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Endopolyploidization and the interstitial invasion of the supergiant trophoblast cells of the field vole *Microtus rossiaemeridionalis*

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ABSTRACT

The supergiant trophoblast cells characteristic of vole placenta prove to be highly invasive being found at the boundary of the decidualized endometrium and myometrium. Their size (100 μm and higher) suggests them to be highly polyploid, though their ploidy was not determined by now. We performed determination of the ploidy level of the supergiant trophoblast cells (SuGT) in order to verify whether the highly polyploid trophoblast cells are capable of deep intrauterine invasion. Anti-Cytokeratin trophoblast immunolabelling were performed to estimate the ways of the SuGT migration. DNA content measurement with help of image analysis was performed at the series of Feulgen-stained sections of the SuGT nuclei. The SuGT were observed to migrate through the endometrial stroma reaching myometrium. Most of the cells corresponded to 2048c-8192c; the maximum level was 16384c comparable to the salivary glands of *Drosophila*. The nuclei contained bundles of non-classic polytene chromosomes. At the final steps of differentiation when SuGT reach myometrium, the bundles of polytene chromosomes disintegrate into multiple separate endochromosomes. The supergiant trophoblast cells in *Microtus rossiaemeridionalis* represent an example of highly polyploid cells capable of deep intrauterine invasion.

1. Introduction

Trophoblast cells in the mammas with hemochorial placenta are characterized by their capability of migration into the uterine wall reaching myometrium. The invasive and phagocytose activity of the trophoblast cells appear to correlate with change of their capacity of proliferation and/or cell reproduction that includes modification of the cell cycle, such a peculiarity being strongly pronounced in rodents (Zybina and Zybina, 1996, 2005; Zybina et al., 2000). In this case, the trophoblast cells migrating into the depth of placenta have, as a rule, an optimal but not the highest, ploidy level characterized of the species (Zybina and Zybina, 2000, 2005).

Meantime, in the vole placenta the so-called supergiant trophoblast cells (Zybina and Zybina, 1985, 1996) or the interstitial giant cells (Stewart and Clarke, 1993a,b, 1999) have been observed. These cells and their nuclei possess an extraordinary large size (more than 100 μm) that suggests very high ploidy level. The enormous cells appear highly invasive; they are capable of penetration into the depth of uterine wall reaching myometrium (Zybina and Zybina, 1985, 1996; Stewart and Clarke, 1993a,b, 1999).

By the present time, there is no agreement regarding the relationship between the trophoblast invasiveness and its capability of proliferation and polyploidization in different mammalian species (Zybina and Zybina, 1985, 1996) as well as between the trophoblast invasiveness and its capability of proliferation (Zybina and Zybina, 1996, 2005; Zybina et al., 2000, 2001, 2002, 2004; Kaufmann and Castellucci, 1997; MacAuley et al., 1998; Kemp et al., 2002). Therefore, in this study, we performed determination of the ploidy level of the supergiant trophoblast cells (SuGT) to verify whether the highly polyploidy trophoblast cells are capable of deep, perhaps interstitial, intrauterine invasion. Besides, this study was aimed to determine the mechanism of genome multiplication of the SuGT.

2. Materials and methods

2.1. Materials

Placentae of the East European field vole were used in this work; this species was initially described as *Microtus subarvalis* (Meyer et al., 1972) but later on, according to the nomenclature codex, was renamed to *Microtus rossiaemeridionalis* (Malygin, 1983). Placentas were supplied by E.D. Skholl (Institute of Cytology RAS). To obtain the placentas, female were caged with males, and the morning on which the vaginal plugs were confirmed was designated by the day 1 post-coitum (dpc). The placentas were fixed with 3:1 mixture of ethanol and glacial acetic acid. The total of 44 fetuses of 16

Abbreviations: SuGT, supergiant trophoblast cells; dpc, day post-coitum.

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pregnant females were used, 24 fetuses being of 8 females at 12th (dpc), in twos fetuses of 2 females being at the 9th, 10th, 11th, 13th and14th dpc. The fixed placentas were dehydrated in a graded series of ethanols and embedded in paraffin; from this material 10 μm sections were made. The series of sections of 18 placentas at 12th dpc were Feulgen stained (hydrolysis in 1N HCl at 60 °C, incubation in the Shiff reagent for 3–4 h). The 10 μm sections of placentas at 9–14 dpc were also stained with Boemer hematoxylin, bromphenol blue to reveal protein and methyl green/pyronin to reveal RNA.

2.2. Image analysis cytophotometry

The DNA content in each SuGT nucleus was measured at the series of sections. The integral optical density of each section was determined with the use of "Videotest" image analyzer composed of the CPT 83 digital CCD-video camera (Chipper, USA) installed on an EC Bimam-13 microscope and computer IBM PC. Input of the image and measurement of the integral optical density was performed using Videotest-Morpho software (St.-Petersburg). An objective of 20 × 0.65 and an interference filter at 550 nm were used. The values of integral optical density were summed over all sections belonging to one nucleus. 106 nuclei of SuGT were analyzed. To assign ploidy of the SuGT nuclei, the data on the DNA content were compared with the data obtained on lymphocyte nuclei that are known to be diploid (2c).

2.3. Immunohistochemistry

Cytokeratin immunolabelling was performed to prove the trophoblast origin of the SuGT within the decidualized endometrium. Immunolabelling for Cytokeratin was carried out according to the slightly modified standard procedure (Mühlhauser et al., 1993). The monoclonal anti-human antibodies Cytokeratin pan were used as the primary antibodies. The deparaffinized sections were incubated with bromelin for 15 min at 37 °C. Endogenous peroxidase activity was quenched by the 15 min incubation with 3% hydrogen peroxide. Non-specific antibody binding was block by incubation for 30 min in rabbit serum, then the sections were incubated with the primary antibodies diluted 1:300 in 0.6% Tris buffer, 1.5% BSA (pH 7.6), then for 30 min with biotynilated rabbit antimouse antibody (1:400), and with Streptavidin-peroxidase (1:400), 3′-3-diaminobenzidine was used as a substrate for peroxidase.

3. Results

The supergiant trophoblast cells are separated from the continuous layer of the primary and secondary giant trophoblast cells and migrate for a large distance of the decidua basalis (Fig. 1). Those are mononuclear cells that stand out by their large size: diameter of their nuclei is mostly 100–200 μm and higher. Meantime, the supergiant trophoblast cells are rather rarely encountered: their number does not exceed, as a rule, 8–10 per placenta of the field vole though they occupy a significant part of the decidualized endometrium.

The SuGT were observed beginning from the 9th day of gestation. They lie as single enormous Cytokeratin-positive cells mostly in the depth of the decidualized endometrium. By the 10–11 dpc they may be encountered mostly at the border of decidua and myometrium (Fig. 1b and c) and, sometimes, reach the border of serosa. Despite their large size, they detach from the continuous layer of trophoblast of the fetal part of placenta and migrate in mesometrial direction. They most probably move via sinuses with the maternal blood and the interstitial spaces (Fig. 2a and b). At the 14th dpc some of SuGT show many signs of degeneration.

To prove SuGT polyploidization, cytophotometric measurement of the DNA content in their nuclei was performed. The SuGT have the ploidy level mostly 2048c-8192c (Fig. 3), the single nuclei reach 16384c. Thus, the supergiant trophoblast cells proved to be highly polyploid. Moreover, their ploidy level proved to exceed significantly the ploidy level of the secondary giant trophoblast cells of the field vole being 16c-1024c (Zybina et al., 1975a, 2003).

In the SuGT nuclei, the bundles of non-classic polytene chromosomes were found (Fig. 4a and b). As compared to the classic polytene chromosomes of Dipterans they appeared to be shortened; their chromatid did not show the tight attachment at their full length. Therefore, they did not show a clear-cut disc pattern that was observed on the classic polytene chromosomes from the salivary glands of *Drosophila*, *Chironomus* and other Dipterans (Beermann, 1962, 1972; Kiknadze, 1972; Zhimulev, 1992). The bundles of the non-classic polytene chromosomes in the supergiant trophoblast cells were tightly attached to the nuclear envelope, some of them also contacted a large single nucleolus. In the most deeply invading supergiant trophoblast cells, the bundles of polytene chromosomes disaggregate into the olygotene chromatin fibers or endochromosomes (Fig. 4c). The nucleoli also prove to disintegrate giving rise to the numerous small nucleoli attached to the thin chromatin fibers. These pictures suggest a transformation of the polytene nucleus into the "polygenomic" one at the late stages of the field vole SuGT differentiation.

4. Discussion

According to the present study, the field vole SuGT prove to be highly polyploid. Their ploidy level – 2000C-16000C – exceeds significantly the ploidy of the primary and secondary giant trophoblast cells in rat, mouse and field vole (Zybina et al., 1975a,b; Zybina and Zybina, 1996). It appears to be the maximum for the trophoblast cell populations studied by the present time and for the mammalian tissues as a whole. Such a ploidy level is comparable to the ploidy of Dipteran salivary gland cells, i.e. cells in which the classic polytene chromosomes were found (Beermann, 1962, 1972; Kiknadze, 1972; Zhimulev, 1992). Interestingly, the present study reveals the polytene chromosomes in SuGT, though they are non-classic ones, characteristic of the giant trophoblast cells in rodents and carnivores (Zybina and Zybina, 1996; Zybina et al., 2001). Both the SuGT and the secondary giant trophoblast cells appear to reach their high ploidy level via endoreduplication, as polytene chromosome formation results from a series of endoreduplication cycles (Nagl, 1978, 1995; Brodsky and Uryvaeva, 1985; Edgar and Orr-Weaver, 2001). Besides, both the SuGT and the secondary giant trophoblast cells, most probably, undergo the same cycles of genome multiplication and rearrangements of the polytene nucleus into the polygenomic one (Zybina and Zybina, 1996). Thus, they first undergo polytenization, and then the non-classic polytene chromosomes disintegrate into endochromosomes.

Meantime, the SuGT are highly invasive. They are capable of migration into the depth of the uterine wall, and most of them penetrate into endometrium, the single cells reaching serosa. Thus, despite of their exclusive size, and the highest ploidy level of their nuclei these cells are capable of the deep invasion of the uterine wall migrating via both lacuna spaces and interstitially. The similar peculiarities were described in *Microtus agrestis*. The individual giant trophoblast cells were lying close to the giant cell layer enclosing the embryo, the others giant cells lying more deeply in the developing decidua as early as at the 6 day of pregnancy in the developing placenta (Stewart and Clarke, 1993b). These cells were generally round and ovoid in shape although some of them appeared to have short broad pseudopodium-like projections. Later on, at 8 day of pregnancy, the cells become much larger, being up to 200–300 μm in size. As a rule, they were located in the more peripheral area of decidua, a part of the interstitial trophoblast cells were adjacent to the myometrium (Stewart and