this thesis with the results of other studies indicate that the changes in expression levels of some, but not all, gene products observed in the endothelium and blood circulation of pre-eclamptic women may be contributed to the interactions between STBM fragments and endothelial cells.

Given that the very basic cause of pre-eclampsia presumably is the presence of the placenta, a link between the placenta and the maternal endothelial dysfunction must be assumed. STBM are found at a higher concentration in the circulation of pre-eclamptic women compared to women with uncomplicated pregnancies. A simple explanation for this could be that factors responsible for the increased shedding of STBM exist within the pre-eclamptic placenta. One might expect such factors to belong to apoptotic pathways. However, the analysis of placental gene expression performed in this thesis revealed no change in the expression of apoptosis genes. Therefore, the increased shedding may be caused by mechanisms other than apoptosis, or maybe the initiating factors are no longer active in the placenta at term. Secreted proteins released from the placenta into the maternal blood stream may also contribute to the endothelial dysfunction.

Certain results of thesis supports the hypothesis of a contribution of STBM to the endothelial dysfunction. But it also demonstrates that the influence of STBM on vascular endothelium alone cannot account for all the pre-eclampsia related changes in measurable blood parameters that have been described through time. Other factors secreted from the placenta may also play a role. Finally, the direct or indirect effect of STBM or other placental factors with cell types such as monocytes and leukocytes may be of importance, too.