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REVIEW

Developmental genes during placentation: insights from mouse mutants

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Abstract **Placenta, a temporary organ first formed during the development of a new life is essential for the survival and growth of the fetus in eutherian mammals. It serves as an interface for the exchange of nutrients, gases and wastes between the maternal and fetal compartments. During the past decades, studies employing gene-engineered mouse mutants have revealed a wide range of signaling molecules governing the trophoblast development and function during placentation under various pathophysiological conditions. Here, we summarize the recent progress with particular respect to the involvement of developmental genes during placentation.**

Keywords developmental gene, placentation, mouse mutant

Introduction

Placenta is essential for the development of a new life in placentalia mammals. It forms the interface between the fetal and maternal environments accounting for the exchange of nutrients, gases and wastes between the mother and fetus. Moreover, placenta is an important source of pregnancy-associated hormones and growth factors conducive to the success of pregnancy establishment and maintainace. In addition, it can protect the fetus from recognition by the maternal immune system (Rossant and Cross, 2001; Watson and Cross, 2005).

The trophoblast layer of the placenta arises from one of the first two cell types differentiated during early embryogenesis in mammals–the outer trophectoderm layer of the blastocyst. It has been shown that two distinct cell lineages are formed by embryonic day (E) 3.5 of development in the mouse embryos: the outer, specialized trophectodermal epithelium, which is destined to form the fetal part of the placenta, and the inner cell mass (ICM), which develops to form the embryo proper (Wang and Dey, 2006). At the time of implantation (E4.5), trophectodermal epithelium starts to differentiate to form different trophoblast cell types (Fig. 1). The trophectoderm cells away from the ICM, also called mural trophectoderm, stop dividing but continue to endoreduplicate their DNA to form the primary trophoblast giant cells that invade into the uterus (Cross et al., 1994). In the postimplantation embryo, more giant cells form from the outer regions of the ectoplacental cone to surround the entire conceptus. However, the trophectoderm cells overlying the ICM, which are polar trophectoderm, continue to proliferate in response to mitogenic signals from the epiblast, and form diploid extraembryonic ectoderm (proximal) and ectoplacental cone (distal) of the early conceptus (Copp, 1979). Thereafter, the extraembryonic ectoderm develops to form the chorionic epithelium, which is lined by a thin layer of mesothelial cells. The allantois from the posterior end of the embryo makes contact with the chorion at about E8.5. This process is termed chorioallantoic fusion or chorioallantoic attachment. Soon after, primary villi begin to develop across the chorionic surface (Cross et al., 2003) and blood vessels from the fetus soon fill in the villous folds. The trophoblast and its associated fetal blood vessels undergo extensive villous branching to create a densely packed structure called the labyrinth (Rossant and Cross, 2001; Watson and Cross, 2005). At the same time, chorionic trophoblast cells differentiate into two layers of syncytiotrophoblast cells. An additional mononuclear cell type called spongiotrophoblast

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cells remain outside the syncytiotrophoblast layer and form a layer between the labyrinth and the outer giant cells. Evidence from histological studies and the continuity of marker gene expression show that the spongiotrophoblast may largely derive from the cells of the ectoplacental cone. By E10.5, a placenta with complete structure has been formed in mice. Along with the course of trophoblast development and placental development, there are two distinct types of trophoblast invasion occurred to ensure the normal progression of pregnancy, endovascular and interstitial trophoblast invasion (Adamson et al., 2002). Endovascular invasion takes place soon after implantation. A subtype of trophoblast giant cell invades into the maternal spiral arteries that bring maternal blood to the implantation site. However, interstitial invasion starts after E12.5. Glycogen trophoblast cell from spongiotrophoblast layer invades interstitially into the uterus (Natale et al., 2006).

Evidence from the gene targeting experiments has shown that placentation is a process regulated by a great deal of signaling molecules and pathways (Supplemental Table 1). In this manuscript, we will primarily outline the pathophysiological significance of several developmental genes, including the fibroblast growth factor (FGF), Wnt, transforming growth factor beta (TGFβ)/bone morphogenetic protein (BMP), and Notch families.

FGF signaling pathway and placentation

FGF was first identified over 30 years ago based on their mitogenic and angiogenic activities from pituitary extracts (Armelin, 1973; Gospodarowicz, 1974). This discovery subsequently led to the identification of a large family of proteins, FGFs, and further characterization and implication of the FGF pathway cascades in various cellular processes including proliferation, apoptosis, cell survival, chemotaxis, cell adhesion, motility, and differentiation (Basilico and Moscatelli, 1992; Baird, 1994).

FGFs are a large family of peptide growth factors composed of 18 distinct multifunctional members in mammals (FGF1–FGF10 and FGF16–FGF23) (Gospodarowicz, 1974; Beenken and Mohammadi, 2009). These FGFs share a high-sequence homology within a central core domain of 120 amino acids (Ornitz and Itoh, 2001), which interacts with the FGF receptors (FGFR) (Eriksson et al., 1991; Zhu et al., 1991; Plotnikov et al., 2000). FGFRs belong to a family of receptor tyrosine kinases (RTKs). By now, five different FGFRs have been identified, known as FGFR1–5. However, most FGF signaling is mediated by FGFR1–4 (Lee et al., 1989; Powers et al., 2000; Schlessinger, 2000; Sleeman et al., 2001). Similar to other RTK receptors, FGFRs are transmembrane proteins composed of an extracellular ligand binding domain and a cytoplasmic domain which contains the catalytic protein tyrosine kinase core (Williams, 1993; Hunter, 2000; Johnson and Schlessinger, 2000). The extracellular ligand binding domains of FGFRs are composed of three immunoglobin-like domains (Schlessinger, 2000). FGFs can also bind to heparin or heparin sulfate proteoglycans (HSPG) with low affinity. HSPG binding of FGF induces FGFR dimerization followed by the transphosphorylation of receptor subunits, leading to the initiation of intracellular signaling events (Ornitz et al., 1996; Robinson et al., 1995; Powers et al., 2000). FGF signal can proceed via three main pathways: Ras/mitogen-activated protein kinase (MAPK) pathway; phospholipase C (PLC)/Ca^{2+} pathway; phosphoinositide-3 (PI3) kinase/Akt(PKB) pathway (Böttcher and Niehrs, 2005).

Evidence from mutant mice showed that FGF signaling cascades play important roles in all four milestone stages of placentation (Table 1). For example, disruption of *Erk2* gene (insertion at exon3, which contains protein kinase subdomains V and VI), which encodes extracellular signal-regulated kinase 2 (ERK2) led to embryonic lethality before E8.0 in mice (Saba-El-Leil et al., 2003). Phenotypic analysis revealed that *Erk2* mutant embryos failed to form the ectoplacental cone and extraembryonic ectoderm, which give rise to mature trophoblast derivatives (placentas) in the fetus, suggesting that Erk2 play an important role in normal

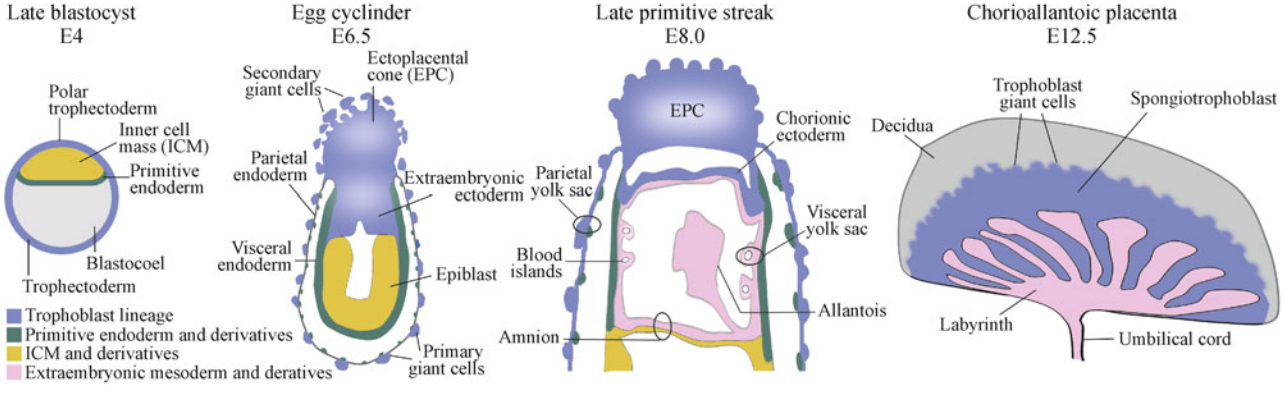

Figure 1 A diagram showing the development of mouse placenta. The development of placenta from embryonic day (E) 4.0-E12.5, showing the origin of the trophoblast, primitive endoderm, ICM and extra-embryonic mesoderm lineages and derivatives.

Table 1 The placenta phenotype of FGF family mutant mice

Gene	Expression in placentas	Placenta phenotype of mutant mice	Reference
Fgfr2	Trophectoderm and its derived extraembryonic ectoderm	Failure of chorioallantoic fusion; Defect of fetal vascular invasion into chorion	Arman et al., 1998; Xu et al., 1998
PLCδ1 /PLCδ3	Placenta trophoblast	Reduced vascularization, proliferation and aberrant apoptosis in labyrinth area	Nakamura et al., 2005
Pkbα (Akt1)	Trophoblast giant cell, spongiotrophoblast, labyrinth, the endothelium of fetal capillaries	Small layer of labyrinth and spongiotrophoblast; marked reduction of glycogen-containing cells in spongiotrophoblast; disordered fetal vasculature with fewer vessels	Yang et al., 2003
Gab1	Labyrinth trophoblast cells and spongiotrophoblast Cells	Severely reduced number of trophoblast cells in the labyrinth region	Itoh et al., 2000
Grb2	Unknown	Defects in chorioallantoic fusion, smaller labyrinth structures	Saxton et al., 2001
Sos1	Trophoblast giant cell, spongiotrophoblast, labyrinth layer	Disorganized spongiotrophoblast and labyrinth trophoblast layers, incomplete embryonic vasculature in labyrinth	Qian et al., 2000
B-Raf	Unknown	Discontinuous spongiotrophoblast and giant trophoblast layers, underdeveloped labyrinth layer (hypocellular areas filled with stroma in labyrinth)	Galabova-Kovacs et al., 2006
C-raf-1	Unknown	Reduced size of spongiotrophoblast and the labyrinth layer, poorly vascularized and abundant mesenchymal cells-contained labyrinth layer	Mikula et al., 2001
Mekk3 (Map3k3)	Unknown	Reduced embryonic blood vessels in the labyrinth layer	Yang et al., 2000
Mekk4 (Map3k4)	Labyrinth, spongiotrophoblast, and giant cell layers	A point mutation exhibited dysregulated placental development with increased trophoblast invasion.	Abell et al., 2009
Mek1 (Map2k1)	Labyrinth layer	Less defined spongiotrophoblast layer, more compact labyrinthine region with fewer blood vessels	Giroux et al., 1999; Bissonauth et al., 2006; Nadeau et al., 2009
Erk2 (Mapk1)	Trophoectoderm and its derivatives including ectoplacental cone, extraembryonic ectoderm and giant cells	Failure to form the ectoplacental cone and extraembryonic ectoderm in ERK2 (Ex3) mutants; Thinner labyrinthine layers, few fetal blood vessels penetrating into the labyrinthine layer in Erk2 (Ex2) mutants.	Hatano et al., 2003; Saba-El-Leil et al., 2003
Erk5 (Mapk7)	Labyrinth layer	Thinner labyrinth layer, less intermixing between embryonic and maternal blood vessels in the labyrinthine region, more apoptosis.	Regan et al., 2002; Sohn et al., 2002; Yan et al., 2003
FRS2α	Extraembryonic ectoderm	Trophoblast stem cells failed to maintenance of self-renewing.	Melillo et al., 2001; Gotoh et al., 2005
p38α (Mapk14)	Diploid trophoblast of the placenta, including the labyrinth, yolk sac	Thinner labyrinth layer, greatly reduced spongiotrophoblast,decreased vascular network within the labyrinth layer	Adams et al., 2000; Mudgett et al., 2000
Shp2 (Ptpn11)	Spongiotrophoblast and labyrinth trophoblast (unpublished data)	Diminished numbers of trophoblast giant cells, and failure to yield trophoblast stem (TS) cell lines.	Yang et al., 2006

trophoblast development in the mouse, particularly in regulating the proliferation of polar trophectoderm cells.

Chorioallantoic attachment is the first step during labyrinth development. Defects in this process are one of the most common causes accounting for midgestation embryonic lethality (Rossant and Cross, 2001). FGFR2 is a transmembrane protein with intrinsic tyrosine kinase activity, serving as a high affinity receptor for several FGF proteins. Deletion of exons 7, 8 and 9 in the *Fgfr2* gene resulted in mutated FGFR2 without the entire immunoglobin-like domain III. In one third of the mutants, chorion and allantois failed to fuse with each other (Xu et al., 1998). GRB2 is one of the adaptor proteins coupling with MAPK signaling pathway and it can transduce the activity of RTKs to the activation of Ras-MAP kinase

pathway. In a hypomorphic mutation of Grb2, mutants have defects in chorioallantoic fusion (Saxton et al., 2001), which is similar to *Fgfr2* mutants. It should be noted that only about one third of *Fgfr2* and *Grb2* mutants exhibit chorioallantoic attachment defects, similar to the case in mice with null mutation of VCAM1, a cell adhesion molecule expressed in the allantois (Gurtner et al., 1995; Kwee et al., 1995) or its ligand α4-integrin expressed on the chorionic mesothelium (Yang et al., 1995; Watson and Cross, 2005), both of which are known to mediate the chorioallantoic fusion. The fusion did occur in the rest of the *Fgfr2* mutants. However, these mutants exhibited defects in the following morphogenesis of the labyrinth.

Soon after chorioallantoic fusion, primary villi begin to develop at specific sites across the chorionic surface (Cross et al., 1994), and fetoplacental blood vessels from allantois concomitantly grow into the chorionic plate generating the fetal components of the placental vascular network to form a mature and functional placenta (Rossant and Cross, 2001; Watson and Cross, 2005). Mutation of many FGF signaling cascade molecules led to developmental defects in the labyrinth and/or the blood vessels of the fetoplacenta (Table 1). As mentioned above, in the absence of FGFR2 and GRB2, the blood vessels from the allantois failed to penetrate into the labyrinth trophoblast thoroughly and the labyrinth of the mutant placentas were smaller than that of their wild type counterparts (Xu et al., 1998; Saxton et al., 2001). These defects were associated with largely reduced trophoblast proliferation in the *Fgfr2* deficient labyrinth. Evidence achieved during the course of establishing trophoblast stem (TS) cell lines from the blastocyst and extraembryonic ectoderm in the presence of FGF4 reinforced the contention that FGF4, which is expressed by the inner cell mass (ICM) is important for trophoblast proliferation (Tanaka et al., 1998). Since FGFR2 is expressed strongly in the diploid trophoblasts during early postimplantation development (Haffner-Krausz et al., 1999), it is conceivable that FGFR2 functions as the main receptor for FGF4 to exert its proliferation-promoting effects. Upon FGFR2 depletion, the chorionic trophoblasts with impaired proliferation cease branching, leading to the insufficient penetration of the chorion by the fetal blood vessels from the allantois. In this respect, it remained elusive how Grb2 deficiency induce the labyrinth developmental defects.

In comparison with compound defects in the processes of chorioallantoic fusion and branching in *Fgfr2* and *Grb2* mutants, mice with depletion of Gab1, which is mainly expressed in the labyrinth trophoblast cells, showed severely reduced numbers of trophoblast cells in the labyrinth region (Itoh et al., 2000); whereas Mekk3 deficiency induced impaired fetal blood vessel formation in the labyrinth (Yang et al., 2000). In addition, gene null mutation mouse studies have revealed that gene targeting of FGF signaling cascade molecules, such as PLCδ1/PLCδ3, Pkbα (Akt1), Sos1, B-Raf, C-raf-1, Mek1, Erk2 (Ex2), Erk5 and Mapk14 (p38α) resulted in labyrinth morphogenesis abnormalities, including defective chorion development and allantois vascularization (Giroux et al., 1999; Adams et al., 2000; Mudgett et al., 2000; Qian et al., 2000; Mikula et al., 2001; Regan et al., 2002; Sohn et al., 2002; Hatano et al., 2003; Yan et al., 2003; Yang et al., 2003; Nakamura et al., 2005; Galabova-Kovacs et al., 2006). However, in most circumstances, it is difficult to distinguish the trophoblast versus fetal vascular contribution to these obvious labyrinth defects in above-mentioned gene mutants, and warrants future in-depth investigation. Nonetheless, it is generally accepted that FGF signaling is essential for normal labyrinth morphogenesis (Fig. 2).

Regarding the major cellular zone in mature murine placenta, the junctional zone is a trophoblast layer linking the labyrinth and maternal decidua basalis, which is composed of two principal trophoblast cell types, the spongiotrophoblasts and the invasive glycogen cells (Coan et al., 2005). The function of spongiotrophoblasts and glycogen cells remains largely unknown. Previous evidence indicated that the trophoblasts within the junctional zones line maternal venous sinuses and synthesize secreted proteins, such as IGF2 and soluble Flt1, suggesting that they could influence fetal-maternal interactions (Redline et al., 1993). Mice with gene null mutation of Akt1, Sos1, B-Raf, C-raf-1, Mek1, p38α exhibited impaired spongiotrophoblast development with substantially reduced numbers of glycogen-containing cells and/or increased numbers of trophoblast

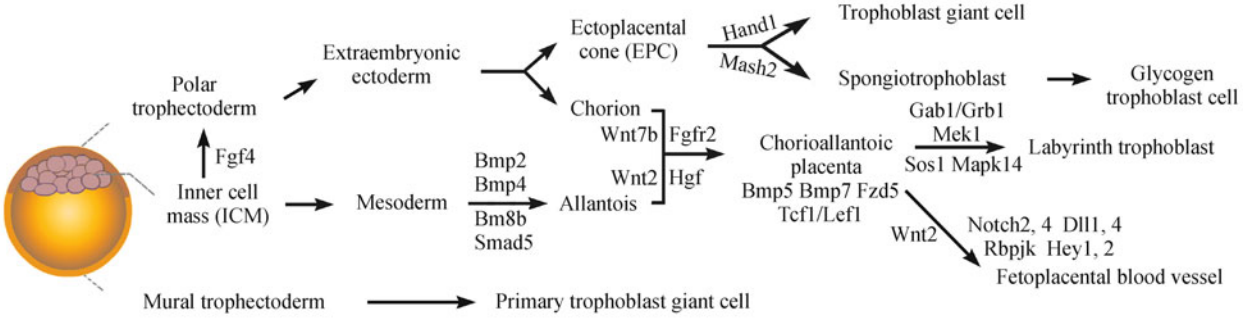

Figure 2 The regulation of developmental genes during trophoblast development and cell lineage specification.

giant cells (Giroux et al., 1999;Adams et al., 2000; Mudgett et al., 2000; Qian et al., 2000; Mikula et al., 2001; Yang et al., 2003; Galabova-Kovacs et al., 2006). These observations collectively pointed toward the fact that FGF and its driven downstream signaling also play an important role in governing the normal development of spongiotrophoblasts and glycogen cells (Fig. 2).

Wnt signaling and placentation

The first *Wnt* gene, *Wnt1*, was initially identified as a preferential insertion site for the murine mammary tumor virus in 1982 (Nusse and Varmus, 1982). Thereafter, the functions and mechanisms of Wnt signaling have been extensively studied. Wnt signaling is highly conserved from invertebrates to vertebrates and plays an important role in regulating cell proliferation, survival, differentiation, polarization, and migration during embryogenesis (Polakis, 2000; He, 2003; Huang and Klein, 2004; Moon et al., 2004; Fuerer et al., 2008). Deregulation of this signaling pathway has been implicated in many human diseases.

Wnts form a family of 19 secreted lipid-modified glycoproteins (Willert et al., 2003) in both human and mouse. They can signal through different downstream pathways including canonical and noncanonical pathways, which are determined by the dependence on β-catenin. With respect to canonical pathway, Wnts bind to frizzled (Fzd) receptors, which belong to a 10 member family of seven-pass transmembrane proteins (Bhanot et al., 1996) and low density lipoprotein receptor-related protein (LRP)5/6 receptors. This binding leads to inactivation of destruction complex, which is composed of glycogen synthase kinase 3 (GSK-3) β, casein kinase I (CKI), axin and adenomatous polyposis coli (APC), and promotes degradation of β-catenin when Wnt ligands are absent. Thus, β-catenin can accumulate in cytosol and translocate into the nucleus, where it can interact with its partner T cell/lymphoid enhancer binding (TCF/LEF) transcription factors to regulate the transcripton of target genes (Gordon and Nusse, 2006; Willert and Jones, 2006; Grigoryan et al., 2008; Huang and He, 2008). The characteristics of noncanonical Wnt pathway is β-catenin-independent and (LRP)5/6 receptors are also not required. Noncanonical signaling includes Wnt/calcium pathway, which utilizes G proteins or intracellular calcium influx with activation of calcium-sensitive kinases PKC and Ca^{2+}/calmodulin-dependent protein kinase II to transduce Wnt-mediated signals (Kühl et al., 2000), and the planar cell polarity (PCP) pathway, which is known to act through Rho-GTPases and JNK to regulate orientation of cellular structures (Mlodzik, 2002). Wnt signaling pathway can be inhibited by direct binding to secreted frizzled-related proteins (sFRPs), which are similar to Fzd receptors in the cysteine-rich domain (Rattner et al., 1997). In addition, canonical Wnt signaling can also be antagonized by Dickkopf proteins (DKKs), which bind to the Wnt co-receptors LRP5/6 (Glinka et al., 1998; Bafico et al., 2001; Mao et al., 2001; Semënov et al., 2001) and interact with Kremen to downregulate LRP receptors (Mao et al., 2001, 2002).

Regarding the physiologic significance of Wnt pathway during placental development, previous studies employing gene targeting mice have provided genetic evidence demonstrating a pivotal role of this pathway in trophoblast functions (Table 2, Fig. 2). For example, Wnt2 was expressed mainly in the allantois (unpublished data) and targeted disruption of *Wnt2* gene caused an impaired development of fetal vascular network in the labyrinth, leading to perinatal embryo demise (Monkley et al., 1996). Moreover, mice with null mutation of *Wnt7b* that is expressed on the chorion died at midgestation stages due to the defect of chorioallantoic fusion and the cells in the mutant chorion plate was disorganized. The α4 integrin protein, required for chorioallantoic fusion, is absent from cells in *Wnt7b* deficient chorion (Parr et al., 2001), suggesting that Wnt7b might regulate placental development by timely controlling the α4 integrin expression. In addition, abnormal labyrinth development, particular a failed chorioallantoic branching was also observed in Fzd5 mutant mice (Ishikawa et al., 2001). However, it still remains unknown where the defects are resided in the chorion and/or the allantoic blood vessels. In this respect, our recent work employing placenta-specific Fzd5 knockout mouse models further confirms that a trophoblast-depletion of Fzd5 receptors derails the normal chorioallantoic branching (unpublished data).

As the cofactors of nucleating β-catenin signaling, the LEF-1/TCF family of transcription factors have also been showed to be essential for normal placenta functions. For example, double targeted inactivation of Lef1 and Tcf1 led to impaired placenta formation in mice (Galceran et al., 1999),

Table 2 The placenta phenotype of Wnt family mutant mice.

Gene	Expression in placentas	Placenta phenotype of mutant mice	Reference
Wnt2	Allantois, fetal blood vessels in the labyrinth (unpublished data)	Defects of vascularization in placenta	Monkley et al., 1996
Wnt7b	Chorionic trophoblast	Defects in chorioallantoic attachment and cell organization in chorionic plate	Parr et al., 2001
Fzd5	Labyrinth trophoblast, yolk sac	Small labyrinth, failure of fetal vascular invasion into the chorion, defects in yolk sac angiogenesis	Ishikawa et al., 2001
Tcf1/Lef1	Placenta trophoblast (unpublished data)	Unknown defect in placenta	Galceran et al., 1999

although detailed phenotypic analysis and underlying mechanisms of this mutant mice remain elusive. Our recent work employing adenoviral vector-mediated DKK1 overexpression demonstrated that silencing nuclear Wnt-β-catenin signaling derail the normal trophoblast development, causing periimplantation pregnancy loss (Xie et al., 2008). Collectively, diversified Wnt signaling pathways are implicated in the morphogenesis of placentas.

Notch family and placentation

Notch family is a conservative developmental gene cluster. Notch receptors that exist at the cell surface interact with single-pass type I transmembrane ligands expressed on neighboring cells. After ligand binding, the proteolytic cleavage of Notch receptor generates the notch intracellular domain (NICD), which further translocates into the nucleus, and forms a complex accompanied with immunoglobulin kappa J region (RBPJk) and other proteins, leading to the transcriptional activation of notch target genes, such as the basic helix–loop–helix (bHLH) transcriptional repressors and the Hes/Hey family (Bray, 2006; Ehebauer et al., 2006).

A wide range of functions have been ascribed to the notch pathway in various developmental events (Bray, 2006; Ehebauer et al., 2006). One of the well-known activities of this signaling pathway is its participation in regulating vasculogenesis and angiogenesis, including that during allantoic development (Table 3, Fig. 2) (Gridley, 2007; Roca and Adams, 2007). The allantois is the developmental anlage for the midgestation formation of umbilical vasculature and entire fetoplacental vascular network in mice (Downs, 2006; Zeigler et al., 2006; Inman and Downs, 2007). Recent studies have demonstrated that gene targeted mutation for the respective notch family members like notch1, Dll4 and Jagged1 all led to placental vascular defects in mice (Gridley, 2007; Roca and Adams, 2007; Gasperowicz and Otto, 2008) (Table 3, Fig. 2). Moreover, there is evidence that notch pathway plays an essential role in specifying the trophoblast cell fate during placental development (Gasperowicz and Otto, 2008). One of the notable examples is the putative notch effector, Mash2, which is known as the proliferation factor of the spongiotrophoblasts (Guillemot et al., 1994; Tanaka et al., 1997). The Mash2 mutant mice lose the layer of spongiotrophoblast cells, with excessive giant cells at the E10.0 (Guillemot et al., 1994; Tanaka et al., 1997). It is well accepted that Mash2 deficient spongiotrophoblast progenitor cells fail to maintain the proliferation potential, and thus differentiate into the final cell type trophoblast giant cells. In addition, RBPJk mutants also exhibited trophoblast cell defects with a shallow development of the spongiotrophoblast layer (Oka et al., 1995). However, the cellular specified defects and underlying mechanisms in many notch family member mutant mice still warrant further investigation.

Table 3 The placenta phenotype of Notch family mutant mice

Gene	Expression in placentas	Placenta phenotype of mutant mice	Reference
Jagged 1	Unknown	Died at E10.0 due to the vascular defects. Large blood vessels were not present in the yolk sac.	Xue et al., 1999
Delta-like 4	Trophoblast giant cells, umbilical and vitelline arteries	At E10.5 no viable null embryos were found. Yolk sac defects. Haploid insufficiency leads embryonic lethality. Vascular remodeling defects in the yolk sacs and the major central placenta arteries undergo degeneration and regression.	Gale et al., 2004; Duarte et al., 2004
Notch 1	Vascular endothelial cells in labyrinth, maternal blood sinus (unpublished data)	Single knockout null embryos died before E11.5, but had no placenta defects. Conditional knockout (with tie2-cre) had yolk sac vascular remodeling failure and placenta blood vessels labyrinth invasion defects.	Swiatek et al., 1994; Limbourg et al., 2005
Notch 2	Allantois, spongiotrophoblast, giant cells, endovascular trophoblasts and mesenchymal derivatives in labyrinth	Delayed entry of maternal blood into the mutant placenta and poor blood sinus formation at later stages.	Nakayama et al., 1997; Hamada et al., 1999; Hamada et al., 2007
Notch 4	Vascular endothelial cells in labyrinth	Single knockout exhibited no obvious mutant phenotype. Notch 1 and Notch 4 double knockout had pale yolk sacs, lacking obvious blood vessels and failture of mutant endothelial cells to invade the labyrinth.	Krebs et al., 2000
RBP-J kappa	Mesodermal derivatives in labyrinth	Died before E10.0 due to the deficiency of chorioallantoic fusion.	Oka et al., 1995; Krebs et al., 2004
Hey1 and *Hey2*	Blood vessels of the allantois and chorionic plate	Complete lack of embryonic blood vessels in the placental labyrinth.	Fischer et al., 2004
Mash2	Ectoplacental cone, chorion and their derivatives in the placenta	Died at E10.0 because of the placental defects. No spongiotrophoblast cells and their precursors, more trophoblast giant cells.	Guillemot et al., 1994; Tanaka et al., 1997

TGFβ superfamily and placentation

TGFβ superfamily is a huge group of growth factors involved in many developmental processes, such as cell proliferation, differentiation and apoptosis. The TGFβ family ligands can form the homo or heterodimers that bind to two types of transmembrane serine/threonine kinase receptors, TGFβR1 and TGFβR2. The ligands bind one type of receptors and then recruit the other type receptor to form a complex. This ligand-receptor complex then stimulates the cytoplasmic-to-nuclear translocation of downstream Smad proteins, where they interact with transcription factor or coregulators and regulate the expression of BMP- or TGFβ/activin-specific target genes (Chang et al., 2002).

Increasing evidence has shown that TGFβ signaling pathway is an important player during trophoblast development and fetoplacentation (Table 4, Fig. 2) (Chang et al., 2002; Pollheimer and Knofler, 2005; Jones et al., 2006). For example, during postimplantation extraembryonic development, BMP subfamily proteins have been shown to play indispensible roles. The *Bmp2* mutant mice exhibited allantoic development and chorioallantoic fusion defects (Zhang and Bradley, 1996). The *Bmp5* and *7* double knockout mice, *Bmp8b*, *Smad1*, *Smad5* knockout mice all showed impaired allantoic development with a brokendown integrity of the allantois and chorion leading to the midgestation death (Chang et al., 1999; Solloway and Robertson, 1999; Ying et al., 2000; Lechleider et al., 2001; Tremblay et al., 2001). Moreover, loss of some TGFβ members led to the yolk sac vasculature defects. For example, more than half of the TGFβ1 null mutant fetuses died owing to the yolk sac defects (Dickson et al., 1995). TGFβR2, P300 and CREB binding protein (CBP) knockout mice had the similar yolk sac vasculature defects (Oshima et al., 1996; Yao et al., 1998; Oike et al., 1999). In addition, Nodal mutants exhibited compounded trophoblast developmental defects spanning labyrinth trophoblasts, spongiotrophoblasts and giant cells (Iannaccone et al., 1992; Ma et al., 2001). Using mouse trophoblast stem cell (TSC) line, recent study has demonstrated that activin can promote the TSC toward a labyrinth cell fate (Natale et al., 2009).

Perspective

Previous gene expression studies and application of gene targeting mouse models have greatly advanced our understanding of the regulatory network during trophoblast development and placental formation. One emerging chal-

Table 4 The placenta phenotype of TGF beta family mutant mice

Gene	Expression in placentas	Placenta phenotype of mutant mice	Reference
TGF-β1	Extraembryonic blood islands of the yolk sac, mesodermal cells of the allantois	About 50% homozygous and 25% heterozygous embryos died around E10.5. The yolk sac vasculature defects.	Akhurst et al., 1990; Dickson et al., 1995
Nodal	Spongiotrophoblasts	Insertional null mutant loss of the diploid spongiotrophoblasts and labyrinth, and had more giant cells. Hypomorphic mutation results in an expansion of the giant cell and spongiotrophoblast layers, and a decrease in labyrinthine development.	Iannaccone et al., 1992; Ma et al., 2001
Bmp2	Extraembryonic mesoderm, allantois.	Died at midgestation. Allantois had delayed development.	Winnier et al., 1995;
Bmp4	Allantois	Died between E6.5-E9.5. Yolk sac defects, reduced blood islands and no allantois.	Zhang and Bradley, 1996; Lawson et al., 1999
Bmp5 and *7*	Allantois, endoderm of the visceral yolk sac.	Died at E10.5. Allantois developed abnormal. Failure of chorioallantoic fusion.	Solloway and Robertson, 1999
Bmp8b	Extraembryonic ectoderm.	Short allantois, no allantois in some more severe mutant.	Lawler et al., 1994;
TGF-β receptor Type II	Extraembryonic blood islands of the yolk sac, mesodermal cells of the allantois	Homozygous embryos died around E10.5. Yolk sac hematopoiesis and vasculogenesis.	Oshima et al., 1996; Ying et al., 2000
Alk1	endothelial cells of fetal vessels	Dilated and fused chorioallantoic vessels.	Lechleider et al., 2001;
Smad 1	Yolk sac, allantois	Mutant embryos died around E9.5 due to the defects of allantois formation. The abnormal allantois failed to fuse to the chorion.	Tremblay et al., 2001; Hong et al., 2007
Smad 5	Allantois, yolk sac	Homozygous mutant died between E9.5 and E11.5. Allantois lacked a well-organized vasculature. Allantois could fuse to the chorion, but was not well-elongated.	Chang et al., 1999
P300	Unknown	Null embryos died between E9.0 and E11.5. The yolk sac was poorly vascularized.	Yao et al., 1998
CBP	Unknown	Homozygous embryos died between E9.5 and E10.5. Decreased erythroid cells and colony-forming cells in the yolk sac.	Oike et al., 1999

lenge is how this enriched knowledge obtained from laboratory animal models can be translated into the clinical investigation and diagnosis in human beings. This is particularly an important issue, since many trophoblast-related pregnancy diseases, such as intrauterine growth restriction and preeclampsia often hamper the pregnancy success in women. In this respect, increasing attention has been paid to explore the pathophysiological significance of the developmental genes during human trophoblast development and diseases (Murphy et al., 2006; Scifres and Nelson, 2009). For example, the expression of many Wnt ligands and receptors has recently been explored in human placental tissues (Peng et al., 2006; Pollheimer et al., 2006; Sonderegger et al., 2007). Moreover, recent findings demonstrate that an elevated circulating level of placenta-derived soluble TGFβ coreceptor, endoglin predicts the disease progress of preeclampsia in women (Levine et al., 2006; Venkatesha et al., 2006). It is worthy of mentioning that increasing evidence support the concept that the quality of embryo implantation determines the success of term pregnancy (Wilcox et al., 1999; Song et al., 2002; Dey, 2005; Ye et al., 2005). A short delay in the attachment of embryos to the endometrial bed during early pregnancy adversely affects later developmental processes. Therefore, many placenta developmental defects maybe seeded very early during implantation.

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