Development and function of trophoblast giant cells in the rodent placenta

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ABSTRACT Trophoblast giant cells (TGCs) are the first cell type to terminally differentiate during embryogenesis and are of vital importance for implantation and modulation of post-implantation placentation. TGCs are mononuclear and polyploid but are heterogenous and dynamic. At least four different subtypes of TGCs are present within the mature placenta that have distinct cell lineage origins. The development of TGCs is complex and requires transition from the mitotic to the endoreduplication cell cycle and is regulated by a wide variety of factors. During early gestation, TGCs mediate blastocyst attachment and invasion into the uterine epithelium, regulate uterus decidualization, and anatomosis with maternal blood spaces to form the transient yolk sac placenta. During later gestation, TGCs secrete a wide array of hormones and paracrine factors, including steroid hormones and Prolactin-related cytokines, to target the maternal physiological systems for proper maternal adaptations to pregnancy and the fetal-maternal interface to ensure vasculature remodeling. The large number of mouse mutants with defects in TGC development and function are giving us significant new insights into the biology of these fascinating cells.

KEY WORDS: *pregnancy, hormone, cell cycle, polyploid, endoreduplication*

The enigmatic life of the trophoblast giant cell

Trophoblast giant cells (TGCs) are the first terminally differentiated cell type to form during embryogenesis in rodents and are of vital importance for embryo implantation and promoting maternal adaptations to pregnancy. They arise from the trophectoderm layer in the blastocyst (Fig. 1), are endocrine in nature and characterized by their extremely large cytoplasm and polyploid nuclei that result from endoreduplication (Zybina and Zybina, 1996). TGCs are best studied in rodents and are usually mononucleated. In mice, there are several subtypes of TGCs with distinct functions that arise at different stages of gestation and in different locations within the placenta (Simmons *et al.*, 2007; Simmons *et al.*, 2008b). Mono-nucleated, bi-nucleated or occasionally multi-nucleated cells with polyploid nuclei have also been identified in the rabbit, vole, fox, human, cow, water buffalo and alpaca placenta (Carvalho *et al.*, 2006; Klisch *et al.*, 2005; Klisch *et al.*, 1999; Klisch *et al.*, 2004; Zybina *et al.*, 1975; Zybina and Zybina, 1985; Zybina *et al.*, 1992; Zybina *et al.*, 2004; Zybina *et al.*, 2002; Zybina *et al.*, 2001). In humans, the polyploid cells are the so-called extravillous cytotrophoblast cells that invade into the uterus (Zybina *et al.*, 2002) and are associated with remodeling of

the spiral arteries (Pijnenborg *et al.*, 1980). In addition to polyploidy, many genes that are involved in TGC development and function in rodents are conserved between rodents and humans, such as transcription factors, proteases and cell adhesion molecules (Cross *et al.*, 2003; Rawn and Cross, 2008). Therefore, studies of TGCs should give insights into human gestational diseases that are associated with extravillous cytotrophoblast cells such as preeclampsia and intrauterine growth restriction (Brosens *et al.*, 1977; Brosens *et al.*, 1972). We review here the development and functions of TGCs focusing on insights from mice, discussing the differences between TGC subtypes and implications of their diverse functions.

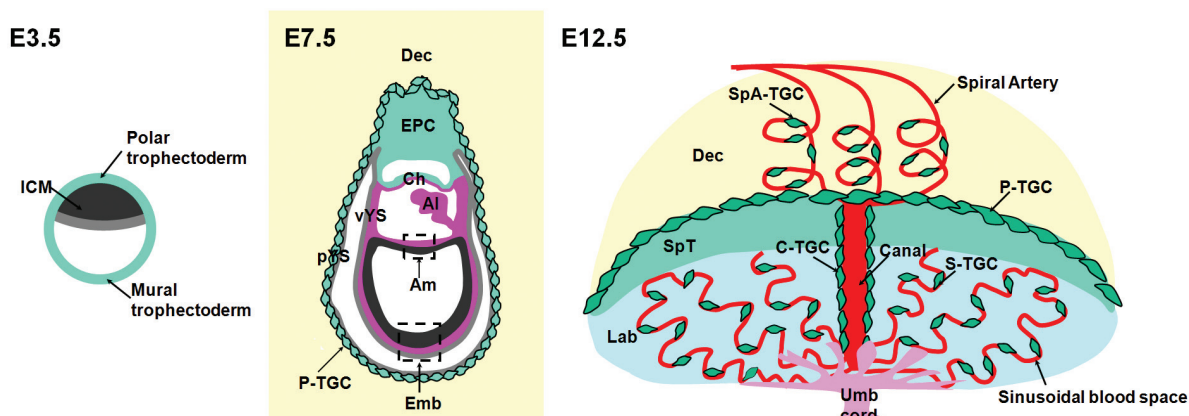## Characteristics, origins and regulation of TGC development

The mature placenta in rodents is composed of three broad zones including the maternal decidua on the outside, the junc-

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Fig. 1. Overview of placental structure in the mouse. *Diagrams depict early development of the mouse conceptus at embryonic days (E) 3.5, 7.5 and 12.5, displaying the components of the mature placenta and different subtypes of TGCs. Abbreviations: Al, allantois; Am, amnion; Ch, chorion; Dec, decidua; Emb, embryo; Epc, ectoplacental cone; ICM, inner cell mass; Lab, Labyrinth; pYS, parietal yolk sac; SpT, spongiotrophoblast; TGC, trophoblast giant cell; Umb Cord, umbilical cord; vYS, visceral yolk sac. C-TGC, maternal blood canal trophoblast giant cell; P-TGC, parietal trophoblast giant cell; S-TGC, sinusoidal trophoblast giant cell; SpA-TGC, spiral artery-associated trophoblast giant cell; Cyan-trophectoderm and trophoblast lineage, Black-inner cell mass and embryonic ectoderm, Gray-endoderm, Red-maternal vasculature, Purple-mesoderm, Yellow-decidua, Pink-fetal blood vessels in labyrinth.*

tional zone and the innermost labyrinth (Fig. 1). The decidua is devoid of trophoblast cells until around mid-gestation when trophoblast cells invade both into the decidua and up the spiral arteries, replacing the endothelium and thereby promoting the transition from endothelial cell-lined to trophoblast cell-lined maternal blood spaces (hemo-chorial) in the placenta (Adamson *et al.*, 2002). The junctional zone consists of spongiotrophoblast cells and a layer of TGCs that line the implantation site. The labyrinth is the region in which nutrient exchange occurs and the bulk of the trophoblast compartment is composed of two layers of multi-nucleated syncytiotrophoblast that arise from cell-cell fusion of post-mitotic cells (Hernandez-Verdun and Legrand, 1975) and not endomitosis or endoreduplication as with TGCs. Four TGC subtypes have been identified in the placenta (Simmons *et al.*, 2007), and include parietal TGC (P-TGC) that line the implantation site and are in direct contact with decidual and immune cells in the uterus, spiral artery-associated TGCs (SpA-TGC), maternal blood canal-associated TGCs (C-TGC), and sinusoidal TGC (S-TGC) that are within the sinusoidal blood spaces of the labyrinth (Figs. 1 and 2). The four subtypes of TGCs are distinguished by their anatomical location and gene expression (Table 1) (Simmons *et al.*, 2007).

Characteristics of TGCs

All TGC subtypes share the characteristics that they are large, have polyploid (usually single) nuclei, and are secretory in nature with their content of golgi and endoplasmic reticulum increasing during differentiation (Bevilacqua and Abrahamsohn, 1988). In contrast to proliferating cells, TGCs undergo rounds of DNA replication without intervening mitoses, a process called endoreduplication, and can accumulate DNA up to 1000C (Zybina and Zybina, 1996). The genome of P-TGCs is 'polytene', a state in which many sister chromatids are synapsed together resulting from multiple rounds of genome replication (Varmuza *et al.*, 1988; Zybina and Zybina, 1996). While the other subtypes of TGCs have polyploid nuclei (Simmons *et al.*, 2007), it is unclear whether their nuclei are polytene. Consistent with their large cytoplasm and extensive rough endoplasmic reticulum, TGCs secrete a variety of proteins including extracellular matrix, cell adhesion molecules, proteinases, cytokines and hormones (see below).

The importance of endoreduplication and polyploidy for TGC function is a matter of speculation. Polyploid nuclei have been identified in many plant and animal cells with secretory or nutritive function such as salivary gland and follicle cells in *Drosophila melanogaster,* and leaf cells in plants (Edgar and Orr-Weaver, 2001). It has been suggested that polyploidy may increase their capacity for protein synthesis. Alternatively, since TGCs have a relatively short lifespan, it may be that endoreduplication and the associated cell hypertrophy allow tissue growth with less time and energy expenditure. An extension of this hypothesis is that because TGCs are post-mitotic, they function without risk of forming

TABLE 1

CHARACTERISTICS AND FUNCTION OF THE FOUR DIFFERENT TGC SUBTYPES IN THE MATURE PLACENTA

Subtype	Location	Temporal appearance	Gene expression	Suggested function
SpA-TGC	Lining maternal spiral arteries bringing blood into placenta	E10.5	*Plf*	Regulate maternal spiral artery remodeling and blood flow into the placenta
P-TGC	Lining implantation site and outer layer of parietal yolk sac	E7.5	*Pl1, Pl2, Plf*	Facilitate implantation and initial maternal vascular connections, regulate decidual cell differentiation, and maternal physiology
C-TGC	Lining canals that bring maternal blood to base of labyrinth	E10.5	*Plf, Pl2*	Regulate maternal vasculature remodeling and maternal physiology
S-TGC	Within maternal blood sinusoids of the labyrinth layer	E10.5	*Ctsq, Pl2*	Modulation of hormone and growth factor activity before they enter fetal and/or maternal circulation, regulate maternal physiology

tumours (Hemberger, 2008). This is important since TGCs have the ability to invade and promote local angiogenesis (see below). Interestingly though, TGC nuclei in voles become fragmented during late gestation with sex chromosomes in each sub-domain, and this suggests that there is some structural order to the polyploid nuclei (Zybina *et al.*, 2005). The formal test of whether or not polyploidy is critical for function is to analyze mutants in which it does not occur. Endoreduplication is compromised in cyclin E1/E2-deficient mice but markers of TGCs are still induced, indicating that endoreduplication and differentiation are not linked (Geng *et al.*, 2003; Parisi *et al.*, 2003).

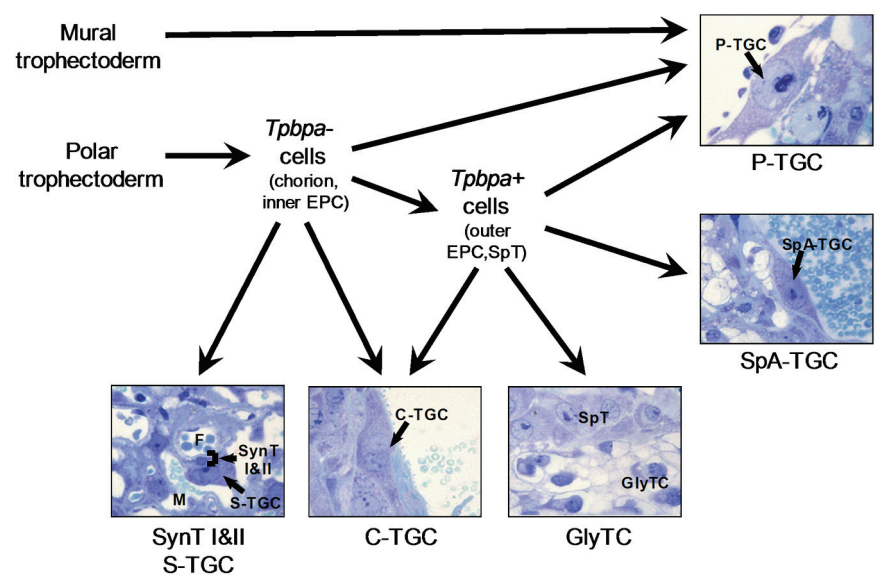### Transformation of the mitotic cell cycle to the endocycle in TGCs

Mechanisms concerning the initiation and maintenance of endoreduplication have been well studied in flies and plants (Edgar and Orr-Weaver, 2001; Larkins *et al.*, 2001), though there are some data from rodent TGCs as well (Fig. 3) (Hattori *et al.*, 2000; MacAuley *et al.*, 1998; Nakayama *et al.*, 1998). The most obvious change in the cell cycle is that S phase is dissociated from M phase. This is not trivial since checkpoint controls normally prevent initiation of DNA replication until completion of mitosis and entry into mitosis is prevented until completion of S phase (Elledge, 1996). TGCs show other changes in checkpoint controls such that they continue through DNA replication even after sustaining DNA damage (MacAuley *et al.*, 1998).

The G2 decision point: mitosis or endoreduplication?

In a mitotic cell cycle, cyclin B/Cdk1 promotes entry into mitosis (Sherr and Roberts, 2004). The Rcho-1 trophoblast cell line has been used to study TGC differentiation as precursor cells that have committed to leave the mitotic cell cycle can be selected as a result in a change in cell adhesiveness even before they have begun to endoreduplicate (MacAuley *et al.*, 1998). The transition occurs in the G2 phase but cyclin B/Cdk1 complex is not activated due to reduced association of cyclin B and Cdk1 (MacAuley *et al.*, 1998). This is likely due to the effect of the Cdk inhibitor p57Kip2 which has recently been shown to inhibit Cdk1 activity during TGC differentiation (Ullah *et al.*, 2008). In the subsequent endocycle, cyclin B expression is suppressed (MacAuley *et al.*, 1998; Palazon *et al.*, 1998). The zinc finger transcription factor Snail regulates the 'G2 decision point' of whether trophoblast cells go through mitosis or enter the endocycle (Fig. 3) (Nakayama *et al.*, 1998). Its precise mechanism is unknown but over-expression of Snail increases expression of the mitotic cyclins A and B (Nakayama *et al.*, 1998).

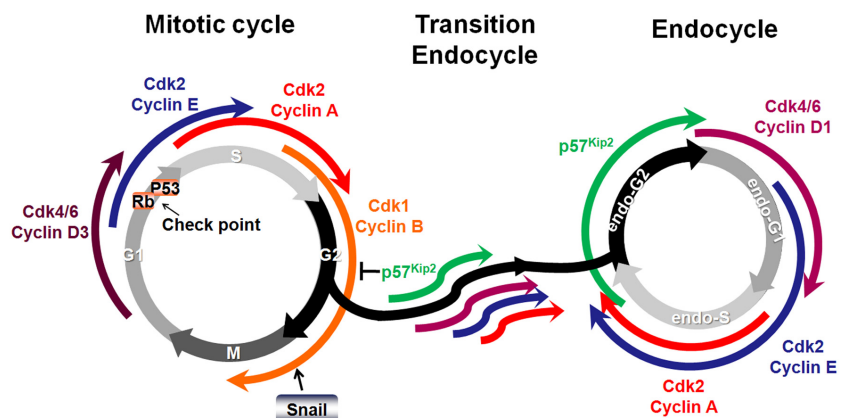#### Resetting the periodic S phases during endoreduplication

During the mitotic cell cycle, biochemical events coincident with mitosis lead to the re-setting of the

origins of replication (Elledge, 1996). This includes degradation of the protein Geminin which otherwise suppresses the firing of origins of replication (McGarry and Kirschner, 1998). Geminin mutant embryos die during pre-implantation development and show ectopic endoreduplication in blastomeres (Gonzalez *et al.*, 2006). Another important mechanism to maintain periodicity during the endocycle involves cyclic expression of p57Kip2, a G1/S Cdk inhibitor (Hattori *et al.*, 2000). When TGC differentiation is initiated, *p57Kip2* mRNA expression appears during the transition endocycle (Fig. 3) (Hattori *et al.*, 2000). During subsequent endocycles, p57Kip2 protein levels fluctuate and this defines two phases: a

Fig. 2. Outline of the trophoblast cell lineage and origins of different TGC subtypes. *Micrographs of different trophoblast cell subtypes show high magnification views of toluidine blue-stained plastic sections of the mid-gestation placenta. C-TGC, canal trophoblast giant cell; EPC, ectoplacental cone; F, fetal capillary; GlyTC, glycogen trophoblast cell; M, maternal sinusoid blood space; P-TGC, parietal trophoblast giant cell; S-TGC, sinusoidal trophoblast giant cell; SpA-TGC, spiral artery-associated trophoblast giant cell; SpT, spongiotrophoblast; SynT-I & II, syncytiotrophoblast cell layers I and II.*

Fig. 3. Overview of the expression and activity of cell cycle regulators *that function during the transition from the mitotic cell cycle to the endocycle associated with TGC differentiation based on published data (Hattori et al., 2000; MacAuley et al., 1998; Nakayama et al., 1998; Ullah et al., 2008).*

TABLE 2

GENES REGULATING TGC DEVELOPMENT

Name	Gene product	Function	Reference
Trophoblast stem cell specification and maintenance			
Eomes	T-box transcription factor	Mutants die at peri-implantation stage, primary TGCs absent	(Russ *et al.*, 2000)
Elf5	Ets transcription factor	Mutants die by E8.5 with extraembryonic ectoderm absent, ectoplacental cone present	(Donnison *et al.*, 2005)
Err2/Errβ	Orphan nuclear receptor	Mutants die by E9.5 with chorion absent, TGC number increased	(Luo *et al.*, 1997, Tremblay *et al.*, 2001a)
Foxd3	Forkhead transcription factor	Mutants ie by E6.5 with TGC number increased	(Tompers *et al.*, 2005)
AP-2γ	AP-2 family of transcription factors	Mutants die by E8.5 with trophoblast stem cells and ectoplacental cone reduced, Primary TGCs reduced	(Auman *et al.*, 2002, Werling and Schorle, 2002)
Erf	Ets domain transcriptional repressor	Mutants die by 10.5 with persistence of ectoplacental cavity, failure in chorioallantoic attachement, expanded TGCs layer	(Papadaki *et al.*, 2007)
Ets2	Ets transcription factor	Mutants die by E8.5 with chorion absent and ectoplacental cone growth reduced and defective trophoblast stem cells self renewal	(Yamamoto *et al.*, 1998, Wen *et al.*, 2007)
Dp1	DP family transcription factor	Mutants die by E12.5 with reduced chorion, fewer TGCs and TGCs with minimal nuclear enlargement	(Kohn *et al.*, 2003)
FGF4	Fibroblast growth factor	Mutants die shortly after implantation with failure to maintain trophoblast stem cells, premature TGC formation	(Feldman *et al.*, 1995, Tanaka *et al.*, 1998)
FGFR2	Fibroblast growth factor receptor 2	Mutants die shortly after implantation with failure to maintain trophoblast stem cells, premature TGC formation	(Arman *et al.*, 1998)
Erk2	Extracellular signal-regulated kinase 2	Mutants die shortly after implantation, extraembryonic ectoderm and ectoplacental cone not formed	(Hatano *et al.*, 2003, Saba-El-Leil *et al.*, 2003)
Shp2	Non-receptor protein-tyrosine phosphatase	Mutants die at peri-implantation stage, trophoblast stem cells not generated	(Yang *et al.*, 2006)
Nodal	Transforming growth factor β superfamily member	Mutants die by E9.5 with spongiotrophoblast layer reduced and TGC number increased	(Guzman-Ayala *et al.*, 2004, Ma *et al.*, 2001)
Activin	Transforming growth factor β superfamily member	Promotes maintenance of cultured trophoblast stem cells	(Erlebacher *et al.*, 2004)
Tgf β	Transforming growth factor β superfamily member	Promotes maintenance of cultured trophoblast stem cells	(Erlebacher *et al.*, 2004)
BMP2	Transforming growth factor β superfamily member	Mutants die by around E8.5 with amnion/chorion defects caused by an open proamniotic canal	(Zhang and Bradley, 1996)
Acvr1b	Activin/Nodal receptor 1B	Mutants die by E9.5 with disorganized extraembryonic ectoderm	(Gu *et al.*, 1998)
Acvr2, Acvr2b	Activin/Nodal receptor 2 and 2B	Compound homozygous mutants die by E8.5	(Song *et al.*, 1999)
Smad1	Intracellular transducer of TGF-β signals	Mutants die by E10.5 with chorion erratically folded and allantois growth defects	(Arnold *et al.*, 2006, Lechleider *et al.*, 2001, Tremblay *et al.*, 2001b)
mTOR	Mammalian TOR (target of rapamycin)	Mutants die shortly after implantation. Mutant trophoblast fails to proliferate *in vitro*.	(Murakami *et al.*, 2004)
Ectoplacental cone and spongiotrophoblast maintenance			
Mash2	Basic helix-loop-helix transcription factor	Mutants die by E10 with smaller ectoplacental cone and lack of spongiotrophoblast, TGC number increased, labyrinth layer reduced	(Guillemot *et al.*, 1994, Scott *et al.*, 2000, Tanaka *et al.*, 1997)
Sp1,3	Zinc finger transcription factors	Sp1-/- die by E10.5, Sp3-/- die postnatally, spongiotrophoblast layer decreased in Sp1/Sp3 compound heterozygous and Sp3-/- mutants	(Kruger *et al.*, 2007)
PPAR β/δ	nuclear receptor peroxisome proliferator-activated receptor *β/δ*; lipid-activated transcription factors	Mutants die by E10.5 with reduced spongiotrophoblast and TGC	(Nadra *et al.*, 2006, Wang *et al.*, 2007)
HIF	bHLH/PAS transcription factors composed of HIFα and HIFβ/Arnt subunits	*Arnt-/-* and *Hif1α-/- Hif2α-/-* die by E10.5 with TGC number increased, smaller ectoplacental cone and reduced spongiotrophoblast	(Abbott and Buckalew, 2000, Adelman *et al.*, 2000, Cowden Dahl *et al.*, 2005)
Cited 1	CBP/p300-interacting transactivator	Mutants die shortly after birth, spongiotrophoblast layer irregular in shape and enlarged	(Rodriguez *et al.*, 2004)
Cited 2	CBP/p300-interacting transactivator	Mutants die by E14.5 with reduced spongiotrophoblast, glycogen trophoblast cells and TGCs	(Withington *et al.*, 2006)
Dnmt3L	DNA methyltransferase 3-like protein	Mutants die by E10.5 with TGCs number increased, spongiotrophoblast and labyrinth reduced	(Arima *et al.*, 2006)
Keratin 8, 18, 19	Cytokeratin-intermediate filaments	K8-/- die by E12.5, K8-/-K19-/- die by E10.5 and K18-/-K19-/- die by E9.5, all with altered TGCs	(Hesse *et al.*, 2000, Jaquemar *et al.*, 2003, Tamai *et al.*, 2000)
Connexin 31	Connexin; Gap junction protein	60% mutants die between E10.5 and 13.5 TGC number increased, spongiotrophoblast and labyrinth decreased	(Kibschull *et al.*, 2004, Plum *et al.*, 2001)
Connexin 31.1	Connexin; Gap junction protein	Mutants die by E14.5, compact spongiotrophoblast with increased thickness	(Zheng-Fischhofer *et al.*, 2007)
Bruce	BIR repeat-containing ubiquitin-conjugating enzyme	Mutants in C57BL/6 background die perinatally with spongiotrophoblast reduced	(Hitz *et al.*, 2005, Lotz *et al.*, 2004)
HOP/NECC1	Homeodomain-only protein/not expressed in choriocarcinoma clone 1 (HOP/NECC1)	Mutants have TGCs number increased, spongiotrophoblast reduced	(Asanoma *et al.*, 2007)
Talin	Cytoplasmic protein associated with integrin-containing cellular junctions	Mutants die by E9.5 with disorganized extraembryonic tissues and the ectoplacental and excocoelomic cavities are not formed	(Monkley *et al.*, 2000)
TGC terminal differentiation			
Hand1	Basic helix-loop-helix transcription factor	Mutants die by E8.5 with smaller ectoplacental cone, TGC number reduced and nuclear size reduced	(Firulli *et al.*, 1998, Riley *et al.*, 1998, Scott *et al.*, 2000)
Stra13	Basic helix-loop-helix transcription factor	Over-expression stimulates TGC differentiation	(Hughes *et al.*, 2004)

TABLE 2 (CONTINUED)

I-mfa	bHLH transcription factor repressor protein	Mutants die by E10.5 in C57B1/6 background TGC number reduced	(Kraut *et al.*, 1998)
Eed	Polycomb group protein	Mutants die by E11.5 with TGC number reduced, TGC nuclear size preferentially small in females	(Wang *et al.*, 2001)
Snail	Zinc finger transcription factor	Over-expression suppresses TGC differentiation	(Nakayama *et al.*, 1998)
Gata2,3	Zinc finger transcription factor	Gata2 -/- die by E10 and Gata3-/- die by E11.5, TGCs with reduced hormone synthesis and indistinguishable cell number	(Ma and Linzer, 2000, Ma *et al.*, 1997)
Sox15	Sry-type HMG box (Sox) transcription factor	Ectopic expression of Sox15 promotes TGC differentiation	(Yamada *et al.*, 2006)
LIF	Leukemia inhibitory factor	Administration promotes TGC differentiation *in vitro* and *in vivo*, genetic reduction in LIF rescues TGC phenotype in Socs3 null mutants	(Robb *et al.*, 2005, Takahashi *et al.*, 2003, Takahashi *et al.*, 2008)
LIFR	Leukemia inhibitory factor receptor	Mutants die perinatally, TGC number reduced, spongiotrophoblast and labyrinth layers disorganized	(Takahashi *et al.*, 2003, Ware *et al.*, 1995)
Socs3	Suppressor of cytokine signaling protein	Mutants die by E13.5 with TGC number increased, spongiotrophoblast and labyrinth layers reduced	(Boyle and Robb, 2008, Robb *et al.*, 2005, Takahashi *et al.*, 2003)
Jak1	Janus kinase 1; tyrosine kinase	Mutants die perinatally, TGC number reduced and spongiotrophoblast cell number increased, labyrinth layer disorganized	(Takahashi *et al.*, 2008)
Stat3	Signal transducer and activator of transcription 3	Mutants die by E7.5 with TGC number decreased	(Takeda *et al.*, 1997)
RXR	Retinoid X receptors	RXRα-/- die by E16.5 and RXRα-/-/RXRβ-/- compound homozygous die by E10.5, TGCs disorganized, spongiotrophoblast reduced, labyrinth layer absent	(Sapin *et al.*, 1997, Wendling *et al.*, 1999)
PTHrP	Parathyroid hormone-related protein	Promotes TGC differentiation *in vitro* Mutants die immediately after birth	(El-Hashash *et al.*, 2005, Karaplis *et al.*, 1994)
Fbw7	F-box protein component of an SCF (Skp1-Cul1-F-box protein-Rbx1)-type ubiquitin ligase	Mutants die by E10.5, TGC number increased TGCs, DNA synthesis increased	(Tetzlaff *et al.*, 2004)
Chm	Choroideremia (CHM); Rab escort protein-1	Mutants die by E11.5, TGC number increased, smaller ectoplacental cone and reduced spongiotrophoblast layer	(Shi *et al.*, 2004)
CCNE1 and CCNE2	Cyclin E1, E2	Cyclin E1-/-E2-/- embryos die by E11.5, TGC nuclei have marked reduction in DNA content though TGC nuclear size not reduced	(Geng *et al.*, 2003, Parisi *et al.*, 2003)
p57Kip2	Cyclin-dependent kinase inhibitor	Mutants die neonatally, spongiotrophoblast layer increased, TGC number not different Defines the length of the gap phase during endoreduplication	(Hattori *et al.*, 2000, Kanayama *et al.*, 2002, Takahashi *et al.*, 2000)
p53	Tumour suppressor protein, transcriptional factor	Mutants die postnatally, TGC number increased, spongiotrophoblast layer reduced	(Komatsu *et al.*, 2007, Soloveva and Linzer, 2004)
Mfn2	Mitochondrial transmembrane GTPase	Mutants die by E11.5, TGC number reduced, smaller TGC nuclear size	(Chen *et al.*, 2003)
Geminin	Nuclear protein that inhibits DNA replication	Mutants die at preimplantation stage, premature endoreduplication of 'trophoblast-like' giant cells at eight cells stage	(Gonzalez *et al.*, 2006)

endo-G2 phase with p57^{Kip2} accumulating upon completion of S phase and an endo-G1 phase with p57^{Kip2} declining several hours before entry into S-phase (Hattori *et al.*, 2000). It is hypothesized that periodic expression of p57^{Kip2} protein promotes alternating S and gap phases during the endocycle.

Altered G1 to S checkpoint

A G1 to S checkpoint is present during the mitotic cell cycle to ensure that chromosomes are intact before replication. At the G1 checkpoint, the Rb tumor suppressor protein is phosphorylated by cyclin D/Cdk allowing the E2F transcription factor to be liberated, which in turn drives the cell cycle into S phase (Sherr and Roberts, 2004). The p53 tumour suppressor protein helps cells to survey genotoxic damage and cooperates with Rb to regulate G1 arrest (Sherr, 2000). During the transition from the mitotic cell cycle to the endocycle in trophoblast cells, cyclin D isoform expression switches from D3 to D1 (MacAuley *et al.*, 1998). Expression of p53 and Rb declines during TGC differentiation whereas their forced over-expression inhibits differentiation (Soloveva and Linzer, 2004). This altered G1 checkpoint control might allow cells to go through repeated S phases without intervening mitoses.

Maintenance of S phase cyclin/Cdk activities

Cyclin E promotes the G1 to S phase transition whereas cyclin A promotes S phase progression (Sherr and Roberts, 2004), and both cyclin A and E are expressed during endo-S phase (MacAuley

et al., 1998). Cyclin E1/E2-deficient mice show a reduced endoreduplication in TGCs, indicating that cyclin E is essential for endoreduplication (Geng *et al.*, 2003; Parisi *et al.*, 2003). Conversely, cyclin E levels are elevated in *Fbw7* (Tetzlaff *et al.*, 2004) and *Cul1* (Wang *et al.*, 1999) mutants due to compromised cyclin E degradation, and TGCs are larger than normal.

Cell lineage origins of different TGC subtypes

In addition to distinct localization and gene expression patterns, the different subtypes have different lineage origins and arise at different times during development (Fig. 2) (Simmons *et al.*, 2007). Some of the P-TGCs arise directly from the ~60 mural trophectoderm cells in the blastocyst in a process called primary TGC differentiation. However, most of the several hundred P-TGCs that are present by mid-gestation, and all of the other subtypes, arise from the polar trophectoderm through so-called secondary TGC differentiation. Historically, it has been hypothesized that secondary TGCs are derived from progenitor cells within the ectoplacental cone and the spongiotrophoblast layers that are *Mash2* and *Tpbpa/4311* positive (Simmons and Cross, 2005). However, lineage-tracing studies have shown that only some TGC subtypes arise from *Tpbpa*-positive precursor cells (Fig. 2) (Simmons *et al.*, 2007). Both P-TGCs and C-TGCs have mixed developmental origins. In contrast, all of the SpA-TGCs originate from *Tpbpa*-positive cells, whereas all of the S-TGCs arise from *Tpbpa*-negative precursors (Fig. 2). While *Tpbpa*-positive precursors are located in the outer ectoplacental cone

starting at ~E8.5 and later in the spongiotrophoblast, the source of the *Tpbpa*-negative precursors is unknown but could be the extraembryonic ectoderm/chorion trophoblast cells, inner ectoplacental cone cells, or both. The chorion has distinct layers of cells by E8.5 that are thought to give rise to the three different trophoblast cell layers in the labyrinth including two multinucleated syncytiotrophoblast layers and S-TGCs (Fig. 1 and 2) (Simmons *et al.*, 2008a).

Regulators of TGC development

A variety of factors regulate TGC development (Table 2). After implantation, the trophoblast lineage is maintained by proliferation of trophoblast stem cells that reside in the polar trophectoderm and that produce the bulk of the trophoblast lineage save for ~60 of the P-TGCs that derive from mural trophectoderm (Fig. 2). The trophoblast stem cell pool is maintained by FGF4/FGFR2 (Arman *et al.*, 1998; Tanaka *et al.*, 1998) and Nodal (Guzman-Ayala *et al.*, 2004) signaling and the AP2γ (Auman *et al.*, 2002), Eomes (Russ *et al.*, 2000), Err2 (Luo *et al.*, 1997; Tremblay *et al.*, 2001a), Foxd3 (Tompers *et al.*, 2005) and Elf5 (Donnison *et al.*, 2005) transcription factors. Mice that are deficient for these factors, in general, show premature TGC differentiation as a consequence of the failure to maintain trophoblast stem cells (Table 2).

Genes involved in maintenance of the ectoplacental cone and/or spongiotrophoblast

There are many genes involved in maintaining the ectoplacental cone and/or spongiotrophoblast (Table 2) and, in general, loss-of-function mutations result in an increase in the number of TGCs. The precise mechanisms of action are established for only a few of these factors and only these will be discussed in detail. The basic helix-loop-helix (bHLH) transcription factor Mash2 plays an essential role in maintenance of the ectoplacental cone and spongiotrophoblast and negatively affects TGC differentiation (Guillemot *et al.*, 1994; Scott *et al.*, 2000; Tanaka *et al.*, 1997). *Mash2* is expressed in the in the chorion, ectoplacental cone and later spongiotrophoblast (Scott *et al.*, 2000). *Mash2*-deficient mice die by E10 due to placenta defects, which include the absence of spongiotrophoblast, an increase of TGCs and a failure of labyrinth formation (Guillemot *et al.*, 1994; Tanaka *et al.*, 1997). A similar phenotype is observed in mutants for the DNA methyltransferase 3-like gene *Dnmt3L* (Arima *et al.*, 2006). Dnmt3L is required for the establishment of maternal methylation imprints, and *Dnmt3L$^{mat-/-}$* mutants die by E10.5 due to an imprinting defect and expression of Mash2 is diminished (Arima *et al.*, 2006).

Oxygen levels can regulate trophoblast lineage cell fate in mice both *in vitro* and *in vivo* (Adelman *et al.*, 2000; Cowden Dahl *et al.*, 2005). Hypoxia promotes *in vitro* differentiation of trophoblast stem cells into spongiotrophoblast cells as opposed to TGCs (Adelman *et al.*, 2000; Takeda *et al.*, 2006). Hypoxia inducible factors (HIFs) are heterodimeric basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) transcription factors composed of HIFβ/Arnt and HIF1α or HIF2α, that are activated by hypoxia (Semenza, 2007). In *HIF1α/HIF2α* double mutants, there are fewer spongiotrophoblast and syncytiotrophoblast cells, and more TGCs (Cowden Dahl *et al.*, 2005). *Arnt* mutant placentas are similar to *HIF1α/HIF2α* mutants (Adelman *et al.*, 2000; Cowden Dahl *et al.*, 2005).

The *Cx31* and *Cx31.1* genes encode for connexin gap junction proteins and are involved in maintaining TGC progenitor cells within the ectoplacental cone and spongiotrophoblast (Kibschull *et al.*, 2005; Kibschull *et al.*, 2004; Plum *et al.*, 2001; Zheng-Fischhofer *et al.*, 2007). *Cx31* is expressed at pre-implantation stages, but is restricted to the ectoplacental cone and extraembryonic ectoderm after implantation and then it persists in spongiotrophoblast (Plum *et al.*, 2001). *Cx31.1* is co-expressed with *Cx31* at post-implantation stages, except that its expression is suppressed in spongiotrophoblast after E11.5 and persists in glycogen trophoblast cells (Zheng-Fischhofer *et al.*, 2007). *Cx31* and *Cx31.1* deficient mice show similar placental defects including excessive TGCs (Plum *et al.*, 2001; Zheng-Fischhofer *et al.*, 2007).

Genes involved in TGC terminal differentiation

A large number of factors are implicated in promoting the terminal differentiation of TGCs though most of the information to date is limited to insights into P-TGCs (Table 2). Only some of these factors will be discussed in detail in which the molecular and cellular function is understood.

Transcription factors. It is well established that bHLH factors play key roles in TGC differentiation. Hand1 plays an essential role in promoting TGC differentiation (Firulli *et al.*, 1998; Riley *et al.*, 1998; Scott *et al.*, 2000). It is expressed in the upper layer of the chorion, ectoplacental cone and all TGC subtypes. In *Hand1* mutants, the ectoplacental cone is smaller, the number of cells lining the implantation site is reduced and they are strikingly smaller than normal P-TGCs. *Hand1* mutants die by ~E8.5 but, in studying *Hand1*-deficient trophoblast stem cells in culture, it appears that *Hand1* is essential for differentiation of all four TGC subtypes (Simmons *et al.*, 2007). Mash2, the bHLH protein that maintains the diploid TGC progenitors, antagonizes Hand1 action (Scott *et al.*, 2000). Stra13, another bHLH factor that is induced by retinoic acid (Sapin *et al.*, 2000), can induce TGC differentiation *in vitro* (Hughes *et al.*, 2004). I-mfa, a bHLH factor interacting protein, promotes TGC differentiation by inhibiting Mash2 (Kraut *et al.*, 1998).

Gata transcription factors are also implicated in TGC development. Gata2 and Gata3 regulate transcription of TGC-specific hormone genes (Ma *et al.*, 1997; Ng *et al.*, 1993; Ng *et al.*, 1994). In addition, Gata2 has been implicated in restricting expression of the *Plpa* gene to P-TGCs that surround the ectoplacental cone (Ma and Linzer, 2000). The results are interesting because they may imply distinct regulatory mechanisms for TGCs that are derived from *Tpbpa*-positive versus *Tpbpa*-negative precursors.

Intercellular signaling pathways. Several signaling pathways are implicated in TGC development and highlight the importance of paracrine interactions. Leukemia inhibitory factor (LIF) is a member of the interleukin-6 cytokine family and has several biological functions (Metcalf, 2003). LIF binds to a low-affinity receptor (LIFR), which in turn forms a high-affinity complex with the gp130 receptor protein. The LIFR-gp130 heterodimer complex transduces the LIF signal through activation of JAK kinase and STAT transcription factors (Metcalf, 2003). Suppressor of cytokine signaling (SOCS) proteins are important negative regulators of JAK-STAT signaling that form a negative-feedback loop—(Metcalf,

2003). LIF is expressed in the uterus of pregnant mice (Shen and Leder, 1992) and promotes TGC differentiation both in cultured Rcho-1 cells and *in vivo* (Takahashi *et al.*, 2003; Takahashi *et al.*, 2008). Deletion of *Lifr* results in placentas with fewer TGCs (Takahashi *et al.*, 2003) and disorganized spongiotrophoblast and labyrinth layers (Ware *et al.*, 1995), resulting in perinatal lethality. *Jak1*-deficient mice also die perinatally and show fewer TGCs with increased spongiotrophoblast cells, suggesting compromised differentiation from spongiotrophoblast to TGCs (Takahashi *et al.*, 2008). In contrast, SOCS3 mutants have excessive TGCs and smaller spongiotrophoblast and labyrinth layers (Takahashi *et al.*, 2008). Interestingly, reduction of *Lif, Lifr* or *Jak1* gene dosage rescues the placental defects and embryonic lethality in *SOCS3* mutant mice (Boyle and Robb, 2008; Robb *et al.*, 2005; Takahashi *et al.*, 2003; Takahashi *et al.*, 2008).

Retinoic acid, the active derivative of vitamin A (retinol), promotes TGC differentiation *in vivo* and *in vitro* (Yan *et al.*, 2001) and several retinoic acid-inducible genes, including *Stra13*, are expressed in the developing placenta (Sapin *et al.*, 2000). When trophoblast stem cells are cultured with retinoic acid, their proliferation is compromised, TGC differentiation is induced but spongiotrophoblast reduced (Yan *et al.*, 2001). Recent studies suggest that retinoic acid preferentially induces differentiation of P-TGCs and suppresses formation of the S-TGC and C-TGC subtypes, providing the first evidence that different TGC subtypes can be differentially regulated (Simmons *et al.*, 2007). Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) form heterodimers that are the receptors for retinoic acid. *RXRα* mutants and *RXRα/RXRβ* compound mutants die by E16.5 and E10.5, respectively, and show placenta defects that are mostly restricted to labyrinth (Sapin *et al.*, 1997; Wendling *et al.*, 1999). Single RAR mutants do not display obvious placental phenotypes suggesting redundancy among RARs in placental development and function.

Several growth factors affect TGC differentiation and/or the expression of TGC-specific genes including Activin (Erlebacher *et al.*, 2004), EGF (Yamaguchi *et al.*, 1995), TGFβ (Erlebacher *et al.*, 2004; Yamaguchi *et al.*, 1995), IGF-I (Kanai-Azuma *et al.*, 1993), IGF-II (Kanai-Azuma *et al.*, 1993), and PTHrP (El-Hashash *et al.*, 2005; El-Hashash and Kimber, 2006). Many of these factors are expressed locally within the uterus or by trophoblast cells themselves. However, in most cases, whether these factors are critical for TGC differentiation *in vivo* has not been established through analysis of mouse mutants.

Functions of TGCs

TGCs have diverse functions that are crucial for implantation and subsequent placental function (Fig. 4). The mural trophectoderm-derived TGCs mediate attachment of blastocyst to the uterine epithelium, induce uterine decidulization, invade into the uterine stroma, and anastomose to form the yolk sac placenta for early

exchange of nutrients and endocrine signals between mother and fetus. After implantation, TGCs produce hormones and cytokines for maintenance of the feto-maternal interface and regulation of maternal adaptations to pregnancy.

Functions of TGCs in establishment of fetal-maternal interface
Adhesion to the uterine epithelium

At the time of implantation, mural trophectoderm cells increase their adhesiveness and become competent to attach to the uterus (Armant, 2005). Meanwhile, uterine epithelial cells are primed by progesterone and estrogen from the ovary and become capable of attaching to the blastocyst (Dey *et al.*, 2004). TGCs also produce progesterone (Yamamoto *et al.*, 1994) that may contribute to regulation of uterine changes. During implantation, trophoblast cells attach to extracellular matrix (ECM) in the receptive uterus, which is composed of fibronectin, laminin, vitronectin and collagen (Sutherland, 2003; Wang and Armant, 2002). As the blastocytst and uterine epithelium attach, the now differentiating P-TGCs express several integrins such as α5β1 (Metcalf, 2003; Schultz and Armant, 1995), α7β1 (Klaffky *et al.*, 2001), α4 β1 (Basak *et al.*, 2002), αIIbβ3 (Rout *et al.*, 2004) and αVβ3 (Rout *et al.*, 2004).

TGCs affect decidualization of the uterine stromal cells

Upon attachment of the blastocyst to the uterine epithelium, the uterine stromal cells at sites of blastocyst apposition undergo proliferation and differentiation into decidual cells, a process called decidualization. The uterine stroma can undergo decidualization in response to even an artificial stimulus such as scratching of the epithelium or intraluminal injection of lectin-coated beads or oil, as long as the uterus has been exposed to appropriate priming by estrogen and progesterone (Dey *et al.*, 2004). TGCs are thought to be indispensible for decidualization because of their production either of progesterone or other signals that affect decidual cell differentiation (Bany and Cross, 2006). These latter signals are inferred from the fact that there are differences in gene expression between the decidua surrounding a normally implanted embryo compared to an artificially induced one, or surrounding mutant mouse embryos that have TGC
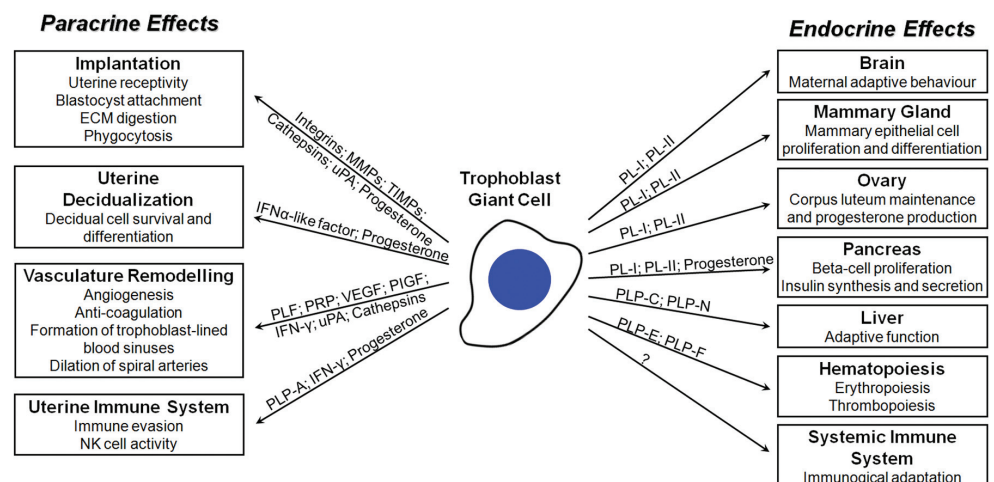

Fig. 4. Summary of the paracrine and endocrine functions of TGCs.

defects (Bany and Cross, 2006). At least one of the TGC signals is a type I interferon (Bany and Cross, 2006).

Invasion into the decidua and anastomosis with the maternal vasculature

After attachment of the blastocyst to the uterine luminal epithelium, P-TGCs invade the uterus by remodeling of the ECM, phagocytosis and cell motility (Cross *et al.*, 1994). They form a transient structure called the parietal yolk sac (Fig. 1) that is the site of exchange for nutrients and gases between the mother and fetus in the early post-implantation conceptus (Cross *et al.*, 1994). It is composed of P-TGCs, parietal endoderm cells and an extensive basement membrane (Reichert's membrane) between them (Welsh and Enders, 1987). The formation of the yolk sac placenta is highly dependent on the ability of the TGCs to penetrate the uterine epithelium and anastomose with maternal blood spaces surrounding the implantation site. During anatomosis, the P-TGCs are highly protrusive, with long cytoplasmic lamina extended to envelope a diffuse network of maternal blood sinuses (Bevilacqua and Abrahamsohn, 1989; McRae and Church, 1990).

The mechanisms of trophoblast invasion are best studied in P-TGCs but SpA-TGCs and glycogen trophoblast cells also invade into the uterus (Adamson *et al.*, 2002; Pijnenborg *et al.*, 1981). TGCs secrete a variety of proteinases that are thought to digest the ECM as well as phagocytosed maternal cells and matrix materials. They include matrix metalloproteinases (MMP-2, -3, -9, -13) and inhibitors of metalloproteinases (TIMP-1, -2, -3, -4) (Alexander *et al.*, 1996; Das *et al.*, 1997; Harvey *et al.*, 1995; Teesalu *et al.*, 1999; Zhang *et al.*, 2003) urokinase plasminogen activators (Teesalu *et al.*, 1998; Teesalu *et al.*, 1999) and cathepsins (Afonso *et al.*, 1999; Deussing *et al.*, 2002; Hemberger *et al.*, 2000; Ishida *et al.*, 2004).

Functions of TGCs after implantation

After implantation, TGCs produce many paracrine and endocrine factors that target various maternal physiological systems to maintain maternal adaptations to pregnancy.

Production of hormones that regulate various maternal physiological systems

TGCs produce a broad range of hormones that regulate several maternal adaptations to pregnancy. In particular, the prolactin/placental lactogen (PL)/prolactin-like protein (PLP) gene family is highly evolved in rodents. There are 23 members in mice and all except for the pituitary prolactin gene are exclusively expressed in the placenta and in TGCs in particular (Simmons *et al.*, 2008b, Wiemers *et al.*, 2003). The expression patterns indicate that the 22 placenta-specific genes have diverse functions (Simmons *et al.*, 2008b).

The PL were first identified in rodents as prolactin-related hormones that stimulate the mammary gland similar to prolactin and indeed they work through the prolactin receptor (Linzer and Fisher, 1999), though it is clear that PL has a variety of other target tissues. TGCs produce PL-I starting soon after implantation until mid-gestation and subsequently PL-II from mid-gestation until term (Talamantes, 1990). In mice in which the pituitary gland is removed as the source of prolactin, secretion of both PL-I (Lopez *et al.*, 1991) and PL-II (Kishi *et al.*, 1988) is elevated and some milk production occurs indicating that the placental lactogens are

partially sufficient to promote mammary development (Thordarson *et al.*, 1989). PL-I and PL-II also have luteotrophic effects on the ovary and support progesterone production (Galosy and Talamantes, 1995; Thordarson *et al.*, 1997). PL-I and PL-II can also increase insulin secretion (Brelje *et al.*, 1993; Fleenor *et al.*, 2000; Nielsen *et al.*, 1999; Sorenson and Brelje, 1997) and stimulate an increase in the number of insulin producing β cells in pancreatic islets. By contrast, progesterone inhibits insulin secretion and β cell division (Sorenson *et al.*, 1993). PL-I affects the liver and induces expression of Na+/taurocholate-co-transporting polypeptide (NTCP) (Cao *et al.*, 2001), which is critical for bile salt transport. Finally, prolactin modulates the response of the immune system to stress (Dorshkind and Horseman, 2001; Dugan *et al.*, 2007; Dugan *et al.*, 2002). It is not yet clear if PL-I and/or PL-II have similar effects.

TGCs secrete several PLPs that regulate hematopoiesis. PLP-E and PLP-F can stimulate megakaryocytopoiesis and erythropoiesis (Bhattacharyya *et al.*, 2002; Lefebvre *et al.*, 2001; Lin and Linzer, 1999; Zhou *et al.*, 2005). PLP-E is expressed at the first half of pregnancy by P-TGCs, whereas PLP-F is secreted later in pregnancy by the spongiotrophoblast layer (Simmons *et al.*, 2008b), suggesting that they function in a sequential manner. PLP-E has been shown to stimulate human and mouse erythroid progenitor cell proliferation and differentiation through activation of the JAK/STAT pathway (Bittorf *et al.*, 2000). Proliferin 2 (PLF2) stimulates an increase in the fraction of long-term culture-initiating cells (LTC-IC) in cultured bone marrow cells (Choong *et al.*, 2003).

Production of paracrine factors that regulate the feto-maternal interface

The vascular bed and repertoire of immune cells in the uterus changes dramatically during gestation. TGCs secrete the PLP cytokines proliferin (PLF) and proliferin-related protein (PRP) that stimulate and inhibit endothelial cell migration, respectively (Jackson *et al.*, 1994). PLF is expressed in the early half of gestation and in all TGC subtypes except for S-TGCs (Simmons *et al.*, 2007). PRP is subsequently expressed in the latter half of gestation and in all four subtypes of TGCs. TGCs also express vascular endothelial growth factor (VEGF) (Voss *et al.*, 2000) and placental-like growth factor (PLGF) (Tayade *et al.*, 2007) in early gestation. Antagonists of VEGF/PLGF are also expressed in the placenta. Flt-1 is a VEGF receptor that can undergo alternative splicing to result in a secreted Flt-1 protein (sFlt-1) that blocks VEGF action. sFLT-1 transcripts are detected in the spongiotrophoblast that lies beneath the P-TGCs (Cross *et al.*, 2002; He *et al.*, 1999), implying a mechanism by which maternal blood vessels are prevented from growing into the junctional zone.

TGCs also produce several factors that can regulate blood flow. First, they secrete PLP-A that *in vitro* can inhibit the ability of NK cells to produce interferon-γ (IFNγ) (Muller *et al.*, 1999). Uterine NK cells are important for spiral artery dilatation through their production of interferon-γ (Ashkar *et al.*, 2000). TGCs also produce interferon-γ during mid-gestation (Platt and Hunt, 1998), and could affect NK cell function directly. Despite the predictions from the expression patterns and *in vitro* activity of PLP-A, *Plpa*-deficient mice have normal pregnancies unless the pregnant female mice are exposed to hypoxia (Ain *et al.*, 2004). Second,

TGCs express adrenomedullin (Montuenga *et al.*, 1997; Yotsumoto *et al.*, 1998), a vasodilator, and endothelial nitric oxide synthetase (eNos/Nos3) (Hemberger *et al.*, 2003), an enzyme that produces the vasodilator nitric oxide (NO) and that is implicated in vasorelaxation during pregnancy (Gagioti *et al.*, 2000). However, since spiral arteries at the feto-maternal interface lack smooth muscle, the targets of these vasodilators are unclear (Cross *et al.*, 2002). Finally, trophoblast cells, like endothelial cells, suppress coagulation of blood whereas blood normally rapidly clots when hemorrhage occurs. P-TGCs, ectoplacental cone and spongiotrophoblast cells express thrombomodulin, a protein that has anti-coagulant effects on maternal blood within the parietal yolk sac and placenta (Isermann *et al.*, 2003; Weiler-Guettler *et al.*, 1996).

The activity of the local immune function is also altered during pregnancy in rodents to prevent the maternal immune system from killing the allogeneic conceptus. The precise mechanisms are unknown. Progesterone produced by TGCs may have some effect since it can stimulate activities of type 2 T helper cells (Th2) (Szekeres-Bartho and Wegmann, 1996) that can secrete cytokines (e.g., IL-10) that have feto-protective effects. There are significant changes in the distribution of NK cells during pregnancy associated with the presence of a normal conceptus (Herington and Bany, 2007). This implies that factors from the conceptus, likely from TGCs, regulate NK cell homing, proliferation and/or survival.

Distinct or overlapping functions of the four different TGCs subtypes?

Based on their distinct locations in the placenta and different gene expression patterns (Simmons *et al.*, 2007; Simmons *et al.*, 2008b), we speculate that the four subtypes of TGCs have distinct functions (Table 1). P-TGCs express the greatest variety of PLPs among all subtypes of TGCs (Simmons *et al.*, 2008b). PL-I and PL-II can act on many maternal physiological systems such as corpus luteum, mammary gland, brain, and pancreas (Soares *et al.*, 2007). The angiogenesis and hematopoiesis related hormones (PLF, PRP, PLP-A, PLP-E, PLP-F) are also expressed by P-TGCs (Simmons *et al.*, 2008b), and may function early in establishing the parietal yolk sac before the circulation into the mature placenta is established. P-TGCs also express progesterone and interferon-γ (Yamamoto *et al.*, 1994; Platt and Hunt, 1998) which are important for decidualization and NK cell function. Thus, the functions of P-TGCs are very broad. In general, SpA-TGCs express factors that regulate cardiovascular functions including formation of blood vessels (PLF and PRP) and blood cells (PLF2, PLP-E, PLP-F), and dilation of spiral arteries (PLP-A by affecting NK cells) (Simmons et al., 2008b). SpA-TGCs also express placenta-specific cathepsins, Cts7 and Cts8, and Cts8 in particular is capable of mediating smooth muscle degradation and blood vessel disintegration to facilitate formation of tropho-blast-lined blood sinuses (Screen *et al.,* 2008). These would facilitate the maternal blood supply to the conceptus. The C-TGCs line the canals, but other than this structural role, it is difficult to imagine their function. S-TGCs produce the least number of PLP hormones (PL-II, PRP, PLP-K) (Simmons *et al.*, 2008b). Their expression of PRP, but not PLF, suggests that growth of endothelial cells into the labyrinth may be inhibited which would be critical for maintaining the hemo-chorial blood space. S-TGCs also secrete cathepsin Q (Simmons *et al.*, 2007), a cysteine protease

with related family members implicated in trophoblast invasion, as described above, and hormone regulation. Some cathepsins can cleave prolactin into peptides that have alternative functions (Clapp *et al.*, 2006; Hilfiker-Kleiner *et al.*, 2007; Piwnica *et al.*, 2006). The location of S-TGCs on the maternal side of the feto-maternal interface implies that they could cleave prolactin-like hormones before they leave the placenta and enter the maternal circulation.

Summary

Recent research has made striking progress in understanding the development and function of TGCs. There are at least four different subtypes of TGCs within the mature placenta, each arising at different times and locations in the placenta, and likely having distinct functions. While we now have a fairly good understanding of the regulation of TGC development, the functions of the different TGC subtypes remain very active areas of investigation and there are key open questions that should guide future studies. First, it will be intriguing to understand more details about the different functions of the TGC subtypes and how they are differentially regulated. Second, TGCs express a wide repertoire of hormones, but the biological function of most of these is unknown. Third, since it appears that TGCs regulate homeostatic physiological systems in the mother, it will be intriguing to see if and what type of physiological changes in the mother can alter the development and/or function of TGCs. There are insights from a variety of experimental animals that alteration of diet can affect placental development. Hypoxia during pregnancy can also alter expression of prolactin-like protein genes and supports the notion that these hormones may mediate responses to pregnancy stressors. These emerging themes will be important to pursue in order to gain better insights into the dialogue between the mother and fetus that occurs during pregnancy.

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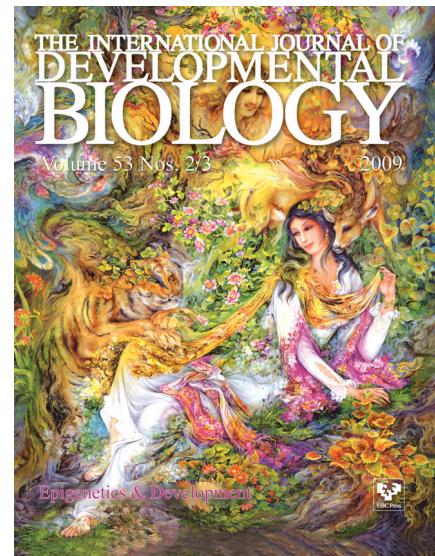**Commitment of hematopoietic stem cells in avian and mammalian embryos: an ongoing story**
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