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Regulatory Pathways Controlling the Endovascular Invasive Trophoblast Cell Lineage

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Abstract

Hemochorial placentation is characterized by trophoblast-directed uterine spiral artery remodeling. The rat and human both possess hemochorial placentation and exhibit remarkable similarities regarding the depth of trophoblast invasion and the extent of uterine vascular modification. *In vitro* and *in vivo* research methodologies have been established using the rat as an animal model to investigate the extravillous/invasive trophoblast lineage. With these research approaches, two signaling pathways controlling the differentiation and invasion of the trophoblast cell lineage have been identified: i) hypoxia/hypoxia inducible factor and ii) phosphatidylinositol 3-kinase/AKT/Fos like antigen I. Dissection of these pathways has facilitated identification of fundamental regulators of the invasive trophoblast cell lineage.

Keywords

Activator protein 1; AKT; FOS like antigen 1; Hypoxia; Hypoxia inducible factor; Phosphatidylinositol 3-kinase; Placentation; Pregnancy; Trophoblast invasion

The maternal-fetal interface is a dynamic site where uterine and placental structures cooperate to promote development of the fetus. The rat, mouse, and human each possess a hemochorial placenta [1–3]. This type of placentation is characterized by erosion of the maternal uterine epithelium and vasculature permitting the direct flow of maternal nutrients to trophoblast cells. In species exhibiting hemochorial placentation, remodeling of the uterine vasculature is critical for the success of pregnancy [4–8]. As gestation progresses, uterine spiral arteries supplying the placenta are modified creatingflaccid, low resistance blood vessels [7–11]. These vessels are the conduit required to meet the nutrient demands of the fetus. Central to uterine spiral artery remodeling is a specialized population of trophoblast cells referred to as invasive trophoblast or alternatively, in humans, as extravillous trophoblast [5, 7, 9, 11]. Defective hemochorial placentation, including impairments in uterine spiral artery remodeling, leads to pregnancy related disorders (preeclampsia, intrauterine growth restriction, preterm birth) that cause significant morbidity and mortality for both mother and fetus [4–7]. In addition, in utero insults have fundamental organizational effects on the developing fetus, which affect postnatal health and susceptibility to adult disease [12, 13].

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The barrier for progress in understanding the invasive trophoblast cell lineage is the availability of appropriate experimental models. Scientific approaches using cell culture systems and animal models that permit molecular dissection of mechanisms are required. Our focus is on pathways that control cell differentiation. Therefore a trophoblast stem (TS) cell is the most appropriate cell culture model. TS cells possess the capacity to proliferate and to differentiate into mature trophoblast cell lineages and are ideal models for investigating mechanisms underlying trophoblast cell development [14–16].

Genes implicated in the regulation of placentation exhibit conservation in their expression patterns and actions (17–22]. The mouse has been an exceptional animal model for elucidating the regulation of most aspects of hemochorial placentation [21, 23]. However, the mouse and human exhibit fundamental differences in the extent of intrauterine trophoblast cell invasion and uterine spiral artery remodeling. In the mouse, the depth of trophoblast cell invasion is limited, whereas in the human the process is robust [8, 24, 25]. Organization of rat and human placentation sites show significant conservation, especially regarding trophoblast cell-directed remodeling of the uterine spiral arteries [24, 26–39]. Both species exhibit deep trophoblast invasion. The rat has many of the advantages of the mouse, including the capacity for genetic manipulation [40]. We have established relevant *in vitro* and *in vivo* research methods using the rat as an animal model to investigate molecular mechanisms regulating trophoblast cell differentiation and invasion [24, 35–39, 41–48]. Blastocyst-derived rat TS cells and Rcho-1 TS cells have proven to be effective *in vitro* culture systems for elucidating regulatory pathways controlling trophoblast cell differentiation (41, 44].

Several "candidate" signaling pathways have been implicated in regulating differentiation of the invasive trophoblast cell lineage [21, 49]; however, the significance of most of these pathways during *in vivo* placentation is unknown. In this short review, we focus on two pathways as probes into the control of the invasive trophoblast cell lineage: i] hypoxia/ hypoxia inducible factor (HIF); and ii] phosphatidylinositol3-kinase (PI3K)/AKT/FOS like antigen 1 (FOSLl). We have established the importance of these pathways using both *in vitro* and *in vivo* experimentation [38, 43, 50–52].

Hypoxia/HIF Signaling Pathway

Oxygenation is critical to tissue morphogenesis. Oxygen deficits can stimulate vascular development, tissue nutrition and growth, and promote cellular specialization. This is also true for placental morphogenesis. Oxygen tensions tend to be lower during early pregnancy and increase following the establishment of the hemochorial placenta [53–59]. Low oxygen tension (hypoxia) is a physiological regulator of hemochorial placentation, especially for *in vivo* development of the invasive trophoblast cell lineage. This *in vivo* response to low oxygen is conserved in rodent and primate placentation [38, 60, 61]. Insights about the role of hypoxia as an intrinsic regulator of placentation have been derived from mutagenesis of genes in the mouse genome controlling cellular responses to O_2 deprivation [56]. Central to the cellular response to hypoxia is the HIF transcription factor complex [62–64]. Phenotypes of placentas with null mutations for several genes encoding components of the HIF signaling pathway, including HIFlA, HIF2A, HIFIB (dimerization partner for HIFIA and HIF2A), prolyl hydroxylase domain protein 2, and von Hippel-Lindau genes, are associated with failures in placentation [65–69]. Through *in vivo* experiments with Holtzman Sprague-Dawley rats and *in vitro* experiments with rat TS cells [44], our laboratory has demonstrated that development of the invasive trophoblast cell lineage is activated by hypoxia (38, 52; Fig. 1).

In vivo exposure of pregnant rats to hypoxia (− 11% oxygen) from gestation day 6.5 to day 13.5 resulted in an expansion of blood vessel density and volume in the uterine mesometrial compartment, a profound increase in the depth of endovascular trophoblast cell invasion, and a reallocation of trophoblast cells within key compartments of the placenta [38). Rat and mouse chorioallantoic placentas are composed of two compartments Uunctional zone and labyrinth zone) associated with essential functions. The junctional zone is situated at the interface with the uterine decidua, produces hormones that modulate the maternal environment, and is the origin of the invasive trophoblast cell population, whereas the labyrinth zone is located at the fetal interface and contributes to bi-directional nutrient/ waste transport between maternal and fetal vascular systems. Maternal hypoxia leads to a preferential expansion of the junctional zone, which is observed in both the rat and mouse [38, P. Bu and M.J. Soares, unpublished results). Responsiveness to hypoxia is most evident during a critical window of pregnancy (gestation day 8.5 to day 9.5) when essential trophoblast cell lineage decisions are being made [38].

In vitro rat TS cells respond to low oxygen tension (−0.5%) by altering their differentiation state [52). Hypoxia activates an epithelial-mesenchymal-like transformation that is associated with a decrease in the expression of E-cadherin *(Cdhl)* and increases in the expression of matrix metalloproteinase 9 *(Mmp9)* and matrix metalloproteinase 12 *(Mmpl2)* and movement through an extracellular matrix (Matrigel; 53). These responses are dependent on activation of the HIF signaling pathway [52).

In summary, there is a plasticity associated with placentation, which is influenced by hypoxia [38, 52, 70]. TS cells are labile and can differentiate in order to meet challenges within the maternal environment (Fig. 1). Their differentiation is sensitive to oxygen tension and dependent on HIF signaling (38, 52].

PI3K/AKT/FOSL1 Signaling Pathway

A signaling pathway involving PI3K/AKT/FOSL1 controls the differentiation of the invasive trophoblast cell lineage and the trophoblast cell-directed uterine spiral artery remodeling phenotype [43, 50, 51]. This has been determined through *in vitro* experiments with Rcho-1 TS cells and *in vivo* experimentation with the Holtzman Sprague-Dawley rat [51].

The PI3K/AKT pathway is a regulator of invasive trophoblast cells [43,51,71–73].Disruption ofPI3K or AKT inhibits the expression of genes associated with the invasive phenotype and trophoblast cell invasion through an extracellular matrix [51]. These actions are mediated, at least in part, through promoting the nuclear accumulation of FOSLl protein [51].FOSLl is a basic region-leucine zipper transcription factor and a contributor to the formation of the activator protein-1(AP-I) transcription factor complex (74). In rat and human placentation sites, FOSLl is expressed in the extravillous/invasive trophoblast cell population [51, 75]. *Fosll* is also expressed in Rcho-1 TS cells induced to differentiate [43, 51]. FOSLl knockdown in Rcho-1 TS cells inhibits the expression of genes associated with the invasive trophoblast cell phenotype, including carcinoembryonic antigen family 4 *(Cgm4),* interleukin 17f *(l/17j), Mmp9,* prolactin family 4-subfamily a-member 1 *(Prl4al),* and semaphorin 6D *(Sema6d)* [51; Kubota, Kent and Soares unpublished results). FOSLl occupies regions of the *Mmp9* promoter in trophoblast cells critical for the regulation of *Mmp9* gene expression [51]. Inhibition of FOSLl expression significantly decreases Rcho-1 trophoblast cell invasion as assessed *in vitro* through Matrigel-coated filters [51). The *in vivo* involvement of FOSLl in regulating trophoblast cell invasion was also assessed following trophectoderm-specific lentiviral shRNA delivery [45, 51]. FOSLl knockdown inhibited endovascular trophoblast cell invasion [51]. These FOSLl actions in trophoblast

cells are consistent with its prominent role in regulating invasion in other cell types [76–79]. FOSLl is also implicated as a potential regulator of human trophoblast cells. Highly invasive trophoblast cells (HTR-8/ SVneo, Swan71) express higher levels of FOSLl versus trophoblast cells with limited invasiveness (BeWo, JEG3) (Renaud, Kubota, Rumi and Soares, unpublished results). A key role for FOSLl in hemochorial placentation has also been derived from mice possessing a null mutation at the *Fosll* locus [80].

The actions of FOSLl are dependent upon its interactions with other proteins, especially members of the basic region-leucine zipper transcription factor family (e.g. JUN, JUNB, and JUND; 75). In preliminary experiments, we have observed that FOSLl interacts with JUN and JUNB in rat trophoblast cells (Rumi, Kubota, and Soares, unpublished results). Based on expression and activity in differentiating TS cells, JUNB is a strong candidate for serving as a partner for FOSLl [43, 81]. JUNB is also expressed in human extravillous trophoblast cells [75]. Additionally, *Junb* null mutations phenocopy *FoslJ* null mutations, both resulting in defective mouse placental development [80, 82].

Collectively, the data indicate that FOSLJ is a key downstream effector of the Pl3K/AKT signaling pathway responsible for development of invasive trophoblast cell lineages integral to establishing the maternal-fetal interface (Fig. 2).

Overview

Our understanding of mechanisms regulating the invasive trophoblast cell lineage is minimal. It is now evident that the processes of trophoblast cell invasion and trophoblast cell-directed uterine spiral artery remodeling are conserved in model organisms, especially the rat. Technological advances have created an unprecedented opportunity to make major progress in elucidating regulatory mechanisms controlling development of the invasive trophoblast cell lineage. With these new tools we have determined that hypoxia/HIP and the PI3K/AKT/FOSLJ signaling pathways participate in the control of the invasive trophoblast cell lineage. It is expected that further dissection of these pathways will lead to the identification of fundamental regulators of differentiation and function of the invasive trophoblast cell lineage.

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References

1. Davies J, Glasser SR. Histological and fine structural observations on the placenta of the rat. Acta Anat (Basel). 1968; 69:542–608. [PubMed: 5760857]
2. Enders AC, Welsh AO. Structural interact ions of trophoblast and uterus during hemochorial placenta formation. J Exp Zool. 1993; 266:578–587. [PubMed: 8371099]
3. Georgiades P, Ferguson-Smith AC, Burton GJ. Comparative developmental anatomy of the murine and human definitive placenta. Placenta. 2002; 23:3–19. [PubMed: 11869088]
4. Kaurmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Bioi Reprod. 2003; 69:1–7.
5. Red-Horse K, Zhou Y, Genbacev O, Prakobpbol A, Foulk R, McMaster M, Fisher SJ. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin lnvest. 2004; 114:744–754.

6. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005; 308:1592–1594. [PubMed: 15947178]
7. Pijnenborg R, Vercruysse L, Hanssens M. The uterine spiral arteries in human pregnancy facts and controversies. Placenta. 2006; 27:939–958. [PubMed: 16490251]
8. Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, Cross JC. Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. Dev Biol. 2002; 250:358–373. [PubMed: 12376109]
9. Whitley GS, Cartwright JE. Cellular and molecular regulation of spital artery remodelling: lessons from the cardiovascular field. Placenta. 2010; 31:465–474. [PubMed: 20359743]
10. Harris LK, Aplin JD. Vascular remodeling and extracellular matrix breakdown in the uterine spiral arteries during pregnancy. Reprod Sci. 2007; 14(Suppl):28–34. [PubMed: 18089607]
11. Harris LK. Trophoblast-vascular cell interactions in early pregnancy: how to remodel a vessel. Placenta. 2010; 31(Suppl):S93–S98. [PubMed: 20060584]
12. Myatt L. Placental adaptive responses and fetal programming. J Physiol. 2006; 572:25–30. [PubMed: 16469781]
13. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of *in utero* and early-life conditions on adult health and disease. N Engl J Med. 2008; 359:61–73. [PubMed: 18596274]
14. Tanaka S, Kunath T, Hadjantonakis AK, Nagy A, Rossant J. Promotion of trophoblast stem cell proliferation by FGF4. Science. 1998; 282:2072–2075. [PubMed: 9851926]
15. Douglas GC, VandeVoort CA, Kumar P, Chang TC, Golos TG. Trophoblast stem cells: models for investigating trophectoderm differentiation and placental development. Endocr Rev. 2009; 30:228–240. [PubMed: 19299251]
16. Roberts RM, Fisher SJ. Trophoblast stem cells. Bioi Reprod. 2011; 84:412–421.
17. Hou, z; Romero, R.; Uddin, M.; Than, NG.; Wildman, DE. Adaptive history of single copy genes highly expressed in term placenta. Genomics. 2009; 93:33–41. [PubMed: 18848617]
18. Wildman DE. Toward an integrated evolutionary understanding of the mammalian placenta. Placenta. 2011; 32(Suppl2):Sl42–Sl45.
19. Cox B, Kotlyar M, Evangelou AI, Ignatcheoko V, lgnatchenko A, Whiteley K, Jurisiea I, Adamson SL, Rossant J, Kislinger T. Comparative systems biology of human and mouse as a tool to guide the modeling of human placental pathology. Mol Syst Biol. 2009; 5:279. [PubMed: 19536202]
20. Hemberger M, Udayashankar R, Tesar P, Moore H, Burton GJ. ELFS-enforced transcriptional networks define an epigenetically regulated trophoblast stem cell compartment in the human placenta. Hum Mol Genet. 2010; 19:2456–2467. [PubMed: 20354077]
21. Maltepe E, Bakardjiev AI, Fisher SJ. The placenta: transcriptional, epigenetic, and physiological integration during development. J Clin Invest. 2010; 120:1016–1025. [PubMed: 20364099]
22. Hunkapiller NM, Gasperowicz M, Kapidzic M, Plaks V, Maltepe E, Kitajewski J, Cross JC, Fisher SJ. A role for Notch signaling in trophoblast endovascular invasion and in the pathogenesis of pre-eclampsia. Development. 2011; 138:2987–2998. [PubMed: 21693515]
23. Rossant, J.; Cross, JC. Extraembryonic lineages. In: Rossant, J.; Tam, PPL., editors. Mouse Development. New York: Academic Press; 2002. p. 155-180.
24. Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: novel endocrine phenotype and regulation. Dev Biol. 2003; 260:176–190. [PubMed: 12885563]
25. Coan PM, Conroy N, Burton GJ, Ferguson-Smith AC. Origin and characteristics of glycogen cells in the developing murine placenta. Dev Dyn. 2006; 235:3280–3294. [PubMed: 17039549]
26. Pijnenborg R, Robertson WB, Brosens I, Dixon G. Trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. Placenta. 1981; 2:71–91. [PubMed: 7010344]
27. Caluwaerts S, Vercruysse L, Luyten C, Pijnenborg R. Endovascular trophoblast invasion and associated structural changes in uterine spiral arteries of the pregnant rat. Placenta. 2005; 26:574–584. [PubMed: 15993707]
28. Dtehend R, Gratze P, Wallukat G, Shagdarsuren E, Plehm R, Brosen Jfl, Fiebeler A, Schneider W, Caluwaerts S, Vercruysst L, Pijnenborg R, Luft FC, Mueller ON. Agonistic auto antibodies to the

ATI receptor in a transgenic rat model of preeclampsia. Hypertension. 2005; 45:742–746. [PubMed: 15699466]

29. Vercruysse L, Caluwaerts S, Luyten C, Pijneoborg R. Interstitial trophoblast invasion in the decidua and mesometrial triangle during the last third of pregnancy in the rat. Placenta. 2006; 27:22–33. [PubMed: 16310034]
30. Vtrlohren S, Niehoff M, Hering L, Geusens N, Herse F, Tintu AN, Plagemann A, LeNoble F, Pijnenborh R, Muller DN, Luft FC, Dudenbausen JW, Gollasch M, Dechend R. Uterine vascular function in a transgenic preeclampsia rat model. Hypertension. 2008; 51:547–553. [PubMed: 18195162]
31. Geusens N, Verlobren S, Luyten C, Taube M, Hering L, Vercruysse L, Hanssens M, Dudenhausen JW, Dechend R, Pijnenborg R. Endovascular trophoblast invasion. spiral artery remodelling and uteroplacental haemodynamics in a transgenic rat model of pre-eclampsia. Placenta. 2008; 29:614–623. [PubMed: 18502502]
32. Hering L, Herse F, Verlohren S, Park JK, Wtllner M, Qadri F, Pijnenborg R, Staff AC, Huppertz B, Muller DN, Luft FC, Dethend R. Trophoblasts reduce the vascular smooth muscle cell proatherogenic response. Hypertension. 2008; 51:554–559. [PubMed: 18195163]
33. Pijnenborg R, Vercruysse L. Shifting concepts of the fetal-maternal interface: a historical perspective. Placenta. 2008; l9(Suppl A):S20–S25. [PubMed: 17967487]
34. Geusens N, Hering L, Verlohren S, Luyten C, Drijkoningen K, Taube M, Vercruysse L, Hanssens M, Dechend R, Pijntnborg R. Changes in endovascular trophoblast invasion and spiral artery remodelling at term in a transgenic preeclamptic rat model. Placenta. 2010; 31:320–326. [PubMed: 20144482]
35. Wiemers DO, Ain R, Ohboshi S, Soares MJ. Migratory trophoblast cells express a newly identified member of the prolactin gene family. J Endocrinol. 2003; 179:335–346. [PubMed: 14656203]
36. Arroyo JA, Konno T, Khalili DC, Soares MJ. A simple *in vivo* approach to investigate invasive trophoblast cells. Int J Dev Biol. 2005; 49:977–980. [PubMed: 16281175]
37. Konno T, Rempel LA, Arroyo JA, Soares MJ. Pregnancy in the Brown Norway rat: a model for investigating the genetics of placentation. Bioi Reprod. 2001; 16:709–718.
38. Rosario GX, Konno T, Soares MJ. Maternal hypoxia activates endovascular trophoblast cell invasion. Dev Biol. 2008; 314:362–375. [PubMed: 18199431]
39. Rosario GX, Ain R, Konno T, Soares MJ. Intrauterine fate of invasive trophoblast cells. Placenta. 2009; 30:457–463. [PubMed: 19344949]
40. Jacob HJ, Lazar J, Dwinell MR, Moreno C, Geurts AM. Gene targeting in the rat: advances and opportunities. Trends Genet. 2010; 26:510–518. [PubMed: 20869786]
41. Faria TN, S-oares MJ. Trophoblast cell differentiation: establishment. Characterization and modulation of a rat trophoblast cell line expressing members of the placental prolactin family. Endocrinology. 1991; 129:2895–2906. [PubMed: 1954876]
42. Sabgal N, Canham LN, Canham B, Soares MJ. Rcho-1 trophoblast stem cells: a model for studying trophoblast cell differentiation. Methods Mol Med. 2006; 121:159–178. [PubMed: 16251742]
43. Kent LN, Konno T, Soares MJ. Phosphatidyl inositol 3-kinase modulation of trophoblast cell differentiation. BMC Dev Biol. 2010; 10:97. [PubMed: 20840781]
44. Asanoma K, Rumi MAK, Kent LN, Chakraborty D, Renaud SJ, Wake N, Lee D-S, Kubota K, Soares MJ. FGF4-dependent stem cells derived from rat blastocysts differentiate along the trophoblast lineage. Dev Bioi. 2011; 351:110–119.
45. Lee D-S, Rumi MAK, Konno T, Soares MJ. *In vivo* genetic manipulation of the rat trophoblast cell lineage using lentiviral vector delivery. Genesis. 2009; 47:433–439. [PubMed: 19444902]
46. Ain R, Konno T, Canham LN, Soares MJ. Phenotypic analysis of the placenta in the rat. Methods Mol Med. 2006; 121:295–313. [PubMed: 16251750]
47. Konno T, Graham AR, Rempel LA, Ho-Chen JK, Alam SM, Bu P, Rumi MA, Soares MJ. Subfertility linked to combined luteal insufficiency and uterine progesterone resistance. Endocrinology. 2010; 151:4537–4550. [PubMed: 20660062]
48. Konno T, Rempel LA, Rumi MA, Graham AR, Asanoma K, Renaud SJ, Soares MJ. Chromosome-substituted rat strains provide insights into the genetics of placentation. Physiol Genomics. 2011; 43:930–941. [PubMed: 21652768]

49. KnOfter M. 2010 Critical growth factors and signaling pathways controlling human trophoblast invasion. Int. J Dev Biol. 2010; 54:269–280. [PubMed: 19876833]
50. Kamei T, Jones SR, Chapman BM, McGonigle KL, Dai G, Soares MJ. The phosphatidylinositol3-kinase/Akt signaling pathway modulates the endocrine differentiation of trophoblast cells. Mol Endocrinol. 2002; 16:1469–1481. [PubMed: 12089343]
51. Kent LN, Rumi MAK, Kubota K, Lee DS, Soares MJ. FOSL1 is integral to establishing the maternal-fetal interface. Mol Cell Bioi. 2011; 31:4801–4813.
52. Chakraborty D, Rumi MAK, Konno T, Soares MJ. Natural killer cells direct hemochorial placentation by regulating HIF dependent trophoblast lineage decisions. Proc Notl Acad Sci USA. 2011; 108:16295–16300.
53. Genbatev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. Science. 1997; 277:1669–1672. [PubMed: 9287221]
54. Caniggia I, Mostathfi H, Winter J, Gassmann M, Lye SJ, Kuliszewski M, Post M. Hypoxia inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFp3. J Clin lnvest. 2000; 105:577–587.
55. Burton GJ, Jaunaiux E. Maternal vascularization of the human placenta: does the embryo develop in a hypoxic environment? Gynecol Obstel Fertil. 2001; 29:503–508.
56. Fryer BH, Simon MC. Hypoxia, HIF and the placenta. Cell Cycle. 2006; 5:495–498. [PubMed: 16552177]
57. Burton GJ. Oxygen, the Janus gas; its effects on human placental development and function. J Anot. 2009; 215:27–35.
58. Rodesch F, Simon P, Donner C, Jauniaur E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol. 1992; 80:283–285. [PubMed: 1635745]
59. Zamudio S. The placenta at high altitude. High Aft Med Biol. 2003; 4:171–191.
60. Zhou Y, Chiu K, Brescia RJ, Combs A, Katz MA, Kitzmiller JL, Heilbron DC, Fisher SJ. Increased depth of trophoblast invasion after chronic constriction of the lower aorta in rhesus monkeys. Am J Obstet Gynecol. 1993; 169:224–229. [PubMed: 8333462]
61. Kadyrov M, Schmitz C, Black S, Kaufmann P, Huppertz B. Pre-eclampsia and maternal anaemia display reduced apoptosis and opposite invasive phenotypes of extravillous trophoblast. Placenta. 2003; 24:540–548. [PubMed: 12744931]
62. Semenza GL. Hydroxylation of HIF-I: oxygen sensing at the molecular level. Physiology. 2004; 19:176–182. [PubMed: 15304631]
63. Dunwoodie SL. The role of hypoxia in development of the mammalian embryo. Dev Cell. 2009; 17:755–773. [PubMed: 20059947]
64. Semenza GL. Oxygen homeostasis. Wiley lnterdiscip Rev Syst Bioi Med. 2010; l:336–361.
65. Gnarra JR, Ward JM, Porter FD, Wagner JR, Devor DE, Grinberg A, Emmert-Buck MR, Westphal H, Klausner RD, Linehan WM. Defective placental vasculogenesis causes embryonic lethality in VHL-deficient mice. Proc Natl Acad Sci USA. 1997; 94:9102–9107. [PubMed: 9256442]
66. Adelman DM, Gertsenstein M, Nagy A, Simon MC, Maltepe E. Placental cell fates are regulated in vivo by HJF mediated hypoxia responses. Genes Dev. 2000; 14:3191–3203. [PubMed: 11124810]
67. Cowden, Dahl KD.; Fryer, BH.; Mack, FA.; Compernolle, V.; Maltepe, E.; Adelman, DM.; Carmeliet, Pt; Simon, MC. Hypoxia-inducible factors 1alpha and 2alpha regulate trophoblast differentiation. Mol Cell Biol. 2005; 25:10479–10491. [PubMed: 16287860]
68. MaUepe E, Krampitz GW, Okazaki KM, Red-Horse K, Mak W, Simon MC, Fisher SJ. Hypoxia-inducible factor-dependent histone deacetylase activity deter mines stem cell fate in the placenta. Development. 2005; 13l:3393–3403.
69. Takeda K, Ho VC, Takeda H, Duan LJ, Nagy A, Fong GH. Placental but not heart defects are associated with elevated hypoxia inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. Mol Cell Bioi. 2006; 26:8336–8346.
70. Ho Chen JK, Bustamante JJ, Soares MJ. Prolactin-like protein-F subfamily of placental hormones/cytokines: responsiveness to maternal hypoxia. Endocrinology. 2007; 148:559–565. [PubMed: 17095594]

71. Qiu Q, Yang M, Tsang BK, Gruslin A. Both mitrogen-activated protein kinase and phosphatidylinositol kinase signaling are required in epidermal growth factor induced human trophoblast migration. Mol Hum Reprod. 2004; 10:677–684. [PubMed: 15235105]

72. Qiu Q, Yang BK, Tsang BK, Gruslin A. EGF-induced trophoblast secretion of MMP-9 and TIMP-1 involves activation of both PI3K and MAPK signaling pathways. Reproduction. 2004; 128:355–363. [PubMed: 15333786]

73. Sonderegger S, Haslinger' P, Sabri A, Leisser' C, Otten JV, Fiala C, Knofier M. Wingless (Wnt) 3A induces trophoblast migration and matrix metalloproteinase-2 secretion through canonical Wnt signaling and protein kinase B/AKT activation. Endocrinology. 2010; 151:211–220. [PubMed: 19887570]

74. Eferl R, Wagner EF. AP-I: a double-edged sword in tumorigenesis. Nat Rev Cancer. 2003; 3:859–868. [PubMed: 14668816]

75. Marzioni D, Todros T, Cardaropoli S, Rolfo A, Lorenzi T, Ciarroela R, Romagnoli R, Paulesu L, Castellucci M. Activating protein family of transcription factors in the human placenta complicated by preeclampsia with and without fetal growth restriction. Placenta. 2010; 31:919–927. [PubMed: 20800894]

76. Kustikova O, Kramerov D, Grigorian M, Berezin V, Bock E, Lukanidin E, Tulcbinsky E. Fral induces morphological transformation and increases *in vitro* invasiveness and motility of epithelilioid adenocarcinoma cells. Mol Cell Bioi. 1998; 18:7095–7105.

77. Milde-Langosch K. The Fos family of transcription factors and their role in tumorigenesis. Eur J Cancer. 2005; 41:2449–2461. [PubMed: 16199154]

78. Verde P, Casalino L, Talotta F, Yaniv M, Weitzman JB. Deciphering API function in tumorigenesis. Fraternizing on target promoters. Cell Cycle. 2007; 6:2633–2639. [PubMed: 17957143]

79. Young MR, Colburn NH. Fra-1 a target for cancer prevention or intervention. Gene. 2006; 379:1–11. [PubMed: 16784822]

80. Schreiber M, Wang ZQ, Jochum W, Fetka I, Elliott C, Wagner EF. Placental vascularization requires the AP-I component Fral. Development. 2000; 127:4937–4948. [PubMed: 11044407]

81. Shida MM, Ng YK, Soares MJ, Linzer DI. Trophoblast-specific transcription from the mouse placental lactogenl gene promoter. Mol Endocrinol. 1993; 7:181–188. [PubMed: 8469232]

82. Schorpp-Kistner M, Wang Z-Q, Angel P, Wagner EF. JunB is essential formammalian placentation. EMBO J. 1999; 18:934–948. [PubMed: 10022836]

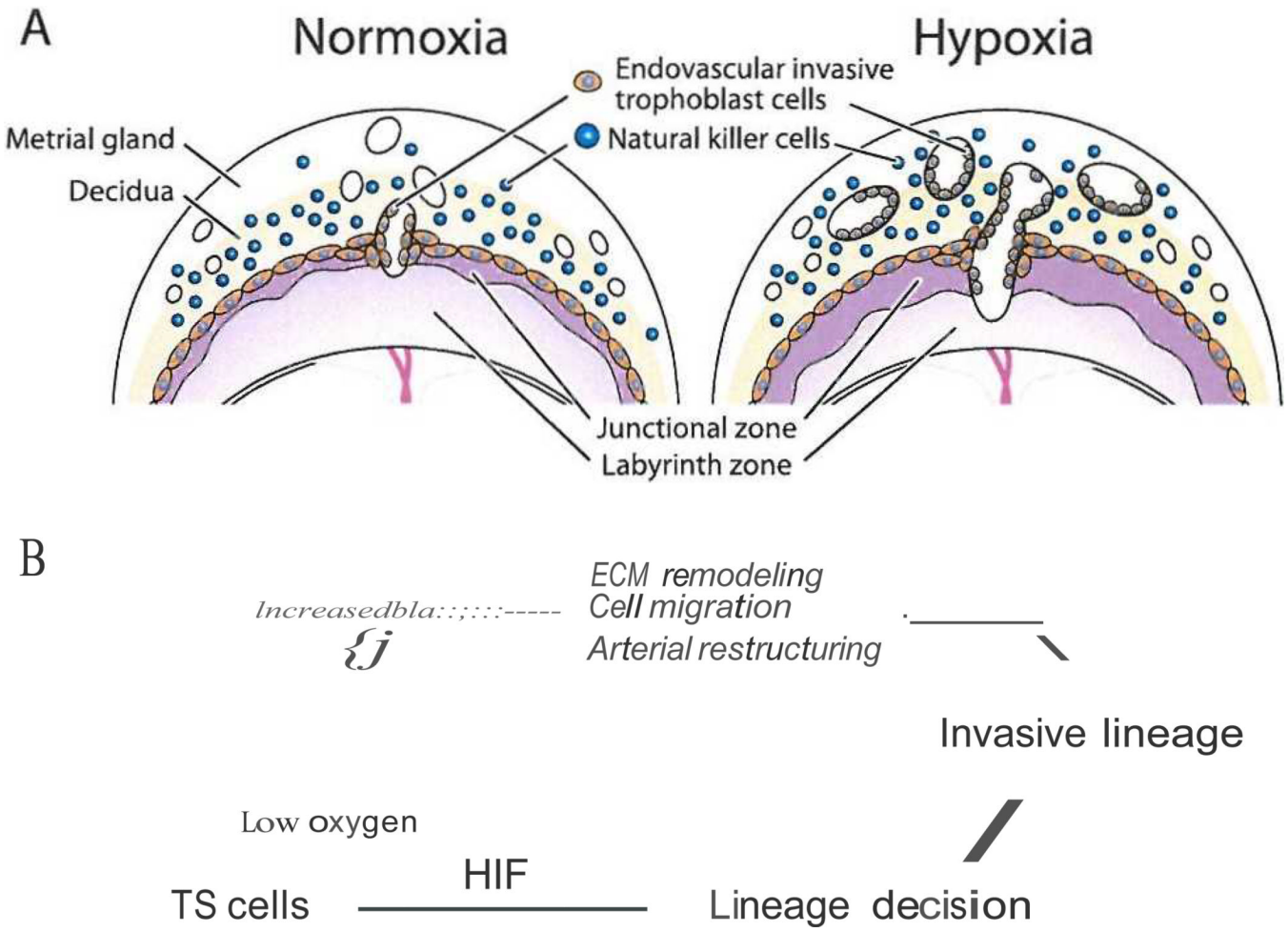

Fig. I.

Hypoxia signaling and activation of the invasive trophoblast cell lineage and trophoblast cell-directed uterine spiral artery remodeling. Panel A} Effects of maternal hypoxia on placentation. Exposure to maternal hypoxia increases uterine vascularity, endovascular invasive trophoblast cell invasion, and cellular allocation to the junctional zone of the chorioallantoic placenta. Panel B) Schematic representation of the effects of oxygen tension on TS cells and HIF-dependent trophoblast cell 1ineage decisions.

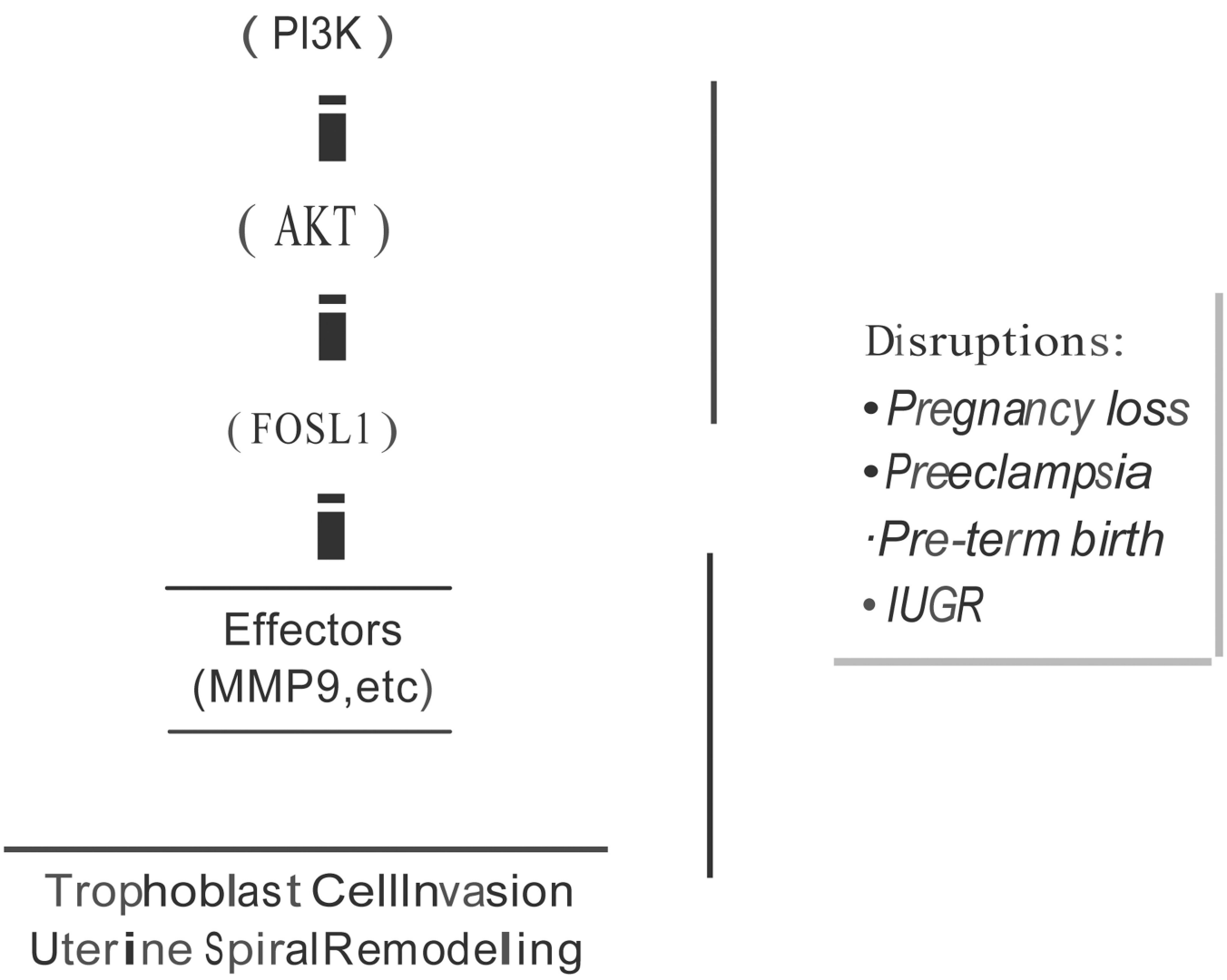

Fig. 2.

PBK/AKT/FOSLI pathway regulating the invasive trophoblast cell lineage and trophoblast cell-directed uterine spiral artery remodeling. Disruptions in the PJ3K/AKT/FOSLI pathway leads to pregnancy related disease, including early pregnancy loss, preeclampsia, pre-term birth, and intrauterine growth restriction (IUGR).