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Villous trophoblast apoptosis is elevated and restricted to cytotrophoblasts in pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR

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Abstract

Human placental villi are surfaced by an outer multinucleated syncytiotrophoblast and underlying mononucleated cytotrophoblasts. Conflicting data have attributed one, or the other, of these villous trophoblast phenotypes to undergo enhanced apoptosis in complicated pregnancies, compared to term, normotensive pregnancies. We use high-resolution confocal microscopy after co-staining for E-cadherin, as a trophoblast plasma membrane marker, and for the cleavage products of cytokeratin 18 and PARP1, as markers for caspase-mediated apoptosis, to distinguish between apoptotic cytotrophoblasts and apoptosis within the syncytiotrophoblast. We test the hypothesis that increased caspase-mediated apoptosis occurs in villi of placentas derived from pregnancies complicated by preeclampsia, intrauterine growth restriction (IUGR), or both. We find significantly elevated apoptosis in villous cytotrophoblasts from women with preeclampsia and/or IUGR, compared to term, normotensive pregnancies. Apoptosis of cytotrophoblasts in villi from complicated pregnancies appears to progress similarly to what we found previously for apoptotic cytotrophoblasts in villi from in term, normotensive pregnancies. Notably, caspase-mediated apoptosis was not detectable in regions with intact syncytiotrophoblast, suggesting strong repression of apoptosis in this trophoblast phenotype *in vivo*. We suggest that the elevated apoptosis in cytotrophoblasts in preeclampsia contributes to the placental dysfunction characteristic of this disorder. We also propose that repression of apoptosis in the syncytiotrophoblast is important to prevent apoptosis sweeping throughout the syncytium, which would result in widespread death of this essential interface for maternal-fetal exchange.

Keywords

Placenta; Apoptosis; Syncytiotrophoblast; Cytotrophoblast; Preeclampsia

1. Introduction

Human placental villi are surfaced by the syncytiotrophoblast, a continuous layer of terminally differentiated, multinucleated epithelial cells. The syncytiotrophoblast is in direct contact with the maternal blood and is involved in the fetal-maternal transfer of gases,

nutrients and wastes. Cytotrophoblasts, mononucleated epithelial cells, underlie the syncytiotrophoblast and reside upon a basement membrane that separates them from the villous stroma. Cytotrophoblasts can divide, differentiate, and fuse with the syncytiotrophoblast, allowing for growth of the syncytium during development and for reepithelization of damaged regions of villi.

Exogenous stimuli that create oxidative and nitrate stress influence placental development and contribute to placental injury. For example, the trophoblast bilayer is denuded in areas where fibrin-type fibrinoid marks sites of injury. These sites are reepithelialized by new syncytiotrophoblast, achieved by fusion of cytotrophoblasts with existing syncytium [1,2]. Oxidative and nitrate stress and placental maldevelopment or injury associate with preeclampsia, with or without intrauterine growth restriction (IUGR) [3–6]. Placental dysfunction commonly results from these maladies, yielding sub-optimal maternal and fetal outcomes in the process [3,7].

Apoptosis is a type of cell death that is often increased in response to stress and is regulated through an extrinsic pathway using cell-surface receptors, through an intrinsic pathway that involves mitochondrial depolarization, and by activation of the unfolded protein response of the ER [8,9]. Apoptosis is typically effected by activated caspase proteases that cleave substrates on the membranes, in the cytoplasm and nucleus, and in the fragmentation and degradation of nuclear DNA. Ultimately, an apoptotic cell undergoes fragmentation, forming multiple, vesicle-enclosed apoptotic bodies.

Immunolocalization of caspase-cleaved substrates and assays for fragmented nuclear DNA by TUNEL assays suggest that, compared to controls, there is an increased level of villous trophoblast apoptosis in pregnancies complicated by preeclampsia [10–12] or IUGR [13–15]. However, only few studies have attempted quantification, and these have reported large variations in the level of apoptosis, likely due in part to the differing methods used and their differing sensitivities and specificities [16,17].

An important, unresolved question is, what is the villous trophoblast phenotype that undergoes increased apoptosis in complicated pregnancies? Multiple previous studies, including our own, did not assign the apoptotic cells to a trophoblast phenotype [12,15,18,19]. Other studies concluded that the increased apoptosis was in localized regions of the syncytiotrophoblast [10,11,13,14,20,21], while others concluded apoptosis was higher in cytotrophoblasts of villi in complicated pregnancies, compared to control [16,22].

One likely contributor to these discrepant results is the difficulty in the definitive identification of villous cytotrophoblasts from the syncytiotrophoblast, because of their close and intertwined anatomical proximity [23,24]. Burton and colleagues [16] highlighted this problem in their electron microscopic study of villi from 6 to 15 weeks' gestation. They noted that cytotrophoblasts with morphological features of apoptosis lost contact with the basement membrane and were often interdigitated into, or even within, the syncytiotrophoblast cytoplasm. We recently found that both non-apoptotic and apoptotic cytotrophoblasts were frequently interdigitated within the syncytiotrophoblast of term villi, and that assignment of the trophoblast phenotype undergoing apoptosis required the use of high-resolution confocal microscopy and co-staining for E-cadherin, a marker of the trophoblast plasma membrane, and for markers of apoptosis [24]. We now apply this approach to test the hypothesis that increased caspase-mediated apoptosis occurs in villi of placentas derived from pregnancies complicated by preeclampsia, intrauterine growth restriction, or both. We further examine the premise that the increased apoptosis is in cytotrophoblasts and not in intact syncytiotrophoblast.

2. Materials and methods

2.1. Study participants, tissue procurement, fixation and sectioning

The Institutional Review Board of the Washington University School of Medicine approved this study. Placentas were obtained from singleton gestations with normotensive pregnancies and from pregnancies with preeclampsia, IUGR, or both. Preeclampsia was defined by the criteria of the American College of Obstetrics and Gynecology [25]. IUGR was defined as birth weight <10th percentile for the gestational age. The clinical characteristics of the patients in these groups are listed in Supplementary table 1, as recommended [26]. Within 20 min of delivery, systematic random sampling of each placenta yielded four tissue samples that were fixed in 10% neutral buffered formalin for 24 h, embedded in paraffin, and sectioned at 10 μ m thickness.

2.2. Immunofluorescence and immunohistochemistry

We previously described methods for tissue deparaffinization, antigen retrieval, DNA staining with DRAQ5, and immunofluorescence detection of E-cadherin and for the caspase-cleaved forms of cytokeratin 18 (cCyt18) or poly-ADP-ribose polymerase (cPARP) by confocal microscopy with a Nikon Eclipse microscope equipped with a C1 laser scanning head and 60 $\times$ objective [24], with typical image size of 1024 $\times$ 1024 pixels covering 212 μ m $\times$ 212 μ m with Z-stack steps of <0.5 μ m. Images were optimized with ImageJ (rsbweb.nih.gov/ij/) using only linear adjustments. To avoid bias in image acquisition, focus was obtained using transmitted light on the upper left-hand portion on tissue sections of each slide, and a confocal Z-stack was acquired, followed by scoring for E-cadherin, DNA and cCyt18. The slide was moved down one field of view and the procedure repeated until >150 cytotrophoblasts had been captured. This was repeated for each of the four tissue samples from each placenta, resulting in >600 cytotrophoblasts (with ~6000 syncytiotrophoblast nuclei) being scored per placenta. Scoring of percent apoptosis using cCyt18 staining was as described [24]. Briefly, a region of E-cadherin staining with a diameter of >5 μ m that outlined a region with cCyt18, with or without DNA, was scored as a single cCyt18-positive cytotrophoblast. Regions of E-cadherin staining of 5 μ m diameter with cCyt18 were scored as derived from a single cytotrophoblast if all were within <50 μ m of each other, and the percent of apoptotic cytotrophoblasts was calculated by dividing the total number of apoptotic cytotrophoblasts by the total number of apoptotic and non-apoptotic cytotrophoblasts and multiplying by 100. Samples were scored by an observer who was blinded to the clinical outcome of the patients until after all tissues had been scored.

For immunohistochemistry, after antigen retrieval tissues were incubated in 5% H₂O₂ in water at 23 $^{\circ}$ C for 5 min and washed with water for 5 min. Blocking, incubation with primary and secondary antibodies and antigen detection were done with the R.T.U. Vectastain Universal Elite kit with ImmPACT DAB substrate (Vector labs, Burlingame, CA), according to the manufacturers' recommendations. Nuclei were counterstained for 30 s at 23 $^{\circ}$ C using Hematoxylin QS (Vector Labs) and images obtained with Nikon E800 microscope with a color Olympus DT digital camera and a 60 $\times$ objective.

Regions with fibrin-type fibrinoid [27] were identified by their characteristic hypocellular, eosinophilic appearance under light microscopy [2].

2.3. Statistical analyses

Comparisons for number of cCyt18-positive regions between term, normotensive and complicated pregnancies were done using the non-parametric Wilcoxon–Mann–Whitney *U* test (Kaleidograph, Synergy Software, Reading, PA) with *p* < 0.05 for significance.

3. Results

3.1. Study population

The demographics of the patient population are shown in Table 1.

3.2. Progression of caspase-mediated apoptosis in cytotrophoblasts in complicated pregnancies

Confocal microscopy using Z-stack image acquisition at $<0.5\ \mu\text{m}$ -thick optical sections allowed definitive distinction of the two villous trophoblast phenotypes when tissues are co-stained for E-cadherin, to identify trophoblast plasma membranes, and DNA, to identify nuclei [24]. Cytokeratin 18, an intermediate filament protein, is expressed in the cytoplasm of cytotrophoblasts and the syncytiotrophoblast but not by cells in the villous stroma [18]. To identify the products of activated caspases, we used the M30 antibody, which recognizes caspase-cleaved cytokeratin 18 (clCyt18) [28] and an antibody that recognizes nuclear poly-ADP-ribose polymerase only after it has been cleaved by caspases (clPARP).

We assessed the localization patterns of clCyt18 by co-staining tissues for E-cadherin, DNA and clCyt18 and examination by Z-stack confocal microscopy. In term, normotensive and complicated pregnancies, clCyt18 was detectable in villous cytotrophoblasts and presumed cytotrophoblast-derived fragments, as identified as regions enclosed by E-cadherin staining. In the term, normotensive and complicated pregnancies, similar patterns of clCyt18 localization were present. First, clCyt18 localized as filaments in E-cadherin outlined cytotrophoblasts which contained both nuclear DNA and long cytoplasmic processes, typically extending for $>20\ \mu\text{m}$ along the basement membrane, suggesting these cells retain extensive attachment to the basement membrane (Fig. 1A). Second, E-cadherin outlined regions expressed clCyt18 puncta (not shown) or as diffuse cytoplasmic staining in cells that contained intact (Fig. 1B) or fragmented (not shown) nuclear DNA. These cytotrophoblasts typically were rounded and lacked extensive cytoplasmic processes, suggesting partial detachment from the basement membrane. Finally, clCyt18 was present in E-cadherin outlined vesicles that lacked detectable DNA, both in vesicles of $>5\ \mu\text{m}$ diameter, which appeared to be undergoing (Fig. 1C), or to have completed (Fig. 1D; see serial confocal sections in Supplementary movie 1) detachment from the basement membrane, or in several neighboring vesicles $\sim 5\ \mu\text{m}$ diameter each (Fig. 1D, E; see serial confocal sections in Supplementary movie 2), which appeared to be detached from the basement membrane and entirely within the cytoplasm of the syncytiotrophoblast (Fig. 1E). Excluding regions with apparent fibrin-type fibrinoid (see below), the predominant pattern of clCyt18 expression in both the term and complicated pregnancies was in the $>5\ \mu\text{m}$ or $\sim 5\ \mu\text{m}$ diameter vesicles that lacked detectable DNA but were outlined by E-cadherin.

Although immunoperoxidase analyses do not allow co-localization of clCyt18 with E-cadherin, the observed patterns of clCyt18 localization were qualitatively similar to those obtained by confocal microscopy. clCyt18 was associated with individual nuclei (Fig. 2C, D) and in regions that were lacked DNA but were in size consistent with the larger (Fig. 2E, F) and smaller (Fig. 2G, H) diameter vesicles we identified by confocal microscopy. Importantly, many of the clCyt18-positive regions identified by immunohistochemistry (*e.g.*, Fig. 2 E–H) would likely be scored as localized regions of apoptosis within the syncytiotrophoblast, although the confocal analysis indicates these are always surrounded by E-cadherin and likely of cytotrophoblast origin.

Caspase-mediated apoptosis also results in the formation of cleaved PARP in the nucleus [9]. In complicated pregnancies, clPARP localization was restricted to cytotrophoblasts, as indicated by co-staining with E-cadherin (Fig. 3A), and clPARP was co-expressed with clCyt18 (Fig. 3B, C). clPARP was not present in regions that did not express clCyt18. Often,

cells expressing cIcPARP displayed fragmented DNA (Fig. 3A, B) or degraded DNA (Fig. 3C). These data are consistent with what we found previously in villi term, normotensive pregnancies and explants exposed to staurosporine [24] and, together, suggest that the full program of caspase-mediated apoptosis is occurring in apoptotic cytotrophoblasts in villi from term, normotensive and complicated pregnancies.

3.3. Higher levels of caspase-mediated apoptosis in cytotrophoblasts in pregnancies complicated by preeclampsia and IUGR

We tested the hypothesis that caspase-mediated apoptosis was elevated in villous cytotrophoblasts in complicated pregnancies (Fig. 4). Regions of fibrin-type fibrinoid were not included in this analysis. Compared to the ~1% basal apoptosis in cytotrophoblasts in term, normotensive control pregnancies ($n = 10$), apoptosis in cytotrophoblasts was significantly increased to ~4% in pregnancies complicated by preeclampsia ($n = 9$), to ~3% in pregnancies complicated with IUGR ($n = 5$), and to ~7% in pregnancies complicated by preeclampsia with IUGR ($n = 5$).

3.4. Caspase-mediated apoptosis is not detectable in intact syncytiotrophoblast but is present in regions with fibrin-type fibrinoid

If caspase-mediated apoptosis occurred in regions of syncytia that were intact, we would expect to identify cIcCyt18 in the syncytia but that were not enclosed by E-cadherin. However such regions were never identified in the term, normotensive or complicated pregnancies. In undamaged syncytia, cIcCyt18 and cIcPARP were always enclosed by E-cadherin. This result is consistent with our finding [24] that there was no detectable caspase-mediated apoptosis in intact syncytiotrophoblast of villi of placentas from term, normotensive pregnancies or in villous explants treated with vehicle control or staurosporine, as an inducer of apoptosis.

Previous electron microscopy studies [2,29] and immunohistochemical studies staining for cIcCyt18 [15,20] indicated that regions of villi with fibrin-type fibrinoid expressed the remnants of apoptosis. We examined regions of fibrin-type fibrinoid by confocal microscopy after staining for E-cadherin, DNA and cIcCyt18 (Fig. 5A–C) and by light microscopy after immunohistochemical staining for cIcCyt18 and co-staining for DNA (Fig. 5D–F). By both approaches, in both term, normotensive and complicated pregnancies, the majority (~90%) of regions that contained fibrin-type fibrinoid expressed cIcCyt18 in a punctate pattern with intact and/or fragmented nuclei. Thus, although the products of caspase-mediated apoptosis are not present in intact syncytium, they are present in regions with fibrin-type fibrinoid.

4. Discussion

Our data using high-resolution confocal immunofluorescence and detection of markers for apoptosis and the plasma membrane identify elevated apoptosis of villous cytotrophoblasts in pregnancies with preeclampsia and or/IUGR, compared to normotensive controls. Our data underscore the ambiguity that can result from analysis of trophoblast apoptosis using routine immunoperoxidase histochemistry, which does not allow for simultaneous detection of markers for the plasma membrane and for apoptosis. In the absence of co-localization, a marker for caspase-mediated apoptosis can improperly identify the apoptosis as occurring within the syncytiotrophoblast, when in reality the apoptotic region is of cytotrophoblast origin. The data further show that areas of intact syncytiotrophoblast do not experience apoptosis, suggesting there is endogenous resistance to caspase activation in the intact syncytium. Yet, cleavage of caspase substrates occurs in regions of villi that are marked by fibrin-type fibrinoid. We speculate that depletion of the villous trophoblast stem cell population in preeclampsia and IUGR limits the ability of villi to respond to needs for

enhanced syncytiotrophoblast formation by cytotrophoblast fusion, whether the needs are directed to villous development or to repair of injury of existing syncytiotrophoblast.

The qualitative morphologies of apoptotic, cIcCyt18-positive villous cytotrophoblasts and E-cadherin outlined vesicles in the complicated pregnancies studied were similar to those in villi from term, normotensive pregnancies [24], and are consistent with the progression of cytotrophoblast apoptosis in term and complicated pregnancies being similar to that of other epithelial cell types [28,30,31]. Our data suggest caspase activation and cleavage of cytokeratin 18 and other substrates occur initially in cytotrophoblasts that are well attached to the basement membrane, resulting in detachment from the basement membrane and rounding of the cell. Condensation, fragmentation, and degradation of nuclear DNA then occur, resulting in the eventual formation of large (>5 μm diameter), cIcCyt18-positive vesicles outlined by E-cadherin that lack detectable DNA. These cIcCyt18-positive vesicles then appear to undergo fragmentation, forming several small (<5 μm -diameter) vesicles outlined by E-cadherin that lack DNA and appear to reside entirely within the syncytiotrophoblast cytoplasm. These vesicles likely represent apoptotic bodies [32]. Because these small vesicles are the predominant localization of cIcCyt18 in term and complicated pregnancies, their generation appears rapid relative to their clearance.

Both our confocal microscope studies and previous EM studies [16] indicate apoptotic cytotrophoblasts and/or cytotrophoblast-derived vesicles can be localized within the syncytiotrophoblast cytoplasm. However, the mechanism by which this occurs is unclear. One possibility is that the syncytiotrophoblast acts as a non-professional phagocyte, similar to what has been seen for endothelial cells [33] and mammary epithelial cells [34], and engulfs the products of cytotrophoblast apoptosis by phagocytosis [35]. It is also unclear how the apoptotic bodies in the syncytiotrophoblast cytoplasm are eliminated, but possible mechanisms include autophagy [36,37] and release by an unknown mechanism into the maternal circulation [38,39]. Indeed, circulating trophoblast fragments have been assigned a role in the pathology of preeclampsia [39,40].

In pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR, villous cytotrophoblast apoptosis is significantly increased compared to term, normotensive pregnancies. In agreement with our *in vivo* results, Crocker et al. [41] previously found an increased susceptibility to apoptosis of primary trophoblasts isolated from patients with preeclampsia or IUGR and cultured *in vitro*. Importantly, cytotrophoblasts are a stem cell population that divides, differentiates, and fuses to replenish the syncytiotrophoblast. Thus, the higher level of apoptosis of villous cytotrophoblasts in complicated pregnancies could limit the availability of cytotrophoblasts, limiting repair of damaged regions of the syncytium, growth of the syncytiotrophoblast needed to cover the increased surface area of villi, or both, in the second half of pregnancy. The end result for either situation would be placental dysfunction and sub-optimal pregnancy outcomes, whether related to secretion, transport, or both.

We find no evidence that caspase-mediated apoptosis occurs in regions of intact syncytiotrophoblast *in vivo*, either in complicated pregnancies described here or in term, normotensive pregnancies described elsewhere [24]. In agreement with our finding of lower apoptosis *in vivo* of intact syncytiotrophoblast compared to cytotrophoblasts, cultured primary human trophoblasts that have differentiated to syncytiotrophoblasts are more resistant to apoptosis than are cytotrophoblasts [42–45]. Similarly, caspase-mediated apoptosis is markedly inhibited in muscle syncytia, compared to the mononucleated precursors [46–48]. Modeling studies suggest that apoptosis rapidly propagates after initiation at a region within a cell [49,50]. This model is supported by the experimental observations that the depolarization of mitochondria in a single region rapidly spreads as a

wave across mononucleated cells [51–54], through the syncytia of myotubes [53], and through the syncytia generated by fusion of HeLa cells [55]. We propose that there is functional significance for the repression of apoptosis in the syncytiotrophoblast. If repression was inhibited, the initiated apoptosis could propagate throughout the syncytium, resulting in a self-destructing syncytiotrophoblast and a resultant nonfunctional placenta.

Thus, we suggest that the products of caspase-mediated apoptosis in regions on villi with fibrin-type fibrinoid are not the result of apoptosis that occurs within a localized region of an intact syncytium that has cytoplasmic continuity with the rest of the syncytium. We postulate instead that the apoptosis in these regions occurs as a secondary response to physical damage and that caspase-mediated apoptosis is initiated only after physical isolation of a region of the syncytium from the rest of the syncytium [24]. Fragments of the apoptotic region could be released into the maternal blood and thereby contribute to the endothelial damage of preeclampsia [38,39].

In summary, we find that there is increased cytotrophoblast apoptosis in pregnancies complicated by preeclampsia, that co-staining for trophoblast plasma membranes and a marker of caspase activity is required to determine if apoptosis is in the cytotrophoblasts or syncytiotrophoblast, and we propose that the induction of caspase-mediated apoptosis is strongly inhibited in the syncytiotrophoblast to prevent catastrophic spreading of apoptosis. Our studies only address apoptosis in villi late in pregnancy and future work will be needed to see if the relative rate of caspase-mediated apoptosis is increased in complicated pregnancies earlier in pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

clCyt18	cleaved cytokeratin 18
clPARP	cleaved poly-ADP-ribose polymerase
IUGR	intrauterine growth restriction

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Fig. 1.

Patterns of clCyt18 in apoptotic villous cytotrophoblasts, as detected by immunofluorescence and confocal microscopy. Shown are maximal projections of confocal Z-stacks, with images oriented with the intervillous space to the left. Indistinguishable patterns of clCyt18 were observed in villi from term, normotensive pregnancies, or in pregnancies complicated by preeclampsia, IUGR, or both. clCyt18 was present in cytotrophoblasts as (A) as filamentous structures with stellate processes in cells that contained nuclear DNA, and, (B) as a diffuse staining pattern in cells with intact or fragmented nuclear DNA. These cells were typically round, suggesting detachment from the basement membrane. The most abundant clCyt18 expressing regions in villi from

normotensive or complicated pregnancies lacked nuclear DNA but were always outlined by E-cadherin (C–E), suggesting a cytotrophoblast origin. These regions were present as (C) large $>5\text{ }\mu\text{m}$ -diameter vesicles or as multiple vesicles of $\sim 5\text{ }\mu\text{m}$ -diameter (D, E; see Supplementary movies 1 and 2). Arrowheads indicate E-cadherin (green) outlining regions expressing clCyt18 (red). Dotted lines indicate apical surface of syncytiotrophoblast, as determined by E-cadherin localization observed in a long exposure.

Fig. 2.

E-cadherin and patterns of clCyt18 patterns in placental villi, as detected by immunohistochemistry and light microscopy. Images are oriented with the intervillous space to the left. (A) IgG control (B) E-cadherin staining identifies cytotrophoblasts that are interdigitated into the syncytiotrophoblast (red arrowhead) or underlying the syncytiotrophoblast (white arrowhead). (C, D) Regions of clCyt18 with apparently associated nuclear DNA (blue arrowheads). (E–H) Regions of clCyt18 lacking associated DNA, present as large structures (green arrowheads) or as small structures (black arrowheads).

Fig. 3. cIPARP expression and DNA fragmentation in apoptotic cytotrophoblasts. Shown are maximal pixel value projections of confocal Z-stacks, with images oriented with the intervillous space to the left. cIPARP was expressed in apoptotic cytotrophoblasts, as identified by co-staining for (A) E-cadherin or (B,C) cICyt18. Insets: (A, B) arrowheads indicate fragmented nuclear DNA. Arrowhead in (C) indicates degraded nuclear DNA in the apoptotic cytotrophoblast. Dotted lines indicate apical surface of syncytiotrophoblast, as determined by E-cadherin localization observed in a long exposure.

Fig. 4.

Apoptosis is increased in villous cytotrophoblasts in pregnancies complicated by preeclampsia. Box plot of percent clCyt18-positive cytotrophoblasts in term, normotensive and pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR. $*p < 0.05$ using the Wilcoxin–Mann–Whitney U test.

Fig. 5.

clCyt18 is expressed in regions with fibrin-type fibrinoid. (A–C) Confocal images of tissues stained for E-cadherin, clCyt18 and DNA. Shown are maximal pixel value projections of confocal Z-stacks. (D–F) Light microscopy images of tissues stained by immunohistochemistry for clCyt18 and DNA. Tissues are from the placentas of (A, D) a normotensive patient and patients with (B, E) preeclampsia or (C, F) preeclampsia with IUGR. Dotted lines in (A–C) indicate the surface of the villous, as determined by E-cadherin localization observed in a long exposure. Blue arrowheads in (C) indicated DNA of fragmented nuclei.

Table 1

Demographic characteristics of the study population.

	Term, normotensive (n = 10)	Preeclampsia (n = 9)	IUGR (n = 5)	Preeclampsia with IUGR (n = 5)
Mean age($\pm$ SD)	31.2($\pm$ 5.6)	30.6($\pm$ 6.1)	27.8($\pm$ 5.8)	23.6($\pm$ 4.6) ^a
Race				
White(%)	7(70%)	3(33.3%)	2(40%)	2(40%)
Black(%)	2(20%)	5(55.6%)	2(40%)	2(40%)
Hispanic(%)	0(0%)	0(0%)	0(0%)	0(0%)
Asian(%)	1(10%)	0(0%)	1(20%)	0(0%)
Other	0(0%)	1(11.1%)	0(0%)	1(20%)
Smoking(%)	1(10%)	1(11.1%)	1(20%)	1(20%)
Mean BMI($\pm$ SD)	29.8($\pm$ 6.7)	33.3($\pm$ 7.6)	27.0($\pm$ 10.9)	31.4($\pm$ 2.5)
Nulliparous(%)	6(60%)	4(44.4%)	3(60%)	3(60%)
Chronic hypertension(%)	1(10%)	4(44.4%)	0(0%)	1(20%)
Pre-gestational diabetes(%)	1(10%)	2(22.2%)	0(0%)	1(20%)
Mean gestational age at delivery ($\pm$ SD)	38.9($\pm$ 1.3)	35.3($\pm$ 5.3)	37.5($\pm$ 3.6)	29.8($\pm$ 2.1) ^a
Mean birth weight (grams, $\pm$ SD)	3454($\pm$ 594)	2595($\pm$ 918)	2399($\pm$ 109)	1200($\pm$ 463) ^b

IUGR, intrauterine growth restriction. SD = standard deviation.

^a $p < 0.05$, ANOVA, compared to term, normotensive.^b $p < 0.05$, ANOVA, compared with term, normotensive and preeclampsia.