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Review
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Review

Novel roles of mechanistic target of rapamycin signaling in regulating fetal growth†

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Abstract

Mechanistic target of rapamycin (mTOR) signaling functions as a central regulator of cellular metabolism, growth, and survival in response to hormones, growth factors, nutrients, energy, and stress signals. Mechanistic TOR is therefore critical for the growth of most fetal organs, and global mTOR deletion is embryonic lethal. This review discusses emerging evidence suggesting that mTOR signaling also has a role as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth. In the fetus, liver mTOR governs the secretion and phosphorylation of insulin-like growth factor binding protein 1 (IGFBP-1) thereby controlling the bioavailability of insulin-like growth factors (IGF-I and IGF-II), which function as important growth hormones during fetal life. In the placenta, mTOR responds to a large number of growth-related signals, including amino acids, glucose, oxygen, folate, and growth factors, to regulate trophoblast mitochondrial respiration, nutrient transport, and protein synthesis, thereby influencing fetal growth. In the maternal compartment, mTOR is an integral part of a decidual nutrient sensor which links oxygen and nutrient availability to the phosphorylation of IGFBP-1 with preferential effects on the bioavailability of IGF-I in the maternal–fetal interface and in the maternal circulation. These new roles of mTOR signaling in the regulation fetal growth will help us better understand the molecular underpinnings of abnormal fetal growth, such as intrauterine growth restriction and fetal overgrowth, and may represent novel avenues for diagnostics and intervention in important pregnancy complications.

Summary Sentence

Emerging evidence suggest that mTOR signaling in the fetal liver, trophoblast, and decidua serves as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth.

Key words: decidua, developmental origins of health and disease, fetal development, insulin-like growth factor, intrauterine growth restriction, kinases, metabolism, nutrition, placenta, placental transport, pregnancy, syncytiotrophoblast.

Introduction

Fetal growth is broadly determined by the genetic growth potential of the fetus and the availability of oxygen and nutrients. Abnormal fetal growth affects 10–15% of all pregnancies in the developed world [1, 2] and occurs when the fetus fails to achieve its genetically determined growth potential (intrauterine growth restriction) or exceeds its growth determined genetically (fetal overgrowth). Abnormal fetal growth is not only associated with increased perinatal morbidity and mortality but also increases the risk of developing obesity, diabetes, and cardiovascular disease in childhood and later in life [3–12]. Thus, understanding the molecular mechanisms regulating fetal growth in normal and complicated pregnancies is of fundamental importance and of significant public health interest. Whereas the role of endocrine factors and nutrients in regulating fetal growth has been the focus of multiple excellent overviews [13–22], this review discusses emerging evidence implicating mechanistic target of rapamycin (mTOR) signaling as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth.

Mechanistic TOR is a serine/threonine kinase that regulates cell survival, metabolism, growth, and proliferation [23–27]. Mechanistic TOR exists in two complexes, mTOR complex (mTORC) 1 and 2, with the protein raptor associated with mTORC1 and rictor associated with mTORC2. mTORC1 regulates protein translation mediated by phosphorylation of S6K1 and 4EBP1 [23–28]. mTORC2 phosphorylates Akt, PKCα, and serum and glucocorticoid-regulated kinase 1 (SGK1), and regulates the actin skeleton, cell-cycle progression, anabolism, and cell survival [29–31]. Deptor is an endogenous inhibitor of both mTORC1 and 2 [32].

It is well established that mice lacking either *mtor* [33, 34], *raptor* [35], or *rictor* [36] die early in development, demonstrating the critical role of mTORC1 and 2 for embryonic development and growth. In contrast, whole-body *deptor* mutant KO mice are viable, fertile, and normal in size [37]. Moreover, there is a wealth of evidence that mTOR signaling plays an important role in the growth of individual fetal tissues and organs such as the intestine [38, 39], beta cell [40, 41], and skeletal muscle [42–45]. Similarly, decreased tissue growth is associated with inhibition of mTOR signaling in the fetal brown adipose [46], brain [47], heart [48], and thymus [49]. Whereas restricted fetal liver growth is not associated with mTOR inhibition following 48 h of starvation in the rat [50], inhibition of fetal liver mTOR signaling has been reported in other animal models of IUGR, including in the naturally occurring runt in pigs [51] and following maternal nutrient restriction in the baboon [52].

In this review, we will summarize recent data suggesting that mTOR signaling in specific tissues plays an important role in regulating overall fetal growth in response to changes in the availability of oxygen, nutrients, and growth factors by influencing global homeostatic systems. The mechanisms involved include mTOR regulation of placental function and influencing the maternal and fetal insulin-like growth factor (IGF) axis by regulating IGF binding protein 1 (IGFBP-1) secretion and phosphorylation. Both IGF-I [53, 54] and IGF-II [55, 56] are key regulators of fetal growth, and both growth factors are abundantly present in the maternal circulation and at the maternal–fetal interface [13] and regulate placental function [13, 57]. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II [58], we will focus on this specific IGF.

First, we will discuss the molecular mechanisms by which mTOR and the amino acid response (AAR) signaling pathway govern the secretion and phosphorylation of IGFBP-1 in the fetal liver. Because changes in the abundance and phosphorylation of IGFBP-1 have profound effects on the bioavailability of IGFs, fetal liver mTOR and AAR signaling link oxygen and nutrient delivery to fetal growth. Second, we will briefly review how trophoblast mTOR responds to a large number of growth-related signals, including amino acids, glucose, oxygen, folate, and growth factors, to regulate trophoblast mitochondrial respiration, nutrient transport, and protein synthesis, thereby influencing fetal growth. Third, we will examine the emerging evidence suggesting that mTOR functions as a decidual nutrient sensor which links oxygen and nutrient availability to increased phosphorylation of IGFBP-1 with preferential effects on the bioavailability IGF-I in the maternal–fetal interface and in the maternal circulation. We will conclude by presenting an overall model placing mTOR signaling as a critical hub in the overall homeostatic regulation of fetal growth and discussing how this model may help us better understand the molecular underpinnings of abnormal fetal growth. Finally, we will briefly speculate how this new knowledge could lead to novel avenues for diagnostics and intervention in important pregnancy complications.

Fetal liver mTOR and AAR signaling pathways link oxygen and nutrient availability to fetal growth

The bioavailability of fetal IGF-I is tightly regulated by IGFBP-1, which is primarily secreted by the fetal liver [59]. Phosphorylation of IGFBP-1 at three serine residues (Ser101, 119, and 169) is known to markedly increase its affinity for binding IGF-I [60], thus affecting the ability of IGF-I to interact with the IGF receptor, resulting in inhibition of IGF-I function [61, 62]. While phosphorylation of human IGFBP-1 does not alter the affinity for IGF-II [58], the affinity of phosphorylated human IGFBP-1 for IGF-I is 6 to 10- fold higher than for the nonphosphorylated protein [62–64] and hypoxia causes increased phosphorylation of IGFBP-1 with up to 300-fold higher affinity for IGF-I [65]. In addition, phosphorylation makes IGFBP-1 more resistant to proteolysis [61, 66]. Functionally, phosphorylation increases the capacity of IGFBP-1 to inhibit IGF-I-stimulated cell proliferation, DNA synthesis, amino acid transport, and apoptosis [67–69]. We have shown that hepatic IGFBP-1 phosphorylation induced in response to hypoxia caused a profound increase in its affinity for IGF-I, resulting in a marked inhibition of IGF-I-dependent cellular proliferation [65, 70]. IGFBPs also influence cell function by mechanisms that are independent of their ability to alter IGF–receptor interaction [71]. For example, IGFBP-1 contains RGD sequences that mediate binding to $\alpha 5\beta 1$ integrin, and this interaction stimulates cell migration independent of IGF-I [72].

There are numerous observations indirectly supporting a mechanistic link between increased IGFBP-1 secretion and restricted fetal growth. For example, mouse fetuses overexpressing *igfbp1* are growth restricted [73–75], clearly demonstrating a cause-and-effect relationship between IGFBP-1 and fetal growth in this species. In addition, IUGR is associated with elevated fetal IGFBP-1 [76] and increased IGFBP-1 phosphorylation at three specific residues in human fetuses [52, 77, 78]. Importantly, using liver tissue from growth restricted and control baboon fetuses we reported that IUGR is associated with increased fetal liver IGFBP-1 abundance and phosphorylation [52].

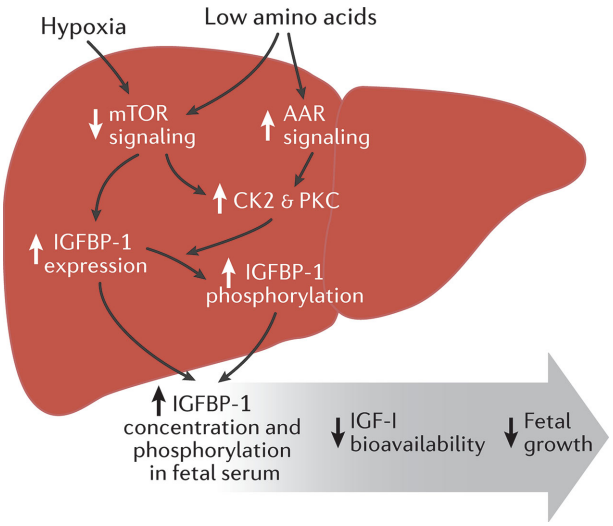

Figure 1. Liver mTOR as a link between decreased oxygen and nutrient availability and restricted fetal growth. Inhibition of fetal liver mTOR signaling and activation of AAR are mechanistically linked to increased IGFBP-1 secretion and IGFBP-1 phosphorylation in primary fetal hepatocytes, and we propose that these changes precede the development of IUGR. Both insulin-like growth factor I (IGF-I) and IGF-II are key regulators of fetal growth. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II only IGF-I is depicted in the figure (see the text). AAR, amino acid response pathway; CK2, casein kinase 2; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I; IUGR, intrauterine growth restriction; mTOR, mechanistic target of rapamycin; PKC, protein kinase C.

It is well established that IGFBP-1 secretion is regulated by nutrient and oxygen availability [79–82]; however, the underlying molecular mechanisms are largely unexplored. Moreover, how the phosphorylation of IGFBP-1 is regulated has, until recently, remained unknown. Based on studies in cultured HepG2 cells and primary fetal baboon hepatocytes, we demonstrated that inhibition of mTOR is required for increased IGFBP-1 secretion and phosphorylation in response to hypoxia [83, 84] and the enhanced IGFBP-1 secretion following decreased amino acid availability [85]. In contrast, IGFBP-1 hyperphosphorylation in response to amino acid deprivation is mediated by activation of the AAR signaling pathway [85] (Figure 1). The AAR signal transduction pathway is activated by limitation or imbalance of essential amino acids [86], resulting in increased levels of uncharged tRNA species, which bind to general control nonderepressible 2 (GCN2) kinase. As a result, the translation initiation factor eIF2α is phosphorylated, which leads to inhibition of global translation, but increased translation of activating transcription factor (ATF) 4. ATF4 increases the expression of a small group of genes involved in transport, metabolism, and oxidative stress [86].

Moreover, it was demonstrated that CK2 and PKC constitute the key kinases, regulated by mTOR and AAR, responsible for IGFBP-1 serine phosphorylation [52, 84, 87] (Figure 1). An important role of CK2 in the phosphorylation of IGFBP-1 was further supported by extensive co-localization between these two proteins in HepG2 cells (Figure 2). CK2 is a ubiquitous kinase that phosphorylates substrates characterized by multiple acidic residues surrounding the threonine or serine residue [88–90]. CK2 exists in tetrameric structures consisting of two catalytic subunits (α or α', in any combination) and two regulatory β-subunits.

Collectively, we propose that inhibition of fetal liver mTOR signaling and activation of AAR result in increased IGFBP-1 secretion and IGFBP-1 phosphorylation and constitute a key molecular link between decreased oxygen and nutrient availability and reduced fetal growth (Figure 1). Preliminary data suggest that these changes in the fetal liver occur prior to the development of IUGR in response to maternal nutrient restriction in nonhuman primates [91].

Mechanistic TOR regulates trophoblast function in response to an array of upstream signals

The placenta constitutes the main interface between mother and fetus and represents the primary site for maternal–fetal exchange. The syncytiotrophoblast, a highly specialized multinucleated epithelial cell layer covering the surface of the chorionic villi, produces a multitude of hormones, mediates nutrient transport, and forms a physical and immunological barrier between the maternal and fetal circulations. Thus, the syncytiotrophoblast is strategically positioned as a large maternal–fetal interface, which determines nutrient supply to the fetus. Moreover, a wide array of cellular signaling pathways in the syncytiotrophoblast modulate and integrate placental growth and function in response to maternal and fetal cues [92].

Upstream signals influencing trophoblast mTOR signaling

mTORC1 integrates a large number of metabolic signals, including hormones and growth factors, such as insulin, IGF-I and EGF, cellular ATP levels, hypoxia, DNA damage, amino acids, glucose, and fatty acids, to regulate cellular metabolism, growth, and proliferation [93, 94]. In contrast to mTORC1, mTORC2 predominantly responds to insulin/PI3K signaling [93]. These signals are likely to regulate mTOR signaling also in trophoblast cells as confirmed for growth factors [95], fatty acids [96], and glucose [97]. In addition, corticosterone administration to pregnant mice has been reported to inhibit placental mTORC1 and mTORC2, as evidenced by a decrease in the ratio of the degree of phosphorylation/total abundance for 4EBP1 and S6K and Ser 473 phosphorylation of Akt [98]. Moreover, adiponectin decreases trophoblast mTOR signaling activity by inhibiting insulin signaling [99–101]. We have recently reported that mTORC1 and mTORC2 are novel folate sensors in the placenta and beyond [102]. Specifically, folate deficiency in pregnant mice caused a marked inhibition of mTORC1 and mTORC2 signaling in multiple maternal and fetal tissues, downregulation of placental amino acid transporters, and fetal growth restriction [103]. In addition, folate deficiency in cultured primary human trophoblast (PHT) cells resulted in inhibition of mTORC1 and mTORC2 signaling and decreased the activity of key amino acid transporters [104]. Folate sensing by mTOR in PHT cells is independent of the accumulation of homocysteine and requires the proton-coupled folate transporter (PCFT, SLC46A1). These findings, which provide a novel link between folate availability and cell function, growth, and proliferation, may have broad biological significance given the critical role of folate in normal cell function.

In summary, trophoblast mTORC1 has an array of upstream regulators, including free fatty acids, amino acids, glucose, ATP, and oxygen (Figure 3), and it is likely that the placental levels of these nutrients are changed in conditions such as placental insufficiency, maternal undernutrition, or obesity [105, 106]. It has been proposed that the placenta integrates a multitude of maternal and fetal nutritional cues with information from intrinsic nutrient-sensing signaling pathways to match fetal demand with maternal supply by regulating maternal physiology, placental growth, and nutrient transport,

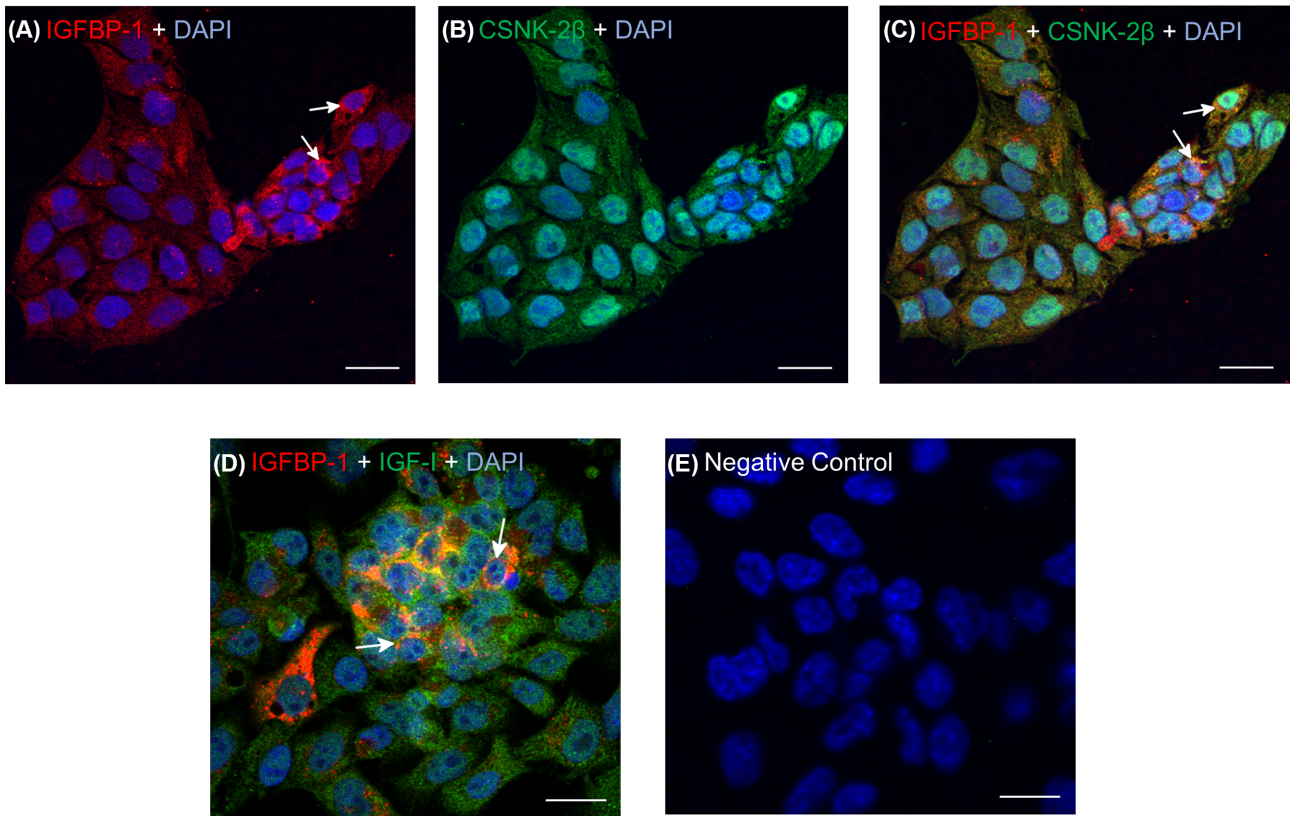

Figure 2. Dual Immunofluorescence staining for the co-localization of IGFBP-1 and CSNK-2β and the co-localization of IGFBP-1 and CSNK-2β. Human hepatocellular carcinoma (HepG2) cells were stained with anti-mouse IGFBP- 1 (monoclonal antibody 6303), anti-rabbit IGF-I, and anti-rabbit CSNK-2β antibodies. Corresponding secondary antibodies were Alexa anti-mouse 660 (shown green) and anti-rabbit 568 (red). Images were captured via confocal microscopy. (A and B) IGFBP-1 (red) is predominantly localized in the perinuclear region of the cells (A, white arrows), whereas CSNK-2β (green) is detected throughout the cell (B). (C) Merged channel image shows co-localization (yellow) predominantly in the perinuclear region (white arrows). (D) Co- localization of IGFBP-1 (red) and IGF-I (green) in HepG2 cells, indicating a positive control, with perinuclear localization of IGFBP-1 signal (white arrows). (E) Dual immunofluorescence with no primary antibodies depicting a negative control where no staining was visualized. Scale bars: 20 μm. Reproduced from [84] with permission. CK2-2β, casein kinase 2-2β; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I.

and that trophoblast mTOR plays a critical role in this homeostatic regulatory loop [92, 107, 108].

Mechanistic TOR regulates key trophoblast functions

mTORC1 is the master regulator of protein synthesis mediated by phosphorylation of p70S6 kinase (S6K1), activating the protein translation initiation, and eiF4E binding protein (4EBP), which allows 5′cap-dependent translation [109]. mTORC1 is also an important regulator of cellular lipid, nucleotide, and glucose metabolism. For example, mTORC1 stimulates de novo lipid synthesis by SREBP activation [110] and promotes a switch from oxidative phosphorylation to glycolysis, thereby shunting glucose into the pentose phosphate pathway and generating critical intermediates such as ribose-5 phosphate needed by growing and proliferating cells [110]. In addition, mTORC 1 inhibits autophagy by phosphorylation of ULK, a key activator of autophagy [111], and stimulates mitochondrial biogenesis mediated by the transcription factor PGC1α [112]. mTORC2, on the other hand, promotes cell proliferation and survival by phosphorylating a number of the AGC protein kinase family members including Akt, PKCα, and SGK1, which regulate cytoskeletal remodeling and cell migration [93].

Most of the information pertaining to mTOR regulation of cell function has been generated in various nonplacental cell lines. However, it is likely that mTOR signaling has similar functions in, for example, PHT cells. It was recently reported that mTORC1, but not mTORC2, is a positive regulator of oxidative phosphorylation mediated by effects of mitochondrial biogenesis [113]. In addition, using human placental villous explants and PHT cells, we have identified a novel role for mTOR signaling as a regulator of nutrient transport in mammalian cells [95, 97, 104, 114–116]. Specifically, we reported that inhibition of both mTORC1 and/or mTORC2 downregulates trophoblast System A and L amino acid transport activity by affecting the plasma membrane trafficking of specific System A (SNAT2) and System L isoforms (LAT1) [95]. Furthermore, it was demonstrated that Nedd4–2, an E3 ubiquitin ligase, is required for the regulation of plasma membrane trafficking of amino acid transporter isoforms by mTORC1, but not mTORC2 [116]. In contrast, regulation of amino acid transporter trafficking by mTORC2 in PHT cells is mediated by the Rho GTPases Cdc42 and Rac1, which influence the actin skeleton (Rosario et al, unpublished observations). The powerful effects of mTOR signaling on nutrient transport are not limited to amino acids. For example, both mTORC1 and 2 are positive regulators of trophoblast folate uptake by modulating the

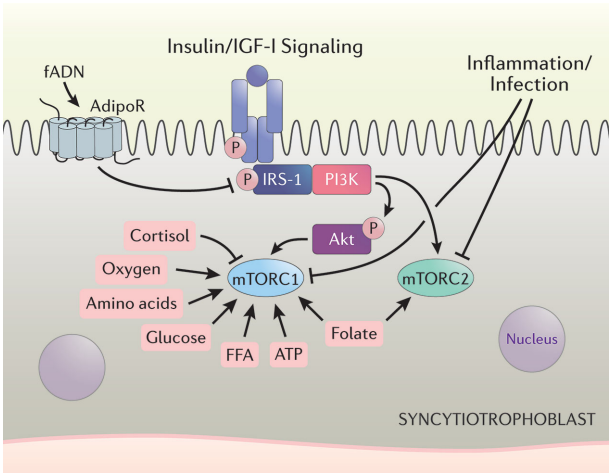

Figure 3. mTORC1 signaling is influenced by a multitude of upstream regulators. AAR, amino acid response pathway; AdipoR, adiponectin receptor; Akt, protein kinase B; ATP, adenosine triphosphate; fADN, full length adiponectin; FFA, free fatty acids, IRS-1, insulin receptor substrate 1; mTORC1, mechanistic target of rapamycin complex 1; mTORC2, mechanistic target of rapamycin complex 2; PI3K, phosphoinositide 3-kinase.

cell surface expression of folate receptor-α (FR-α) and the reduced folate carrier [104].

Taken together, a diverse set of metabolic signals impinges on trophoblast mTOR signaling, which regulates key placental functions, which in turn influence fetal growth and development. For example, mTOR regulation of trophoblast oxidative phosphorylation influences ATP availability with potential profound effects on all active transport processes. In addition, mTOR directly regulates placental transport of amino acid and folate, thereby affecting the fetal availability of these critical nutrients and fetal growth. Moreover, mTOR regulates placental protein synthesis directly and indirectly (by modulating ATP availability) with expected consequences for placental growth.

Placental mTOR signaling and abnormal fetal growth

A consistent relationship exists between changes in placental mTORC1 signaling and altered fetal growth in women and across a range of animal models of IUGR and fetal overgrowth (Table 1). Specifically, placental mTORC1 is altered in pregnancy complications associated with abnormal fetal growth and in animal models where maternal nutrient availability has been altered experimentally. Placental mTORC1 activity is inhibited in human IUGR [117, 118] and activated in placentas of large babies born to obese mothers [119]. Furthermore, placental mTORC1 activity has been reported to be decreased in hyperthermia-induced IUGR in the sheep [120], in response to a maternal low protein diet in the rat [121] and maternal calorie restriction in the baboon [122]. In general, placental nutrient transport, specifically placental amino acid transport, is regulated in the same direction as mTOR signaling (Table 1). However, Sferruzzi-Perri and co-workers reported that undernutrition in pregnant mice resulted in inhibition of placental mTOR signaling, using S6K phosphorylation as a functional readout, but increased transplacental amino acid transport [123]. The reasons for this contrasting finding remain to be established but may be related to the moderate calorie restriction used in the study of Sferruzzi-Perri et al [123].

Mechanistic TOR functions as a decidual nutrient sensor and regulates IGFBP-1 secretion and phosphorylation

In the maternal compartment, the decidua is a major site of IGFBP-1 synthesis and secretion. Locally in the placental barrier IGFBP-1 inhibits trophoblast invasion [124, 125]. Furthermore, the decidua constitutes the major source for maternal circulating IGFBP-1 in pregnancy [126, 127]. Serum IGF-I concentrations are decreased in mothers delivering IUGR babies [128] and most [128–138], but not all [139–142], studies show that IUGR or low birth weight is associated with increased maternal serum IGFBP-1 levels. Because maternal IGF-I is a powerful positive regulator of placental function and growth [143–145], alterations in the maternal IGF-I/IGFBP-1 levels in pregnancies complicated by IUGR may directly contribute to the restricted fetal growth.

Hypoxia and leucine deprivation markedly increased IGFBP-1 phosphorylation and decreased IGF-I bioactivity in cultured human endometrial stromal cells decidualized in vitro [146]. Moreover, IGFBP-1 phosphorylation is increased in decidualized stromal mesenchymal cells in human IUGR [147]. We examined decidua and maternal plasma collected at delivery from appropriate-for-gestational age (AGA) and IUGR pregnancies and maternal plasma collected in late first trimester from women who later delivered an AGA or IUGR infant. It was demonstrate that decidual mTOR is markedly inhibited, AAR is activated, and IGFBP-1 abundance and phosphorylation are increased in IUGR [147]. Moreover, IGFBP-1 hyperphosphorylation in maternal first trimester plasma is associated with the development of IUGR [147].

This data suggest that the decidua functions as a nutrient sensor linking limited oxygen and nutrient availability to increased phosphorylation of IGFBP-1, mediated by mTOR and AAR signaling. Hyperphosphorylation of maternal plasma IGFBP-1 may serve as a novel early biomarker of IUGR. These observations are consistent with the possibility that IGFBP-1 phosphorylation constitutes a link between decreased decidual oxygen and nutrient availability and reduced fetal growth, mediated by diminished IGF-I bioavailability, resulting in inhibition of trophoblast invasion [125, 148, 149] and placental function (Figure 4).

Interestingly, compelling data generated in a genetic mouse model of spontaneous preterm birth, involving a conditional deletion of the tumor suppressor *p53* in uterine tissues, implicate activation of mTORC1 signaling in spontaneous and inflammation-induced preterm birth [150–152]. Specifically, decidual *p53* deficiency resulted in premature decidual senescence mediated by mTORC1 activation, leading to preterm birth and fetal death, outcomes that were prevented with mTORC1 inhibitors [150–152].

Mechanistic TOR signaling as a critical hub in the overall homeostatic control of fetal growth: the example of IUGR

The evidence presented above suggest that mTOR signaling in the fetal liver, trophoblast, and decidua serves as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth. This concept can be illustrated using IUGR as an example (Figure 5). The most common cause of IUGR in Western societies is believed to be placental insufficiency due to a lack of normal gestational increase in uteroplacental blood flow caused by suboptimal trophoblast invasion. It is often assumed that the failure

Table 1. Examples of studies reporting placental mTORC1 signaling and amino acid transport capacity in association to maternal nutrition and fetal growth.

	Placental mTORC1 activity	Placental amino acid transport activity	References
Human IUGR	Decreased	Decreased	[115, 158, 159]
Low protein diet in the rat with IUGR	Decreased	Decreased	[121]
Maternal nutrient restriction in the baboon with IUGR	Decreased	Decreased	[122]
Human GDM with fetal overgrowth	Increased	Increased	[184, 185]
Human obesity with fetal overgrowth	Increased	Increased	[183]
High fat diet mouse with fetal overgrowth	Increased	Increased	[101, 186, 187]

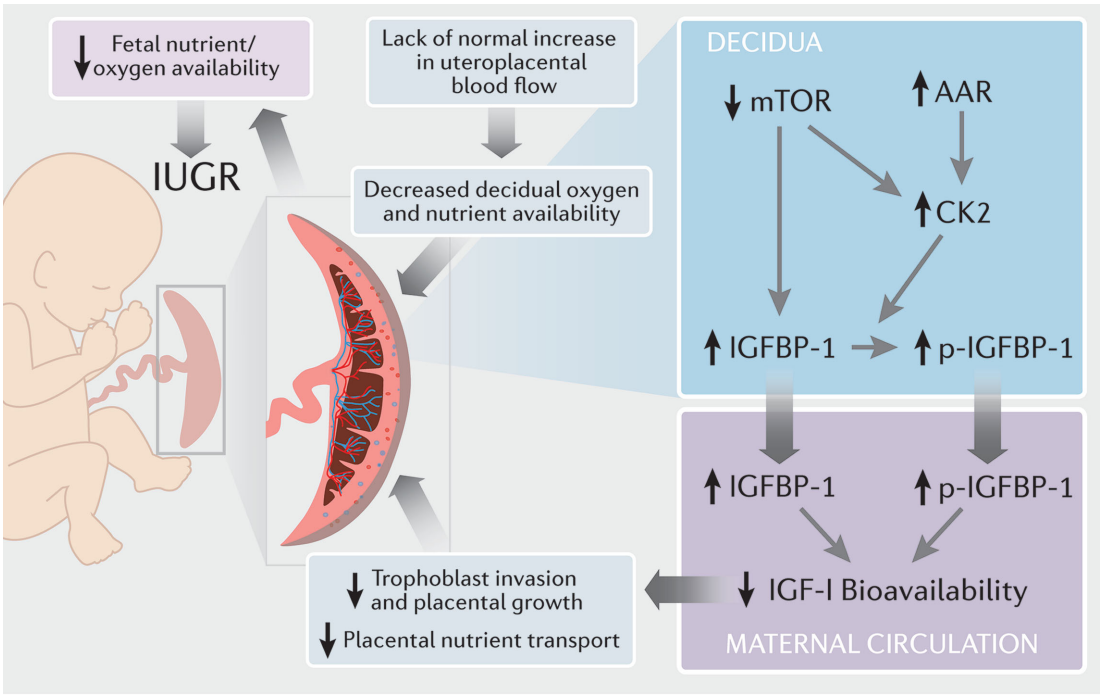

Figure 4. Decidual nutrient sensing. A model linking decreased nutrient and oxygen availability in the decidua in early pregnancy to the development of IUGR. Both insulin-like growth factor I (IGF-I) and IGF-II are key regulators of placental function, and both growth factors are abundantly present in the maternal circulation and at the maternal–fetal interface. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II only IGF-I is depicted in the figure (see the text). AAR, amino acid response pathway; CK2, casein kinase 2; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I; IUGR, intrauterine growth restriction; mTOR, mechanistic target of rapamycin.

of uteroplacental blood flow to increase normally directly causes the restricted fetal growth. However, an array of adaptive responses in the decidua, trophoblast, and fetus as a consequence of the initial change in uteroplacental blood flow, some of which are mediated by inhibition of mTOR (Figure 5), are likely to play important roles.

One consequence of the lack of normal increase in uteroplacental blood flow is that nutrient and oxygen availability decreases in the decidua, trophoblast, and, ultimately, in the fetus, which inhibits mTOR signaling in these tissues. In the decidua, mTOR inhibition results in the increased release of hyperphosphorylated IGFBP-1, which effectively binds IGF-I, decreasing the bioavailability of this important growth factor at the maternal–fetal interface and in the maternal circulation. Because IGF-I promotes placental growth [143–145] and function, specifically amino acid and glucose transport in cultured trophoblast cells [114, 153–157] and across the placenta in vivo [143], the result is a decreased placental growth and placental nutrient transfer contributing to the development of IUGR. The predominant placental response to a lack of normal increase in uteroplacental blood flow is inhibition of mTORC1 and mTORC2 signaling [115, 158, 159], downregulation of placental nutrient transport, including decreased activity of amino acid [160–165] and folate transporters [166], decreased mitochondrial function [113], and protein synthesis, which directly contributes to decreased fetal nutrient availability and IUGR.

A regulatory loop involving mTOR inhibition and IGFBP-1 phosphorylation—similar to what is present in the decidua—exists in the fetal liver. Inhibition of fetal liver mTOR signaling and activation of AAR result in increased IGFBP-1 secretion and IGFBP-1 phosphorylation, which may occur prior to the development of IUGR [91], and constitute a key molecular link between decreased oxygen and nutrient availability and reduced fetal growth.

Trophoblast mTOR signaling may regulate placental secretion of factors that influence maternal and/or fetal physiology. This hypothesis is supported by our preliminary observations linking trophoblast mTOR signaling to fetal liver IGFBP-1 secretion and phosphorylation [167]. Specifically, incubation of HepG2 cells, an established model for fetal hepatocytes, in conditioned media from PHT cells in which raptor (mTORC1 inhibition) or rictor (mTORC2

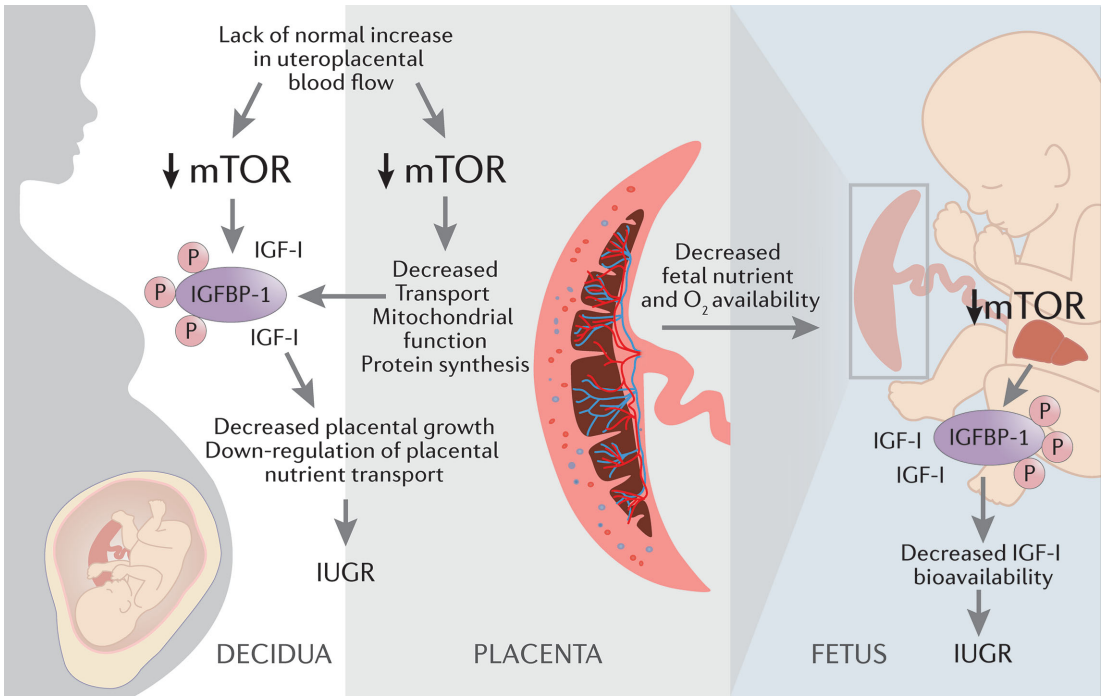

Figure 5. A model placing mTOR signaling as a critical hub in the overall homeostatic regulation of fetal growth. IGFBP-1 binds both IGF-I and IGF-II. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II only IGF-I is depicted in the figure (see the text). IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I; IUGR, intrauterine growth restriction; mTOR, mechanistic target of rapamycin.

inhibition) had been silenced, caused an increase in IGFBP-1 secretion and phosphorylation [167].

The proposal that decidual, trophoblast, and/or fetal liver mTOR signaling plays an important role in regulating fetal growth has yet to be systematically tested in rigorous animal experiments involving approaches for tissue-specific, inducible targeting of the genes in the mTOR pathway. However, when *s6k1* expression was rescued in the placenta of *s6k1–/–* mice using tetraploid embryo complementation, the fetal growth restriction in *s6k1–/–* mice was completely rescued [168, 169], strongly implicating a key role of trophoblast mTORC1 signaling in determining fetal growth in the mouse. Furthermore, some MTOR gene variants in humans may be associated with major functional deficits resulting in early pregnancy growth failure and miscarriage, which is supported by reported associations between a single nucleotide polymorphism in the MTOR gene and recurrent spontaneous abortion [170]. Moreover, an important role of mTOR in the regulation of fetal growth is further supported by animal experiments showing that administration of the mTORC1 inhibitor rapamycin at embryonic day 11 in mice causes spontaneous abortions and fetal lethality around embryonic day 16 [171]. The fact that MTOR gene variants yet to be associated to birth weight in large GWAS studies [172, 173] may suggest a marginal role for mTOR signaling in regulating fetal growth in women. However, a lack of association between variants in a particular gene and a phenotype in GWAS studies cannot be taken as evidence that the gene in question is unimportant in determining the phenotype. This point is best illustrated by efforts in the past 20 years to identify genes responsible for the heritability of type 2 diabetes: the total number of associated variants explains only a small proportion of the heritability of this disease. More importantly, however, no gene variants in, for example, insulin (INS), insulin receptor (INSR), PI3-kinase (PIK3CA), GLUT 2 (SLC2A2), or GLUT 4 (SLC2A4) have been associated with type 2 diabetes risk even in the most recent GWAS study involving more than 600,000 subjects [174], which cannot lead to the conclusion that insulin, the insulin receptor, PI3 kinase, Glut 2, and Glut 4 are inconsequential for the regulation of glucose homeostasis. In analogy, no firm conclusion with respect to the importance of mTOR signaling in the regulation of fetal growth can be drawn from the fact that no genetic variant at the MTOR locus has been shown to associate with birth weight in GWAS.

One important implication of this model is that intervention strategies to alleviate or prevent IUGR must take mTOR-mediated adaptive responses in the decidua, trophoblast, and fetal liver into account and are unlikely to be successful if they attempt to correct isolated fetal deficits associated with IUGR. Based on the concept that decreased fetal amino acid availability represents a key mechanism underpinning the development of IUGR, maternal amino acid supplementation has been contemplated [175, 176] as a strategy to prevent and treat IUGR. It is possible that the positive effects of maternal supplementation with branched chain amino acids on fetal growth that has been reported in animals with normal sized and IUGR fetuses [45, 177, 178] may be due to activation of mTOR in the decidua, trophoblast, and the fetus. Given the recent successful development of in vivo trophoblast-specific gene targeting approaches in mice [179–181], it may be possible to design interventions that activate placental mTOR signaling in IUGR in the future. Drug discovery aiming at identifying mTOR activators or inhibitors of DEPTOR may lead to the development of drugs useful in IUGR. Because mTOR activation promotes cancer cell proliferation, survival, metabolic transformation, and metastasis, a number of drugs have been developed for use in cancer [182]. Albeit speculative, it is possible that some of these drugs could be considered in selected cases of fetal overgrowth in maternal obesity and gestational diabetes, conditions associated with placental mTOR

activation and enhanced placental function [183]. Identifying biomarkers for IUGR in early pregnancy could improve the clinical management of these patients by allowing early intervention, preventing some of the perinatal complications associated with this condition. IUGR is associated with inhibition of mTOR signaling and increased IGFBP-1 phosphorylation in the decidua, and our data suggest that IGFBP-1 hyperphosphorylation in first trimester maternal plasma may serve as a novel predictive IUGR biomarker.

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