Placenta 59 (2017) 46–56

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Dynamic expression of TET1, TET2, and TET3 dioxygenases in mouse and human placentas throughout gestation

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ARTICLE INFO

Article history:
Received 13 June 2017
Received in revised form
7 September 2017
Accepted 18 September 2017

Keywords:
5-Methylcytosine
5-Hydroxymethylcytosine
DNA methylation
Trophoblast
Placenta development

ABSTRACT

Introduction: Throughout pregnancy, the placenta dynamically changes as trophoblast progenitors differentiate into mature trophoblast cell subtypes. This process is in part controlled by epigenetic regulation of DNA methylation leading to the inactivation of 'progenitor cell' genes and the activation of 'differentiation' genes. TET methylcytosine dioxygenases convert 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) during DNA demethylation events. Here, we determine the spatio-temporal expression of TET1, TET2, and TET3 in specific trophoblast cell populations of mouse and human placentas throughout gestation, and consider their role in trophoblast cell differentiation and function.

Methods: In situ hybridization analysis was conducted to localize *Tet1*, *Tet2*, and *Tet3* mRNA at key stages of mouse placental development. The distribution of 5-mC and 5-hmC in these samples was also evaluated. In comparison, expression patterns of TET1, TET2, and TET3 protein in human placentas were determined in first trimester and term pregnancies.

Results: In mouse, *Tet1-3* mRNA was widely expressed in trophoblast cell populations from embryonic (E) day 8.5 to E12.5 including in progenitor and differentiated cells. However, expression became restricted to specific trophoblast giant cell subtypes by late gestation (E14.5 to E18.5). This coincided with cellular changes in 5-mC and 5-hmC levels. In human, cell columns, extravillous trophoblast and syncytiotrophoblast expressed TET1-3 whereas only TET3 was expressed in villus cytotrophoblast cells in first trimester and term placentas.

Discussion: Altogether, our data suggest that TET enzymes may play a dynamic role in the regulation of transcriptional activity of trophoblast progenitors and differentiated cell subtypes in mouse and human placentas.

1. Introduction

The transition from progenitor to differentiated cell requires dynamic epigenetic regulation. DNA methylation is a stable epigenetic mark that is generally associated with transcriptional silencing. Occurring mainly at CpG dinucleotides, DNA methylation is catalysed by DNA methyltransferases (DNMTs). Conversely, the Ten eleven translocation (TET) family of methylcytosine dioxygenases actively demethylate cytosines by targeting 5-methylcytosine (5-mC) for oxidation to generate 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC) [1,2]. In general, 5-hmC is transient in the epigenome and found at a lower levels than 5-mC [3]. However, some cells contain high levels

Abbreviations: 5-caC, 5-carboxylcytosine; 5-fC, 5-formylcytosine; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; DNMT, DNA methyltransferase; E, embryonic day; EPC, ectoplacental cone; ES cells, embryonic stem cells; EVT, extravillous cytotrophoblast; GlyT cells, glycogen trophoblast cells; ICM, inner cell mass; SpT, spongtiotrophoblast; SynT, syncytiotrophoblast; TET, Ten eleven translocation methylcytosine dioxygenase; TGCs, trophoblast giant cells.

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https://doi.org/10.1016/j.placenta.2017.09.008
0143-4004/

of 5-hmC, such as embryonic stem cells (ESCs) [4] and Purkinje neurons [5]. While the role of 5-mC in transcriptional regulation is well studied, the function of 5-hmC is less clear [6,7]. Nonetheless, DNA modified with 5-hmC is generally present in euchromatin suggesting that it promotes transcriptional activity [8,9]. Trophoblast cells have hypomethylated DNA relative to the embryo [10], which is necessary for normal placental development and therefore, a successful pregnancy. Gene knockout studies indicate that TET enzymes are critical for differentiation of a wide range of cell populations including ESCs [11], blood [12], and skeletal cells [13]. However, the role of TET enzymes in trophoblast differentiation and function is unclear.

While architecturally different, both humans and mice have hemochorial placentas that comprises of three main layers: an outer maternal layer containing decidual cells and vasculature that brings maternal blood to and from the conceptus; a 'junctional' region that attaches the placenta to the uterus and consists of trophoblast cells with invasive and/or endocrine functions; and an inner layer composed of highly branched placental villi consisting of outer trophoblast cells that are bathed in maternal blood and surround an inner core of stroma and fetal capillaries [14]. Placenta villi are where maternal and fetal blood come in close proximity for nutrient and waste exchange.

During human placental development, extravillous cytotrophoblast (EVT) cells differentiate from progenitors in cell columns and invade into the uterine spiral arteries [15]. Villous formation results when the chorionic plate gives rise to stem villi, which branch to form terminal villi with a continuous layer of multinucleated syncytiotrophoblast cells that directly contact maternal blood [15]. Beneath these cells lie a progenitor population of cytotrophoblast that fuse to form syncytiotrophoblast cells [15]. In mice, primary trophoblast giant cells (TGCs), which provide a barrier between the maternal decidua and the conceptus, the ectoplacental cone (EPC), and the chorion form by embryonic (E) day 8.0 [16]. The EPC contains progenitor cells of the junctional zone including spongiotrophoblast (SpT) cells, glycogen trophoblast (GlyT) cells and some TGC subtypes [17,18]. Alternatively, the chorion contains syncytiotrophoblast progenitor cells [19]. Upon chorioallantoic attachment at E8.5, branching morphogenesis is initiated and villi form the labyrinth layer [19]. The labyrinth is functional by E10.5 when maternal and fetal blood circulations are established and separated by a trilaminar layer of trophoblast cells including the sinuisoidal-TGCs that contact maternal blood, and a bilayer of syncytiotrophoblast cells that surround fetal capillaries [14,20]. Defects in mouse and human placenta development have deleterious effects on fetal development [14].

Though some differences exist, mechanisms of epigenetic regulation are largely thought to be analogous in mouse and human placentas. Both models contain imprinted genes that are regulated by DNA methylation and are critical for placental development (e.g. *Igf2*, *H19*, *Mest*, *Cdkn1c*, etc.) [21]. As in the mouse, 5-mC, 5-hmC, and histone methylation marks are present in human trophoblast cells during gestation [22]. In the early mouse embryo, patterns of 5-mC and 5-hmC differ between progenitor and differentiated trophoblast cells [23] suggesting differential regulation of methylation as cells become specified. In ESCs [11] and the embryo proper [24], TET enzymes promote differentiation, though similar evidence is lacking in the trophoblast lineage.

Placental development is divided into the 'development phase' when trophoblast cell proliferation and differentiation occurs, and the 'mature phase' when the placenta increases in size and blood volume due to cell morphogenesis and dilation of blood vessels [25]. Indeed, the transition from the 'development' to 'mature' phase is marked by a transcriptional change of ~4000 genes including cell cycle, cell growth, and morphogenesis genes [26]. It is hypothesized that DNA methylation machinery may be implicated in this process, although the extent to which TET enzymes are involved is unknown. Loss of *Tet* expression in the *Tet1* knockout [27] and the *Tet1/2* double knockout [28] mice results in postnatal and prenatal growth restriction, respectively, which suggest a role in placental development and function. To better understand epigenetic dynamics in trophoblast cells between early and late pregnancy, we investigate the spatiotemporal expression of TET1, TET2, and TET3 in correlation with nuclear 5-mC and 5-hmC levels in specific trophoblast cell subtypes throughout mouse and human placental development.

2. Methods

2.1. Mouse dissections

This research was regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). C57Bl/6 mice (http://jaxmice.jax.org) were mated to generate conceptuses at E8.5, E10.5, E12.5, E14.5, E16.5, and E18.5. Noon of the day that the copulatory plug was detected was designated E0.5. At least three placentas per time point were assessed. C57Bl/6 adult male brains were dissected for use as a positive control. Tissue was fixed overnight in 4% paraformaldehyde (PFA)/1x phosphate buffered saline (PBS) at 4 °C. Samples were processed for cryosectioning (10 μm) or paraffin sectioning (7 μm).

2.2. Human placentas

First trimester placentas (N = 4) and decidua basalis samples (N = 3) were collected between 7 and 11 weeks gestation with informed written consent and the approval of the Joint UCL/UCLH Committees on the Ethics of Human Research (05/Q0505/82) from patients undergoing surgical termination of pregnancy under general anesthesia for psycho-social reasons. Gestational age was confirmed by ultrasound measurement of the crown rump length of the embryo. Term placentas (N = 3) were collected from normal term singleton pregnancies delivered by elective caesarean section at 39-weeks gestation with informed written consent of the patients and permission of the Cambridgeshire 2 Research Ethics Committee (03/360). All tissue samples were fixed in 4% PFA/1x PBS and embedded for paraffin sectioning.

2.3. In situ hybridization

Synthesis of digoxigenin-labelled sense and anti-sense probes was performed by PCR amplification of cDNA templates using DIG RNA Labelling Mix (Roche, Cat. No. 11277073910) and gene-specific primers (Supplementary Table 1). In situ hybridization was performed as previously described [29]. Briefly, for probes against *Tet1*, *Tet2,* and *Tet3*, cryosections were rehydrated in 1x PBS under RNase-free conditions, treated with 30 μg/ml proteinase K (Roche) in 50 mM Tris/2.5 mM EDTA for 10 min at room temperature, acetylated with 0.25% acetic anhydride (Sigma) in 0.1M triethanolamine (Sigma) buffer for 10 min, and hybridized with ~50 ng of DIG-labelled riboprobe per section in 200 μl hybridization buffer (Amresco) overnight at 55 °C. Sense probes were generated as negative controls, and were used in conditions similar to above. For probes against *Ctsq* and *Tpbpα*, the same protocol was performed with the following modifications. Dewaxed and rehydrated paraffin sections were treated with 30 μg/ml proteinase K for 20 min at room temperature and probe hybridization occurred at 65 °C overnight. All posthybridization washes, antibody detection of DIG-