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Genome Modifications Involved in Developmental Programs of the Placental Trophoblast

Tatiana G. Zybina

Abstract

The placental trophoblast cells give an example of profound genome modifications that lead to whole-genome multiplication, aneuploidy, under-replication of some genes or their clusters as well as, by contrast, gene amplification. These events are included into program of differentiation of functionally different cell lineages. In some cases the trophoblast cell differentiation involves depolyploidization achieved by non-mitotic division. Aneuploidy may be also accounted for by the unusual mitoses characteristic of Invertebrates and plants; in mammalian it may result from hypomethylation of centromere chromosome regions. The giant (endopolyploid) trophoblast cells organization includes "loose nucleosomes" accounted for by the non-canonical histone variants, i.e. H2AX, H2AZ, and H3. 3 . In the human extravillous trophoblast cells that, like murine TGC, invade endometrium, there occured significant changes of methylation as compared to non-invasive trophoblast cell populations . Meantime, some genes show hypermethylation connected with start of trophoblast lineages specification. Thus, despite the limited possibilities of chromosome visualization trophoblast cells represent an interesting model to investigate the role of modification of gene copy number and their expression that is important for the normal or abnormal cell differentiation.

Keywords: trophoblast, genome, chromatin, chromosomes, histones, polyploidy, aneuploidy, gene amplification

1. Introduction

The placental trophoblast gives examples of genome multiplication included in the program of their lifespan during embryogenesis [1–8]. The degree of ploidy varies between different trophoblastic cell lineages and among different mammalian species indicating that increase of chromosomes or gene copies is required for functional activity of the cell or is dictated by the lifestiyle of a species [3, 5, 9]. Besides euploidy, the trophoblast cells show aneuploidy examples that varies between different mammalian species and may reflect both differences in the genome structure and, again, requirements of species-specific way of life. Here we would like to match the above-mentioned genome peculiarities with the cell nucleus organization of the trophoblast cells that may underlie their specific functions.

2. Poly- and aneuploidy, their origin and significance

Recently, a notion dominates that the multifold genome multiplication is achieved by modified cell cycles. Among them the shortest one leading to the highest levels of ploidy is characterized by alternating DNA synthesis (S) and Gap (G) phases in the absence of intervening mitoses, karyokinesis, and cytokinesis; a series of these shortened cycles allows cells to achieve high level of ploidy that may exceed 1000c [10–13].

The trophoblast cells that form a barrier between semiallogenic fetal (trophoblastic) and maternal (decidua) tissues probably require mechanism(s) to sustain maternal-fetal tolerance achieved by different mechanisms. For example, the trophoblast cells secrete a range of cytokines and chemokines thereby contributing to the process of immune regulation at the placental–maternal interface [14, 15]. On the other hand, as we stated previously, the TGC multifold genome multiplication also may protect their genome from mutagenic effect of the DNA of the phagocytosed maternal cells [16, 17]. Besides, some of the TGC functions of a barrier may be performed due to their giantism. TGC produce enormous keratin-positive sprouts that allow them to phagocytose accumulations of decidual cells and simultaneously to sustain the continuous TGC layer at the border with decidua [18]. Destruction of the cytokeratin 8 and 19 results in disruption of integrity of the murine giant trophoblast cell layer [19], which result in embryo death.

In distinct from the primary and secondary TGC, the low-ploid trophoblast cells in rat and mouse placenta show high proliferative activity and, being protected by a TGC barrier, accumulate a great bulk of cells that differentiate into a range of trophoblast cell subtypes, some of them form placental barrier supplying embryo by nutrition and oxygen; other subtypes are involved in glycogen storage, hormone production and deep intrauterine invasion [20–26].

The lifespan of the endoreduplicated TGC ends in depolyploidization via non-mitotic division of the giant nucleus or nuclear whole-genome fragmentation. In this case, division is achieved without complete chromosome condensation and their arrangement in metaphase plate, spindle formation and poleward chromosome movement. DNA content as well as nucleoli, heterochromatin and gonosomal chromatin bodies distributed into "subnuclei" according to their ploidy levels [3, 27–30]. By now, it is possible to consider it as variant of so called "polyploidy cycle" [31–33] that implies alternation of diploid and polyploid state in a cell lineage. It should be noted that such a phenomenon is fairly rare encountered in the cell lifespan and may be found in the "ancient" organisms like Protists [31–33] and some Invertebrates [34]. In the multicellular Invertebrates and Plants a wast majority of the differentiated cell types are endopolyploid [35, 36]. In Vertebrates, most cells are diploid, and the mammalian trophoblast cells, probably, represent an example of a recapitulation to some ancient forms of cell cycle and cell lifespan similar to protists and Invertebrates.

As to depolyploidization in TGC of rodent placenta, it should be emphasized that they do not belong to the complete polyploid cycle because they do not give rise to the cells capable of self-reproduction because they cease DNA replication shortly before the birth that probably prevents a massive proliferation of semiallogenic embryonic cells inside the maternal tissues.

As we stated in our previous paper, depolyploidization probably may result in aneuploidy in the trophoblast cells [5] because such a way of cell division, most probably does not ensure precise distribution of all chromosomes into dauther cells. Surprisingly, aneuploidy combined with polyploidy were recently reported as a factor of adaptation to the stressful conditions [37, 38]. Hepatocytes represent a cell

type capable of high mitotic activity [39]. In the polyploid cells multipolar mitoses are encountered that may result in cells of lower ploidy, some of them were aneuploid [40]. Thus, in mice with knockdown of the genes *E2f7* and *E2f8* that regulate polyploidy in the liver, the amount of polyploid hepatocytes reduced fourfold; interestingly, nearly all hepatocytes became euploid [36]. Therefore, aneuploid cell resulted from polyploid ones. These mice were bred to tyrosinemia mice. As a result, although tyrosinemic mice were more susceptible to morbidities and death, they developed regenerating nodules similar to the control mice. Notably, the nodules in tyrosinemic livers were generated by aneuploidy; moreover, the mutation of *E2f7* and *E2f8* were inactivated [40]. The authors state that polyploid hepatocytes are necessary for the formation of aneuploid cells that can facilitate adaptation to chronic liver diseases.

In the placental trophoblast wide variability in different mammalian species show aneuploidy. In rodents, TGC of rat and mice undergone genome segregation via nuclear fragmentation, showed a great number of DNA values intermediate between ploidy classes [27]. It may be accounted for the deviation from the regular chromosome distribution rather than S-phase because at this developmental stage TGC do not proceed cell cycle and DNA replication that would be a reason of intermedial DNA content values. In contrast, in another rodent, field vole *Microtus rossiaemeridionalis*, TGC demonstrate the clear-cut classes of ploidy from 1c to 16 c [28]. Much more DNA content variability was found in the silver fox placenta [41, 42]. By contrast, silver fox placenta, especially its invasive trophoblast, shows a notable fluctuation of ploidy and a variability of patterns of polyploidization within the same cell lineage including aneuploidy and genome multiplication pathways (endoreduplication, classic endomitosis, depolyploidization).

It may reflect the necessity of different strategies that may be useful for maintaining the lengthy pregnancy [5, 42]. In the silver fox placenta, upon polyploidization, a considerable deviation from $(2^n)c$ was found, with a tendency to $2^n \times 3c$, and there were a great variety of intermediate values suggesting a significant incidence of aneuploidy [41]. We suggested that it may serve a source of genome variability, in particular, hetero- and homozygosity that may be useful to select a more specific response to stress factors. Unlike small rodents such as mouse, rat and field vole, whose pregnancy do not exceed 30 days, in the fox placenta aneuploid trophoblast cells may have a protective effect during 6 months of intrauterine development [5].

3. Underreplication and amplification of some genes and clasters regulate the giant trophoblast cells differentiation

The cells undergone endoreduplication and formation of the classic polytene chromosomes are known to underreplicate a significant amounts of DNA [43, 44].

Recently it has been found underreplication (UR) of some chromosome regions and genes in the murine giant trophoblast cells. TGCs of the mouse placenta contain 47 regions, totaling 138 Megabases, where genomic copies are underrepresented [45]. UR domains originate from a subset of late-replicating heterochromatic regions containing gene deserts and genes involved in cell adhesion and neurogenesis. Interestingly, both size and degree of depletion of UR domains gradually progresses during early gestation. Thus, all UR domains at 9.5th day are also present at 8.0th day, and UR domains at 9.5 gestation day are also more numerous, larger and more depleted. However, unlike between 8.0th and 9.5th day, where the degree of depletion expanded, there were no significant change from 9.5th and 16.5th day.

Notably, 8-10th day of gestation in mice corresponds to the placenta formation whereas at 10-16th days well-developed placenta functioning takes place. The authors [45] note that the increase in UR domain size and degree of underrepresentation from 8.0th to 9.5th day is linked to the "robust" endocycles of early gestation [46].

Besides, it should be mentionned that, during the late stages of TGC lifespan, new underreplicated regions are also formed but they are more stochastic, less reproducible, and significantly smaller than those conserved between all stages [45]. Notably, underreplication of TGC coincide with period of the most significant stages of TGC invasion and anchoring to endometrium and is integrated in its developmental program.

The above-mentioned data also show that UR domains are formed from a specific class of late-replicating heterochromatic regions that contain mainly non-coding DNA, suggesting that UR domains are not simply a byproduct of late-replicating heterochromatin, but are a precisely regulated subset of DNA sequences. The authors come to conclusion that presence of UR domains in *Drosophila* endo-reduplicated cells and in murine TGC is an example of convergent evolution. In this case UR contributes to accelerating the cell cycles that makes possible fast rate of development both in flies and in mice [45].

The underreplication in the endoreduplicated trophoblast cells not only may fasten the cell cycles but also be important for the TGC specific functions. Thus, Hannibal et al. [45] also note that UR domains are enriched for specific classes of genes involved in cell adhesion and neurogenesis. It is still difficult to find an explanation for the UR of specific genes and gene clusters. It can only be assumed that a certain number of gene copies is optimal in a given cell type. UR of separate genome regions at the background of its multiple duplication makes it possible to fine-tune the number of functioning copies necessary for performing specific functions.

In some cases, significance of UR was clearly demonstrated. Thus, downregulation of genes that regulate cell adhesion, junction and related cytoskeleton rearrangements is necessary for trophoblast EMT transition and invasion in both mice and humans [47, 48, 58]. Upregulation of genes in the SLIT/ROBO neuronal guidance system in the human placenta has been found to be bound with preeclampsia [49]. It is possible, placenta oxygenation requires precise specific function of SLIT/ROBO signaling achieved by UR of its genes.

Therefore, significance of endoreduplication is not only multifold genome duplication itself and enlargement of the cell that may be of significance for TGC barrier function but also a possibility of a fine regulation of a number of functional gene copies to provide cells capabilities to accomplish some functions necessary at the precise stages of development.

4. Amplification of some genes significant for the pregnancy also takes place in TGC

The mammalian polytene chromosomes may also undergo amplification of specific gene cluster. In the murine placenta TGC, five amplified regions were found using whole-genome sequencing and digital droplet PCR [50]. All the gene clusters are known to play key roles in mammalian placenta development and maintenance: the prolactins that regulate trophoblast cell lineage differentiations [51], serpins [52] and cathepsins [53] that promote trophoblast invasion, as well as (NK)/C-type lectin complex that play a crucial role in the feto-maternal cross-talk [54–56].

Therefore, amplification at selective genomic regions is another important mode of genome regulation in placental TGCs.

5. Unusual chromatin structure of TGC

Besides the non-classic polytene chromosomes in rodent placenta TGC [5, 26], some details of unusual chromosome structure have been revealed recently in the endoreduplicated TGC of mice. In the course of differentiation of TSC into TGC, expression of most genes encoding canonical histone were downregulated [1] By contrast, genes encoding non-canonical histones - H2AX, H2AZ and H3.3 did not show downregulation. The micrococcal nuclease digesion assay as well as nucleosome stability assay using a microfluidic devise showed that chromatin progressive loosening of chromatin in the course of TSC differentiated. Experiments combining H3.3 knockdown and overexpression showed that variant H3.3 resulted in formation of the loose nucleosomes in the murine TGC [1].

The presence of H2AZ and H3.3 in the genome potentially correlated with actively transcribed genes, indicating that H2AZ and H3.3 were necessary for creating relaxed and transcriptionally active chromatin structures [57–59]. Therefore, H2AX, H2AZ, and H3.3 histone variant may be responsible for the formation of a loose nucleosome structure that was unique to TGCs [1].

Interestingly, knockdown of H3.3 variant in the differentiationg TSCs significantly decreased the number of cells containing more than 4n DNA content compared to the control cells [1]. Therefore, switch to the non-canonic histone variants seems to be a prereqisite of the trophoblast cell endoreduplication. On the other hand, loose chromatin organization may, like underreplication, promote fastening the modified cell cycle that allow reach multifold (up to 512c and higher) genome multiplication and formation a giant trophoblast cell layer at the border with semiallogenic maternal tissue.

The unusual chromatin status is revealed, in particular, in the organization of the inactive X-chromosome of the murine TGC [60]. Thus, investigation of the precise temporal and lineage-specific X-inactivation status of several genes in postimplantation mouse embryos showed stable gene silencing in most lineages, with significant levels of escape from XCI mainly in one extra-embryonic cell type - TGCs. It has been found that the *Xist* RNA-coated X chromosome has a highly unusual chromatin content in TGCs, presenting both heterochromatic marks such as H3K27me3 and euchromatic marks such as histone H4 acetylation and H3K4 methylation. This unusual combination of silent and active features is likely to reflect, and might underlie, the partial activity of the X chromosome in TGCs. However, some key loci seem to require dosage compensation in TGC that probably points out to combination of the relaxed and silenced gene expression as a specific mode of gene activity regulation in a condition of chromatin unusual organization in TGC.

6. Hypomethylation of human and rodent placenta

Methylation status provides some new insight in the understanding of the trophoblast cell organization that underly their unique features. The human placental trophoblast shows general global hypomethylation [61]. It is possible that the loose-nucleosome structure of TGC in murine placenta and global thryphomethylation in human placenta are similar phenomena. In human placenta, genome-wide hypomethylation, coupled with gene-specific hypermethylation of tumor-suppressor genes, is a common feature of human cancers [64]. Interestingly, the placenta parallels human cancers in both the overall decreased level of genomic DNA methylation and the specific hypermethylation of several tumor suppressor genes [61–64].

Such a parallel with carcinogenesis may be connected with the trophoblast invasive pathways. Inhibition of DNA methylation by 5-azacytidine treatment disrupts trophoblast invasive and migratory potential *in vitro* [65] and proper placental development *in vivo* [66]. Thus, threatment of BeWo cell with DNA methyltransferase inhibitor, 5′-aza-2′-deoxycytidine (AZA) resulted in conversion to non-migratory phenotype. AZA was found to increase mRNA level of E-cadherin and plakoglobin, components of cell junction structures - zonula adherens and desmosomes [65]. AZA treatment also resulted in decrease their gene promoter activity and protein levels. Increases in plakoglobin and E-cadherin promoter activity and inhibition of BeWo cell migration was also achieved with small interfering RNA-mediated depletion of both *DNMT-3a* and *DNMT-3b* [65].

Meantime, beside the trophoblast invasion, some DNA methylation (locus-specific and/or repeat-based) is important for differentiation of the functionally different trophoblast lineages forming the placental barrier and performing other placental specific functions. Thus, most homeobox genes were hypomethylated in the human placenta throughout gestation. Nevertheless, three homeobox genes, *TLX1*, *HOX10* and *DLX5* showed progressive methylation and decrease of their expression in the course of pregnancy – from first to third trimester. Using siRNA treatment the key role of *TLX1, HOXA10* and *DLX5* in trophoblast proliferation, differentiation and apoptosis was demonstrated [67]. It cannot be ruled out that progressive methylation of some homeobox genes promotes trophoblast differentiation into highly proliferative ones that, in turn, gives rise to villous cyto- and syncytiotrophoblast and the invasive trophoblast lineages. The data suggest an important role of several homeobox gene methylation in gene expression in the course of placenta formation.

In mice, *Dnmt3L* is expressed at high levels in the chorion, containing a multipotent trophoblast stem cell population. Disruption of *Dnmt3L* disturbs placental development including spongiotrophoblast and labyrinth malformation, leads to excess of TGC and defective attachment of the chorion to the ectoplacental cone. Excessive TGC development indicates that such a phenotype is bound to the hypomethylation. This is associated with an arrest of proliferation of the extraembryonic tissue [68]. It may be suggested that *Dnmt3L*-mediated *de novo* methylation is connected with initiation of differentiation of trophoblast into multiple lineages that imply maintenance of high level of mitotic proliferation regulated by Mash2 expression as well as syncytiotrophoblast formation demonstrated by *GCM1* expression [68]. It suggests that *Dnmt3L*-mediated *de novo* methylation is critical for proper placental development in mice [61].

Demonstration of placenta-specific hypomethylation of the *DNMT3L* gene supports a role in human placental development [61] probably bound to the start of differentiation of trophoblast into a range of lineages with different proliferative capacity.

7. Hypomethylation and chromosome decondensation may involve genome rearrangement

Thus, the trophoblast cell population are prone to hypomethylation that may be a prerequisite of some other cytogenetic effects. Thus, prolonged culture of normal chorionic villus cells involves chromatin decondensation and rearrangements that mimics the ICF syndrome, i.e. immunodeficiency, centromeric region instability, and facial anomalies [69]. Thus, untreated cultures from normal chorionic villus or amniotic fluid-derived samples displayed dramatic cell passage-dependent increases in aberrations in the juxtacentromeric heterochromatin of chromosomes 1 or 16

(1qh or 16qh). By passage 8 or 9, 82 ± 7% of the chorionic villi metaphases from all eight studied samples exhibited 1qh or 16qh decondensation and 25 ± 16% had rearrangements in these regions. At early and late passages, chorionic villi DNA was hypomethylated, and amniotic fluid DNA was hypermethylated both globally and at Sat2. *DNMT1*, *DNMT3A*, or *DNMT3B* RNA levels did not differ significantly between chorionic and amniotic cultures, or late and early passages. Sat2 hypomethylation may favor 1qh and 16qh anomalies because the chorionic villi cultures, with their Sat2 hypomethylation, displayed 1qh and 16qh decondensation Therefore, hypomethylation characteristic of the embryonic and trophoblast cells may affect the structure heterochromatin regions leading to chromosome rearrangement that may be a source of genome variability at the stage of differentiation of trophoblast cell population.

8. Conclusion

The data of the present paper indicate that the multifold genome multiplication is not the only peculiarity of the placental trophoblast cell lineages. There are a range of other peculiarities that, most probably, result from cell cycle modification or play a role connected with genome duplication. Thus, underreplication and gene amplification may result in fine orchestration of gene copies necessary at a definite trophoblast cell line in order to accomplish its function. "Loose nucleosomes", most probably, represent one of peculiarities of endoreduplication; it cannot be ruled out that lack of mitoses do not require chromosome to proceed all levels of condensation and packaging of chromosomes that makes possible fastening of replication cycles and high level of transcription of different factors regulating formation of the provisory organ – placenta. Unmethylation of chromosomes including centromere regions may contribute to chromosome rise of aberrations that may be a source of genetic variability and selection of the optimal genetic structure that may have an adaptive effect during pregnancy.

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Conflict of interest

The author declares no conflict of interests.

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