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Original contribution

Abnormal expression of transcription factor activator protein-2α in pathologic placentas☆,☆☆

Rachel M. Sheridan MD[a,*], Jerzy Stanek MD, PhD[a], Jane Khoury PhD[b], Stuart Handwerger MD[c]

[a]*Department of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA*
[b]*Department of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA*
[c]*Department of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA*

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Summary Recent studies from our laboratory have indicated that the transcription factor activator protein-2α plays a critical role in the differentiation of human villous cytotrophoblast cells to syncytiotrophoblast cells. However, little is known about the expression of activator protein-2α in placentas from pathologic pregnancies. This study compares the expression of activator protein-2α in placentas from high-risk pregnancies to gestational age–matched controls. Paracentral sections from grossly unremarkable areas of 10 placentas from each group of pregnancies complicated by mild preeclampsia, severe preeclampsia, diabetes mellitus, chronic hypertension, and fetal growth restriction and 10 control cases of placentas from normal pregnancies matched for gestational age were double immunostained for activator protein-2α and E-cadherin. The total numbers of cytotrophoblast cells and syncytiotrophoblast nuclei and the numbers of activator protein-2α–positive and activator protein-2α–negative nuclei in both of these cell types were counted by 2 pathologists blinded to disease status, in 10 representative ×40 high-power fields for each placenta. Abnormal placental maturation in most of pathologic pregnancies was evidenced by a 1.5- to 1.7-fold lower expression ratio of syncytiotrophoblast cell to cytotrophoblast cell. Activator protein-2α in syncytiotrophoblast cells was lower in mild preeclampsia, diabetes mellitus, hypertension, and fetal growth restriction ($P < .0001$ in each instance) and was higher by 2-fold in severe preeclampsia, although this increase was not statistically significant ($P = .3$). Because activator protein-2α has been shown to be critical for villous cytotrophoblast cell differentiation, our findings suggest that abnormalities in the activator protein-2α cascade of transcription factors and/or signaling molecules may contribute to the pathogenesis of the abnormal maturation in placentas in certain types of high-risk pregnancies. The different pattern of activator protein-2α expression in mild and severe preeclampsia clearly suggests that these conditions may have 2 independent pathogenic mechanisms.

1. Introduction

The trophoblast layer of the human placental villus consists of multinucleated syncytiotrophoblast cells (STBs) and mononuclear cytotrophoblast cells (CTBs) [1]. The STBs,

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* Corresponding author.
E-mail address: Rachel.Sheridan@cchmc.org (R. M. Sheridan).

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which are in direct contact with maternal blood, perform many critical functions throughout pregnancy, including regulation of substrate and gas exchange between mother and fetus and synthesis and secretion of many hormones that modulate maternal and fetal growth and metabolism [1,2]. The underlying mononuclear CTBs, which are located between the STB and the basement membrane of the trophoblast layer, fuse with the syncytium, therefore being the source of STB layer [1]. Extravillous CTBs, on the other hand, invade the underlying endometrium and maternal vascular bed, migrating up the uterine spiral arteries that supply the implantation site [1].

At present, much is known about the regulation of trophoblast development in the mouse [3] but less about the differentiation of human villous CTB. In particular, the function of transcription factors and signaling molecules that are involved in the terminal differentiation of human villous CTB to a STB phenotype is incompletely understood. Furthermore, although abnormalities in the development of the trophoblast layer have been observed in several pathologic conditions of pregnancy associated with higher maternal and fetal morbidity and mortality, including preeclampsia (PE) and fetal growth restriction (FGR) [4,5], little is known about the transcriptional regulation of trophoblast differentiation in these conditions. Earlier studies from our laboratory demonstrated that the transcription factor AP-2α (activator protein-2α, also called TFAP2A) plays an important role in terminal differentiation of villous CTB to a STB phenotype [6-8]. AP-2α messenger RNA and protein are induced during the initial stage (first 2 hours) of in vitro CTB differentiation. Global gene profiling indicated that most of the 100 most induced genes during in vitro differentiation of human CTB have 1 or more putative AP-2α binding sites in the proximal 1 kilobase of their promoters [8]. AP-2α also transactivates the human placental lactogen [9], human chorionic gonadotropin (hCG)-α and hCG*β* [10] promoters, and the promoters of other genes that are induced during spontaneous differentiation of primary cultures of villous CTB. In addition, AP-2α was shown by other investigators to stimulate the expression of the genes for aromatase cytochrome P-450 [11], germ-cell alkaline phosphatase [12], and 17*β*-hydroxysteroid dehydrogenase type 1 [13] in human trophoblast cell lines during CTB differentiation. Silencing of AP-2α activity in primary cultures of villous CTB undergoing spontaneous differentiation by an adenovirus that expresses a dominant/negative AP-2α mutant markedly blocked the expression of the messenger RNAs of human placental lactogen, hCG, corticotropin releasing hormone, pregnancy-specific glycoprotein 1, and other genes normally induced during the differentiation process [8].

Recent findings by Kotani and coworkers [14] indicated that overexpression of AP-2α or AP-2γ inhibits the migratory and invasive capacities of a transformed cell line of human first-trimester trophoblast cells (HTR-8/SVneo cells). The increase in the AP-2α protein in the cells led to changes in protease, metalloproteinase, and plasminogen activator inhibitor-1 levels. These in vitro studies demonstrated that AP-2α suppresses trophoblast cell migration and invasion. They also reported that the expression of AP-2α, as well as AP-2γ, was higher in 12 PE placentas as compared with gestational age–matched control placentas. The increase in the AP-2α proteins was demonstrated by immunohistochemical analysis and immunoblotting of placental tissue. The increase in AP-2α expression was noted in both STBs and extravillous trophoblast cells.

In this study, we examined the expression of AP-2α by immunohistochemistry in STB and CTB in placentas from pregnancies complicated by mild PE, severe PE, chronic hypertension (HTN), diabetes mellitus (DM), FGR, and gestational age–matched control group (CG) placentas.

2. Methods

2.1. Inclusion criteria

The protocol for this research was approved by the institutional review boards of Cincinnati Children's Hospital Medical Center and the University of Cincinnati. Archival paraffin blocks of placentas from patients with mild PE, severe PE, chronic HTN, DM, and FGR were selected from the tissue repository of the Department of Pathology of the University of Cincinnati Medical Center. The selected cases for each group were matched for gestational age but, otherwise, were randomly selected from the database. A total of 10 placentas from singleton deliveries were chosen from each group according to the standard diagnostic criteria. All placental reports and archived hematoxylin and eosin (H&E) slides were re-reviewed, and relevant gross, placental weight, and histologic findings were recorded. Clinical data were extracted from electronic medical records and diagnoses confirmed by chart review. The diagnosis of *mild PE* was based on the presence of blood pressure greater than 140/90 mm Hg but less than 160/110 mm Hg, measured in at least 2 different occasions more than 12 hours apart, in a woman who was normotensive before 20 weeks' gestation, as well as the presence of proteinuria (>300 mg and <5 g/24 h) [15]. *Severe PE* was defined as blood pressure greater than 160/110 mm Hg and urinary protein excretion greater than 5 g/24 h. *Chronic HTN* was defined as a blood pressure elevation of greater than 140 mm Hg systolic or greater than 90 mm Hg diastolic in the absence of proteinuria that was detected before pregnancy or by the 20th week of gestation [15]. Placentas from diabetic pregnancies included 5 pregestational DM and 5 gestational DM, diagnosed according to White's classification [16]. Placentas from infants whose weights were below the 10th percentile for gestational age and whose mothers did not have history of PE, chronic HTN, or DM were included in the FGR group [17]. There was no mutual overlap of conditions among the groups studied. Cases of pregnancy-induced HTN with superimposed PE as