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# Review: Placental homeobox genes and their role in regulating human fetal growth

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ABSTRACT

The regulation of fetal growth is multifactorial and complex. Normal fetal growth is determined by the genetically predetermined growth potential and further modulated by maternal, fetal, placental, and environmental factors. The placenta provides critical transport functions between the maternal and fetal circulations during intrauterine development. Formation of this interface is controlled by several growth factors, cytokines and transcription factors including homeobox genes. This review summarizes our current knowledge regarding homeobox genes in the human placenta and their differential expression and functions in human idiopathic fetal growth restriction (FGR). The review also describes the research strategies that were used for the identification of homeobox genes, their expression in FGR, functional role and target genes of homeobox genes in the trophoblasts and the hormonal regulators of homeobox gene expression in vitro. A better understanding of molecular pathways driven by placental homeobox genes and further elucidation of signaling pathways underlying the hormone-mediated homeobox gene developmental programs may offer novel strategies of targeted therapy for improving feto-placental growth in idiopathic FGR pregnancies.



## 1. Introduction

During pregnancy, the placenta is the principal site of metabolic, respiratory, excretory and endocrine action. These functions provide essential support for the growing fetus. The generation of distinct trophoblast cell types within the placenta is required to implement the complex biological processes of implantation, maternal–fetal exchange and maternal tolerance to fetal-parental antigens. Failure in any one of these functions is associated with a range of complications associated with human pregnancy disorders, including missed abortion, miscarriage, fetal growth restriction (also known as intrauterine growth restriction), and pre-eclampsia [1,2,3].

The placenta is derived from two major cell lineages [4,5]. Cytotrophoblast stem cells originate from the trophectoderm, the outermost epithelial cell layer of the blastocyst, which triggers attachment and implantation in the receptive maternal endometrium. Stromal cells and blood vessels of the placenta are derived from the extra-embryonic mesoderm. The cytotrophoblast stem cells differentiate into either the villous or the extravillous cytotrophoblast lineages. Villous cytotrophoblasts are found in the floating villi, which are present in the intervillous space, and these cytotrophoblasts remain attached to the villous basement membrane to form a monolayer of epithelial cells. These mononuclear cytotrophoblasts proliferate, differentiate and fuse to form the multinucleated syncytium of the definitive placenta, the syncytiotrophoblast, which covers the entire surface of the villus. The syncytium is multi-functional and its primary roles include absorption, exchange and transport of nutrients and gases from the maternal to the fetal circulation as well as the production of pregnancy hormones. The multinuclear syncytium is the primary site of endocrine activity in the placenta and secretes hormones such as human chorionic gonadotropin (hCG), human placental lactogen (hPL) and placental growth hormone (PGH), all of which are important for placental growth and/or maternal adaptation to pregnancy [6,7,8].

The extravillous cytotrophoblast lineage is derived from cytotrophoblast cells of the anchoring villi, which proliferate, detach from the basement membrane and aggregate into multilayered columns of non-polarized cells that rapidly invade into the uterine wall. This extravillous cytotrophoblastic invasion is confined to the decidua, the inner third of the myometrium, and the associated

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spiral arterioles. Extravillous cytotrophoblast cells give rise to interstitial cytotrophoblasts, which invade the stroma of the decidua and parts of the myometrium, and to endovascular cytotrophoblasts that migrate and invade along the wall of the uterine spiral arterioles. The consequence of these processes is the remodeling of the uterine spiral arterioles to provide low resistance, high flow blood vessels, allowing increased blood flow to the placenta to cope with the increasing nutritional demands of the rapidly growing fetus [9]. Thus, differentiation of cytotrophoblast cells is fundamental to normal human placental development. In particular, modification of the maternal vessels by extravillous cytotrophoblasts, which replace the maternal endothelium, is thought to be critical for the successful progression of pregnancy, since reduced invasion of interstitial and endovascular cytotrophoblasts is associated with pre-eclampsia and fetal growth restriction [10,11]. Thus, it is evident that elucidation of the key molecular mechanisms controlling trophoblast cell lineage commitment and identification of regulators of cytotrophoblast differentiation in humans is crucial for our understanding of normal and pathological placental development.

## 2. Transcriptional control of placental development

Growth factors and signaling molecules are the cues to which a cell responds, either by maintaining or altering its state of differentiation. However, it is the transcription factors that act within the cell nucleus which determine how these cues are interpreted and what the cellular response will be. Transcription factors achieve this by regulating expression of their target genes within the cell. Numerous transcription factors play essential roles in cellular development and differentiation of a variety of cell types, including the trophoblast cell type in the placenta [5,12]. Transcription factors are categorized into a few large families such as the zinc finger, leucine zipper, helix-loop-helix, helix-turn-helix and homeobox genes [13,14] and also include the ligand-activated nuclear receptor superfamily. Our interest is in one of the largest and most important groups of transcription factors called the superfamily of homeobox genes. Several homeobox genes potentially control commitment and differentiation and were identified in human invasive extravillous cytotrophoblasts [15,16]. Our present knowledge of homeobox transcription factors that are involved in human feto-placental growth is summarized below.

## 3. Homeobox genes

Homeobox genes (also known as homeotic genes) were originally discovered in the fruit fly *Drosophila melanogaster*, where they act as transcriptional regulators to control embryonic development [reviewed in Refs. [17,18,19]]. Homeobox genes contain a highly conserved 183 base pair homeobox sequence, which encodes a 61 amino acid motif called the homeodomain. Structural analyses reveal that the homeodomain consists of an evolutionarily conserved helix-turn-helix motif that binds to DNA. The specificity of this binding allows homeodomain proteins to activate or repress the expression of batteries of down-stream target genes [20]. Homeobox genes are subdivided into the “clustered” homeobox genes known as “HOX” genes, the “non-clustered” divergent or orphan HOX-like genes, as well as several distinct classes of atypical homeodomain containing genes. Homeobox genes are grouped together into subfamilies based on criteria such as their functional and structural characteristics. These subfamilies of homeobox genes are essential for the control of specific aspects of placental growth and differentiation [3,12,21].

Homeobox genes are directly or indirectly involved in a variety of developmental disorders, diseases and cancers [reviewed in Ref. [22]]. Deregulation of specific homeobox genes in cancer and other diseases provides support for the idea that homeobox genes are crucial for normal mammalian development. Furthermore, characterization of homeobox genes involved in disease progression may lead to a greater understanding of the developmental mechanisms that are disrupted in a variety of disease states. Normal homeobox gene expression is altered in various disease states. For example, decreased expression of Cdx2 is detected in the intestinal epithelium of patients with colorectal cancers and decreased Meox2 expression is detected in brain endothelial cells of patients affected by Alzheimer’s disease [22,23]. Thus, homeobox genes could be used as disease markers or potential therapeutic targets of diseases, such as cancer, diabetic wound healing, lymphedema, Alzheimer’s disease, and stroke due to atherosclerosis [24,25,26].

## 4. Homeobox genes in human feto-placental development

Several homeobox genes affect placental and embryonic development. Murine knockout models provide genetic proof that homeobox genes play a pivotal role in murine placental development. For example, targeted disruption of Dlx3, Esx1 and Tgif-1murine homeobox genes result in placental maldevelopment and growth restricted phenotypes in embryos [27,28,29]. Therefore, understanding the expression and localization of homeobox genes in human placental development and their role in pregnancy pathologies including fetal growth restriction (FGR) is highly important.

Our approach to understanding the molecular pathways of homeobox genes and their role in feto-placental growth was based on the following strategy: (i) identifying the spatio-temporal expression pattern of homeobox genes in human placental development that have been identified as regulators of cell fate decisions during embryonic development; (ii) determining whether specific homeobox gene expression levels are altered in placentae from FGR-affected pregnancies compared to placentae from gestation-matched uncomplicated pregnancies; (iii) creating in vitro models using manipulated placental cultured cells that “mimic” homeobox gene expression changes detected in FGR placentae (the manipulation involves the use of loss- or gain-of-function phenotypes by RNA interference systems or gene overexpression plasmids); (iv) identifying the targets of homeobox genes using in vitro cell culture models; and (v) identifying hormonal regulators of homeobox genes. Similar strategies have been proven successful in identifying transcriptional control of endocrine functions during murine placental development [reviewed in Ref. [3]]. Therefore, identification of the homeobox target genes, their targets and hormonal regulators in specialized cell types of the human placenta could reveal important molecular pathways responsible for feto-placental growth. Using the strategy described above, we undertook extensive analyses of homeobox genes in placentae from FGR-affected and uncomplicated, gestation-matched control pregnancies.

## 5. Homeobox genes in the human placenta

### *5.1. Spatio-temporal expression patterns of homeobox genes in the placenta*

We and others have identified the potential importance of homeobox genes *DLX3*, *DLX4*, *GAX*, *ESX1L, HLX* and *TGIF-1* in the human placenta [15,27,30,31,32,33]. These homeobox genes are also expressed in the embryo and play pivotal roles in embryonic development [29,34,35]. More specifically, we demonstrated that homeobox gene *HLX* is expressed primarily in proliferating cytotrophoblast cell types during early placental development [31]. We also determined the expression profile of homeobox gene *DLX3* and