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Immunohistochemical expression of MTA1 and MTA3 in placental tissue of normal and preeclamptic pregnancies

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Summary

Purpose: The aim of this preliminary study was to evaluate and compare MTA1 and MTA3 antigens expression in normal and preeclamptic placentas in order to demonstrate their possible functional relationship during pathogenesis of preeclampsia. *Materials and Methods:* A series including 20 paraffin-embedded placentas, ten of which originated from normal patients and ten from preeclamptic patients, that were examined by immunohistochemistry using the polyclonal antibodies MTA1 and MTA3. *Results:* The results of this study showed a positive nuclear staining reaction against MTA1 and MTA3 in both normal and preeclamptic placentas. However, in preeclamptic chorionic villi, cytotrophoblast and syncytiotrophoblast cells demonstrated increased expression of MTA1 and MTA3 than in normal ones. *Conclusion:* The present observations indicate a potential role for MTA1 and MTA3 for normal human placental function, playing an essential role in the pathogenesis of preeclampsia. Nevertheless, the precise relationship between these antigens' expression and pathological pregnancies remains to be elucidated.

Key words: metastasis associated antigens; MTA1; MTA3; placenta; placental tissue; normal; preeclampsia.

Introduction

Preeclampsia is thought to be a multisystemic disorder that originates in early pregnancy, leading to significant maternal and fetal morbidity and mortality [1, 2]. It is estimated that approximately 5-7% of pregnancies are complicated by preeclampsia [3]. Moreover, hypertensive disorders of pregnancy are accounting for more than 60,000 maternal deaths worldwide [4]. Although many scientific advantages have been demonstrated during the recent years, the precise pathogenesis of these pregnancy-associated disorders remains still quite unclear [5, 6].

The invasion of the extravillous trophoblast into the myometrium displays a phenotype very similar to cancer cells, with their capacity of proliferation, migration, angiogenesis, and immune tolerance using similar molecular mechanisms [7-9]. These changes are often accompanied by an epithelial-mesenchymal transition (EMT) that has been described to play an essential role during embryogenesis [10] and normal trophoblast development [10, 11]. The EMT is accompanied by a shift in gene expression, most apparently by that of cell adhesion molecules such as integrins and E-cadherin [11]. The expression of these cell adhesion proteins is predominantly regulated by nuclear transcription factors such as *metastasis associated antigens 1 and 3* (MTA1 and MTA3) [8].

MTA3 is a modulator of nuclear gene transcription and functions as a transcriptional repressor by recruiting factors of the histone deacetylation procedure [12]. MTA1 is an integral part of the nucleosome remodeling and histone deacetylation complex {nucleosome remodeling and deacetylating (NuRD)} [13, 14]. Being a member of the cluster NuRD, MTA1 regulates the transcription of the target, modifying the acetylation of chromatin accessibility and cofactors in the target DNA [7, 15]. Meanwhile MTA1 and especially MTA3 have been demonstrated in various gynecological cancers [16], including uterine carcinomas [17, 18], as well as in placental tissue [19-21].

Recently expressions of MTA1 and MTA3 have been demonstrated in human placental tissue and chorionic carcinoma cells [19]. Moreover, MTA3 expression was significantly downregulated in preeclamptic placenta, as compared to normal control placenta, with the use of gene expression microarray and qRT-PCR [20]. It is now clear that MTA1 and MTA3 play important roles during placental development, especially within the view that MTA1/MTA3/SNAIL, and E-cadherin are part of a transcriptional regulation network. However, there are still only limited data regarding the expression of metastasis-associated-antigens, especially of

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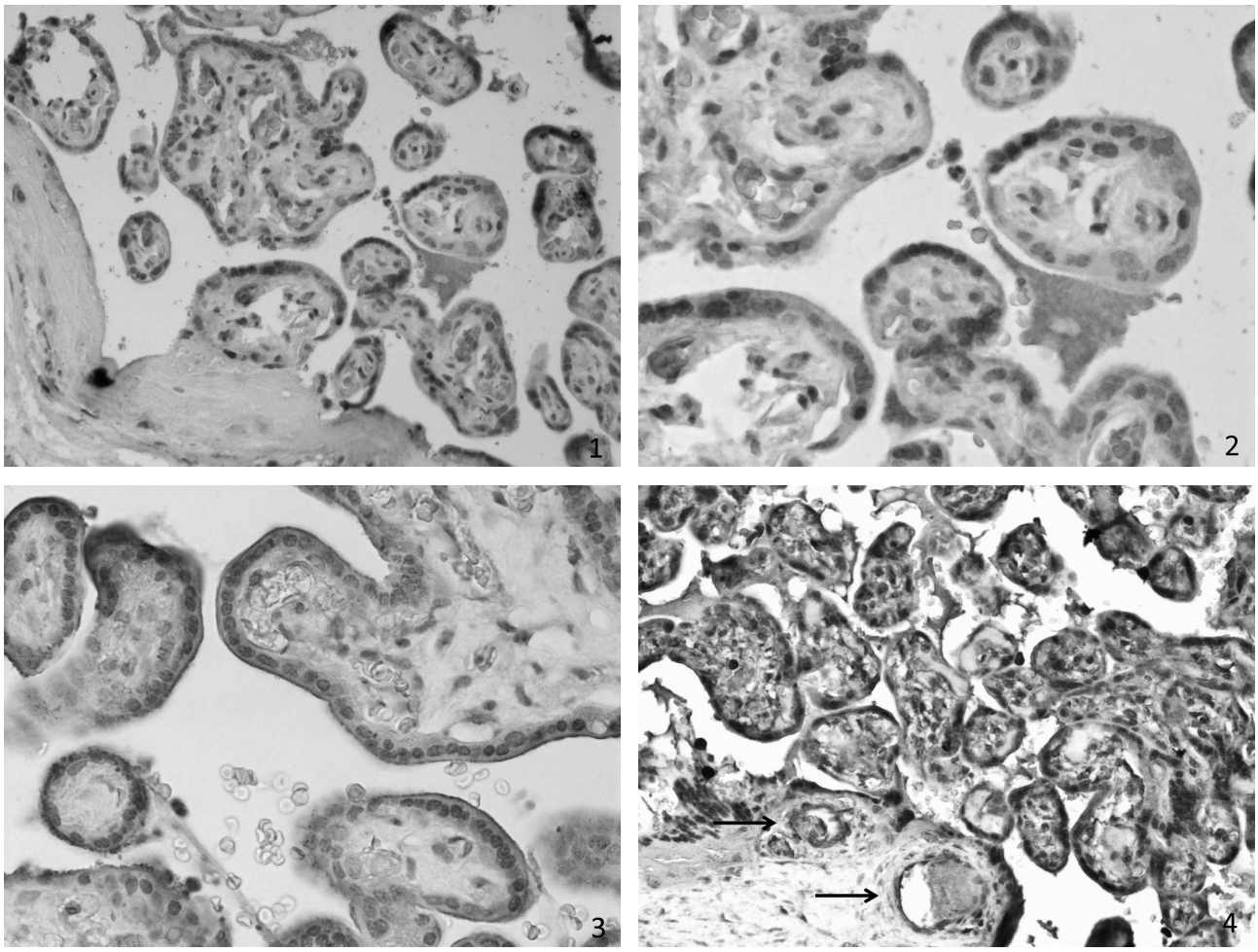

Figures 1-4. — MTA1 and MTA3 expression in normal and preeclamptic human placenta tissues. Placental tissue was immunohistochemically analyzed for MTA1 and MTA3 expression. Normal (Figure 1, ×20) and preeclamptic (Figure 2, ×40) syncytotrophoblast cells demonstrating a positive staining intensity for MTA 1. Additionally, normal (Figure 3, ×40) and preeclamptic (Figure 4, ×20) syncytotrophoblast cells also express MTA3. Staining for MTA3 can further be observed in vascular endothelial cells (Figure 4, →).

MTA1, in preeclamptic placental tissue. Therefore the present authors evaluated in this preliminary study the immunohistochemical labelling of MTA1 and MTA3.

Materials and Methods

Placenta tissues were derived from placentas of ten women with normal and uncomplicated pregnancies and ten patients with preeclampsia who delivered at the Obstetrics and Gynecology Clinic of the University of Evros, Greece. Tissue specimens of normal pregnancies were obtained during the course of an elective cesarean section for breech presentation during the 38th week of gestation to avoid any influencing factors due to the physiological stress during normal delivery [22, 23].

Tissue specimens were fixed in formalin and embedded in paraffin according to standard histological procedures. Four-micron (μm) sections of representative blocks from each case were deparaffinized, rehydrated, and treated with 0.3% H2O2 for five minutes in methanol to prevent endogenous peroxidase activity and were immunostained by the peroxidase method according to the manufacturer's recommendations. After antigen retrieval and endogenous peroxidase blockage, sections were then incubated at room temperature for 60 minutes with the indicated primary antibodies MTA1 rabbit polyclonal antibody (dilution 1:800) and MTA3 rabbit polyclonal antibody (dilution 1:500). After washing with PBS, the slides were incubated in diluted biotinylated antiserum secondary antibody for further 30 minutes at room temperature (ten ml PBS, 50l horse serum). Bound antibody complexes visualization was performed stained for ten minutes with diaminobenzidine chromogen. Sections then were briefly counterstained with Mayer's haematoxylin, mounted, and examined under a microscope. Control slides were incubated for the same period with nonimmunized rabbit serum (negative control). A positive control was always run in the assay.

Results

The specificity of the MTA antibodies has previously been confirmed in ovarian, placental, and endometrial tissues [17-19, 24]. The results of this preliminary study showed a positive staining nuclear reaction against MTA1 and MTA3 in both normal and preeclamptic placentas (Figures 1-4). MTA3 expression appeared to be absent in fibroblasts within the villous structures and in mesenchymal cells, but could be observed in vascular endothelial cells (Figure 4, →). However, in pleeclamptic chorionic villi, cytotrophoblast and syncytiotrophoblast cells demonstrated increased MTA1 and MTA3 expression than normal ones.

Discussion

Since MTA1 and MTA3 have been shown to play an important function in invasion and metastasis of human cancer cells, the aim of this study was to investigate the expression of these proteins in normal and preeclamptic placental tissue. The authors demonstrated that MTA1 and MTA3 are expressed in human trophoblast cells at a rather high level, suggesting an important function during pathogenesis of preeclampsia.

The expression level of MTA1 and MTA3 transcriptional regulators suggests an important role during gene regulation of trophoblast cells. The exact target genes of MTA1 and MTA3 in trophoblast cells remain unclear, and it can only be suggested that MTA1 and MTA3 are involved in the regulation of similar transcription clusters, as recently shown in human cancer cells [24, 25]. Because the target proteins of MTA1 themselves exhibit transcriptional silencing or activating potencies, it can be concluded that whole gene expression clusters are influenced by MTA1 expression, either positively or negatively. Such target genes include both estrogen receptors [24, 25], which themselves are of essential importance during implantation, since they are expressed in normal endometrial tissue [26, 27], as well as during normal and pathological gestation [28-30]. Further target genes comprise the nuclear transcription factors SNAIL and SLUG [8, 24].

Preeclampsia is a multisystemic disorder and, although recent advantages have contributed to our understanding, the precise pathological mechanisms are still quite unclear. Insufficient placental implantation and a failure of an adequate trophoblast invasion into spiral arteries are considered to be one of the major progression steps during pathogenesis of preeclampsia [31-33]. In pathological pregnancies, an imbalance between angiogenetic factors is thought to result in systemic vasospasm and a subsequent endothelial damage [5, 33-35]. Angiogenesis is of crucial importance in placental development and homeostasis [36] and chemokines are important mediators of neovascularisation [37]. Since MTA might have immunomodulatory functions [8], the notion is strongly suggested that these antigens are additionally involved in the angiogenic processes. Moreover, the placenta of preeclamptic women is also hypoxic [9]. Interestingly, MTA1 and MTA3 can be induced by hypoxia [21], suggesting a major contribution of these metastasis associated antigens in the pathogenesis of preeclampsia.

Recently an expression was demonstrated in human placental tissue and chorionic carcinoma cells [19]. In the placenta, invasive trophoblast cells and syncytiotrophoblast cells showed a strong positive staining reaction against MTA1 and MTA3 in the nuclei of the cells [19]. Recently MTA3 was shown in cytotrophoblast cells with a decrease during differentiation into syncytiotrophoblast and invasive extravillous trophoblast [38]. Moreover, MTA3 expression was significantly downregulated in preeclamptic placenta as compared to normal control placenta with the use of gene expression microarray and qRT-PCR, whereas CGB5 (the gene for hCG) and SNAIL were upregulated in preeclamptic placental tissue [20]. Interestingly, MTA1 and MTA3 are induced by hypoxia, upregulating the HIF1a expression and HIF1a target gene in trophoblast cells [21]. These data are highly suggestive that MTA1 and MTA3 play critical roles in trophoblast function and differentiation [21], with MTA3 repressing hCG and SNAIL synthesis with this deregulation associated to preeclampsia [20].

Conclusion

In summary, the present observations indicate a potential role of MTA1 and MTA3 in normal human placental function, playing an essential role in the pathogenesis of preeclampsia. However, the precise relationship between these antigens and pathological pregnancies remains to be elucidated.

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