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Genes regulating implantation and fetal development: a focus on mouse knockout models

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1. ABSTRACT

Timely and efficient regulation of blastocyst implantation and fetal growth are essential for the successful reproduction of viviparous mammals. Disruptions in this regulation can result in a wide variety of human gestational complications including infertility, spontaneous abortion, fetal growth restriction, and premature delivery. The role of several groups of factors, including cytokines, hormones, transcription factors, extra-cellular proteinases, and angiogenic factors has been suggested in both implantation and regulation of fetal growth. Due to the inherent difficulties of studying implantation and fetal development in humans, much of our knowledge of the genes involved in these processes has been derived from animal models. The critical genetic loci involved in blastocyst implantation and fetal growth will be discussed with a focus on those genes with available mouse knockout models.

2. INTRODUCTION

Mammalian reproduction represents a highly orchestrated and intricate process. The spatial and temporal regulation of a fetal-maternal crosstalk is required for a successful pregnancy (1-5). After fertilization, the developing blastocyst must invade and establish itself in the maternal endometrium, from which it will eventually gain the crucial protection and nourishment required for development throughout gestation. Successful blastocyst implantation is contingent on two early and independent processes. While being transported through the fallopian tube, the embryo must undergo developmental changes, which culminate in the ability to implant and become invasive. In conjunction, and just as important, the uterine endometrium must undergo physiological and functional changes that allow it to become receptive to the implanting blastocyst. Thus, the process of blastocyst implantation requires the coordinated expression of a multitude of both

maternal and embryonic factors including cytokines, growth factors, hormones, adhesion molecules, and prostaglandins. Disruption in the regulation of this crosstalk can result in lowered fertility due to decreased implantation efficiency. Several gene products, such as interleukin-1 (IL-1) and leukemia inhibitory factor (LIF) appear very important for this process (6, 7). In addition, blastocyst implantation is likely to be influenced by embryo-regulated preimplantation programming which is likely to begin at a two cell embryo stage (8, 9).

After successful implantation, the major embryo-derived structure that regulates fetal growth is the placenta (10-13). In humans and mice, a hemochorial placenta forms (14). In this type of placenta, the placental trophoblast cells are in direct contact with the maternal blood supply. Especially in humans, the placenta is marked by a deep trophoblast invasion into the maternal endometrium. Accompanying this invasion is the lining of decidual vascular endothelial cells by invading trophoblasts. These processes establish an efficient maternal fetal vascular exchange, crucial to the nutrient needs of the fetus. Many diseases of the neonate and the adult are thought to have their origins in the placental regulation of fetal metabolism and development (15). Placentation is regulated by both embryonic and maternal tissues. As in implantation, this process requires the coordinated expression of both fetal and maternal genes. Much progress has been made into understanding the regulation of trophoblast cells differentiation, especially the many transcription factors involved. In addition, various secreted factors are also important in promoting the invasion of the trophoblast into the maternal decidua. Among these, the matrix metalloproteinases (MMPs), which are expressed by invading trophoblasts , disrupt the decidual extracellular matrix (16-18). Additionally, the tissue-inhibitors of metalloproteinases (TIMPs), which are expressed by the decidual stroma and regulate the actions of MMPs, are particularly well studied. Angiogenic factors secreted by trophoblast cells as well as uterine natural killer (uNK) cell-derived IFN-γ play an important role in remodeling the maternal decidual arteries and in establishment of an efficient fetal-maternal vascular exchange (19). In addition, uNK cell-derived IFN-γ has been suggested to limit the invasiveness of trophoblast into the deciduas (20). To maintain a pregnancy-compatible uterine milieu, IL-10, a potent anti-inflammatory cytokine produced at the fetal-maternal interface (20-27), is expressed during early and through mid-gestation (27). Along with its variety of roles in the regulation of local uterine immunity, IL-10 exerts a positive role in trophoblast survival, and regulation of placentation (Sharma S, Unpublished data).

To complement and extend this knowledge, the use of gene-ablated or knockout mice has proved to be a powerful tool furthering our understanding of gene function. To date, a wide range of knockout mice strains have been created, many with ablation of genes whose expression is associated with reproduction. However, a major concern with the interpretation of data from mouse knockout models is the phenomenon of redundancy. Lack of a certain gene product(s) from conception is often accompanied by partially compensatory gene expression of genes whose products have related or overlapping functions, thus obscuring the phenotypic consequences of many gene knockouts. This appears especially true with respect to genes involved in implantation, placentation, and fetal growth. With rare exceptions, most gene knockout models currently available result in only subtle reproductive defects, which may only be apparent upon encounter with a second insult in the form of inflammatory signals or other stressors. Nonetheless, gene knockout mice have provided crucial insights into the functional requirements of specific genes in the genetic networks involved in reproduction. This review will attempt to provide an overview of the dynamics of gene expression found in the embryonic and maternal reproductive tissues involved in the processes of implantation and fetal development and, with focus on studies of major models of gene ablated laboratory mice.

3. DISCUSSION

3.1. Gene expression in blastocyst implantation

Initiation of successful establishment of pregnancy involves two fundamental elements, developmental changes in the embryo which prepare it for implantation and maternal recognition of pregnancy. Unless those elements are assured, maternal recognition of pregnancy will not take place and implantation can not succeed. The process of maternal recognition of pregnancy initiates shortly after fertilization and involves secretion of specific immunomodulatory factors that allow for maternal immune receptivity of pregnancy, particularly molecules such as pre-implantation factors (PIF) (8, 9, 28).

Implantation is a dynamic process where at each stage multiple processes are involved, such as adhesiveness, invasiveness, or maternal receptivity. Models that could dissect each phase and identify the governing elements involve would be very useful but are not currently available. It should be noted that even within the endometrium distinct regions can be identified- those that are intimately related to implantation and have different morphology and hormonal and cytokine secretion patterns than those at distant sites, as examined by endometrial microarray analysis (28), further illustrating the significance of specific embryo derived factors. Upregulation of immune-related genes IL-15Ralpha, IL15, DAF, IDO, Lpn, NKG5, IRF-1, and NKAT2 is found in secretory versus proliferative human endometrium using RT PCR in endometrial biopsies from healthy women. These genes may be involved in uNK cells proliferation blocking their cytolytic activity, T cell inhibition, and inhibition of the classical complement pathway (29). Differences in expression patterns in human endometrium between LH+2 to LH+7 days were examined using gene arrays (30). This reveals major changes especially in the expression of PP-14 (glycodelin), (31), osteopontin (32), IGFBP-3 (33, 34), crystallin alphaB (35), GPx-3, claudin-4, and SLC1A1 genes. Interestingly, expression of 153 genes was found to be elevated compared to 58 genes that showed decreased expression during the endometrial implantation window.

Differential expression of genes also occurs in the decidua and chorionic villi. As examined by gene array analysis of first trimester elective abortuses (36), these studies revealed 124 genes with high expression in both decidua and villi, 49 of which are in the decidua and other 75 genes were of the chorionic villi origin. These differentially expressed genes were further grouped in categories of cell growth-related factors, hormones/cytokines, cell adhesion molecules, signal transduction molecules, apoptosis-related factors, and cytoskeleton/extracellular matrix proteins. The most relevant genes for the decidua were IGFBP-1 and 4. In the chorionic villi, 11 upregulated genes were cell growth related, others were hCG, SP-1, GH1,2, activin, inhibin hormones and LIF receptor.

Differences are also found between early and late mouse placental gene expression. Genes up-regulated in late-stage placenta reflected the transition to a specialized organ with less proliferative activity, acquisition of endocrine functions, and adapted for metabolic exchange. These gene groups were specific for growth arrest, hormones, glycoproteins, and vascular differentiation. The genes displaying decreased expression were involved in general cell metabolism. These observations illustrate the difficulty of fully understanding the significance of each individual gene contribution versus an integrated view or reproductive process.

3.2. Gene expression (silencing) in the embryo

Gene expression in the morula and blastula revealed an interesting trend. The number of genes upregulated is lower than the number down regulated. Among the upregulated genes are those known to be involved in critical regulatory processes (Mist1, Id2, Hd1, and Requiem). The downregulated genes include CREB-binding protein, Per3, zinc finger protein 217, Krox-25, and miwi1 (37). This indicates that gene expression is tightly controlled, and therefore advanced differentiation of the embryo as occurs in the blastocyst stage requires greater downregulation of previously expressed genes than in less differentiated states. This pattern compares well to the placenta where in advanced gestation there is a decrease in the number of genes expressed after major differentiation to syncytiotrophoblast has already occurred (see below).

In a mouse pregnancy model, the blastocyst can continue for 2 weeks in the presence of progesterone, and can be subsequently activated with estrogen. Analysis of 20,000 genes showed that only 229 were differentially expressed. These included cell cycle, cell signaling, and energy metabolic pathways, especially heparin-binding epidermal growth factor-like moiety (38**).** Recently, human embryo gene profile was also examined in the first 3 days following fertilization *in vitro*. Gene expression was found to be largely downregulated, similar to the mouse. There is also evidence that the embryonic transcriptome is already established at 2 cell stage. (39). The gene expression during mouse embryo development at the post implantation E12.5 stage revealed profound differences in gene expression between the placenta and embryo (40).

These studies illustrate again that the placenta has a greater range of gene expression than the embryo which may be considered counterintuitive considering that the embryo is undergoing the establishment of major organs and other developmental functions of the developing embryo.

4. MOUSE KNOCKOUT MODELS OF REPRODUCTIVE-RELATED GENES

The vast array of genes expressed during implantation and fetal development and their spatial and temporal regulation makes dissection and significance of the function of each gene difficult. The laboratory mouse provides a valuable model for the study of the regulation of reproductive processes. Gene knockout mice can provide valuable insights into the genetic networks involved in these processes. A wide array of gene knockout mice have been created for many genes involved in reproductive processes.

4.1. Cytokines

Interleukin-1 (IL-1) has been one of the most extensively studied cytokines involved in implantation. The complete IL-1 system consists of two soluble agonists, IL-1alpha and IL-1beta, a soluble antagonist, Interleukin-1 receptor antagonist (IL-1ra), as well as two membrane-bound receptors Interleukin-1 receptor type I (IL-1RtI) and type II (IL-1RtII) (41). Signaling only occurs through the IL-1RtI, while the IL-1RtII is inert and serves only to compete with IL-1RtI for IL-1alpha or IL-1beta binding (42). The antagonist IL-1ra functions in preventing IL-1alpha or IL-1beta signal transduction by competitive binding to IL-1RtI (43-46). The expression of IL-1beta, IL-1ra, and IL-1RtI has been found in preimplantation embryos and maternal endometrial epithelium of both humans and mice (47-56).

Direct evidence of a role for the importance of the IL-1 system in implantation comes from studies of mice receiving recombinant IL-1ra prior to implantation. IL-1ra administration blocked blastocyst implantation in these animals (57). However, matings between mice deficient for IL-RtI showed no apparent implantation failure and only a mild reduction in total litter sizes (58).

Leukemia inhibitory factor (LIF) is a cytokine of the IL-6 family, which includes IL-6 and IL-11 among others (59). It signals through an heterodimeric receptor complex containing a LIF-specific LIF receptor (LIF-R) chain and a promiscuous gp130 chain which is a common subunit for all receptor complexes of the IL-6 family (60-62). A wide range of hormones, cytokines and growth factors have been shown to induce the expression of LIF, including TGF-alpha, TNF-beta, IL-1, estrogen, epidermal growth factor (EGF), and platelet-derived growth factor (63-68). LIF expression has been reported in a variety of organs, including uterus, embryonic blastocyst, thymus, lung, cardiac muscle, kidney, liver, and skin (69-76). In these organs, LIF exerts pleiotropic effects, generally regulation of cell differentiation and proliferation (77).

Among the major cells producing LIF in the endometrium are T cells, uterine NK cells, as well as endometrial epithelium itself (78-82). Expression of LIF during murine pregnancy appears to increase in the endometrium at gd 1, drops by gd 3, and peaks again at gd 4 just prior to implantation (68, 83). In humans, endometrial LIF expression increases during the secretory phase of the menstrual cycle, peaking during the implantation window. LIF expression is markedly reduced in the proliferative phase (84, 85).

A critical role for LIF in reproduction has been elucidated from studies involving mice harboring a null mutation for the LIF gene. These mice ovulate and exhibit successful fertilization, but nonetheless are incapable of successful pregnancy due to an absolute failure of blastocyst implantation (86). Although LIF and LIF-R is expressed in both blastocyst and endometrial epithelium during the peri-implantation period, maternal LIF expression appears to be crucial. While $LIF^{-/-}$ mothers are unable to accept the implanting blastocyst, $LIF^{-/-}$ and $LIF\text{-}R^{-/-}$ embryos are able to implant in $LIF^{+/+}$ mothers (87, 88). Furthermore, maternal LIF expression appears to be independent of trophoblast involvement as pseudopregnant female mice express endometrial LIF (68). There has also been evidence for a link between LIF and human reproductive success. Several studies have shown a correlation between low levels of LIF in uterine flushings of women during the secretory phase of the menstrual cycle and infertility (89). Moreover, certain deleterious point mutations of the LIF gene have been found in infertile women (90-92).

The mechanisms of LIF's role during implantation are not well understood. LIF has been demonstrated to induce the expression of various factors that are associated with successful implantation, such as Insulin-like growth factor binding protein 3 (IGFBP-3), chorionic gonadotropin, EGF, COX-2, urokinase-type plasminogen activator (uPA), and MMP-9 (93-95). These factors collectively regulate blastocyst adhesion, and trophoblast invasiveness.

Another member of the IL-6 family of cytokines is interleukin-11 (IL-11). Like LIF, IL-11 exhibits pleiotropic effects in many organ systems (96, 97). IL-11 signals through a heterodimeric receptor complex consisting of the common gp130 chain shared by other IL-6 family members and the IL-11-specfic receptor IL-11Ra (98). Uterine expression of IL-11 in both mouse and human peaks during the peri-implantation period (99, 100).

Matings of $IL\text{-}11Ralpha^{-/-}$ mice showed that while they experience successful fertilization, embryo attachment, and initial implantation, embryos die soon thereafter (101). Moreover, the success of pregnancy with the transfer of $IL\text{-}11Ralpha^{-/-}$ embryos into $IL\text{-}11Ralpha^{+/+}$ mothers and the failure with the transfer of $IL\text{-}11Ralpha^{+/+}$ embryos into $IL\text{-}11Ralpha^{-/-}$ mothers confirmed a crucial maternal function for IL-11 in early pregnancy. Histological studies of matings between $IL\text{-}11Ralpha^{-/-}$ mice show a failure of decidualization associated with an overly aggressive invasion of trophoblast into uterine tissue. Indeed, even oil-induced decidualization responses were defective in these mice. These data suggest that IL-11 is crucial for the initiation of uterine decidualization.

Colony Stimulating Factor-1 (CSF-1), also known as macrophage CSF (m-CSF), regulates the differentiation, proliferation, and survival in many cell types (102). This cytokine is highly expressed in the uterine luminal glandular epithellium beginning at gestational day (gd) 3 in murine pregnancy, peaking around gd 14.5 (103, 104). In humans, CSF-1 is expressed throughout the menstrual cycle in humans and is highly expressed in the decidua during the first trimester (105).

A function for CSF-1 in the regulation of pregnancy was first discovered from examinations of osteopetrotic (op/op) mice. These mice possess a frame shift mutation which renders them functionally null for the CSF-1 gene (106). Ovulation rates in op/op mice are dramatically reduced and matings between these mice display reduced implantation and fetal survival. In humans, low serum levels and reduced production of CSF-1 from $CD4^{+}$ T cells are associated with recurrent spontaneous abortion (107). While these results point to a crucial role for CSF-1 during murine and human pregnancy, the mechanism of action are currently unknown.

Interferon-gamma (IFN-gamma) is a cytokine mainly expressed by T cells, NK cells, and NKT cells and can regulate the transcription of about 0.5% of the genome (108). During normal pregnancy, uterine IFN-gamma is expressed mainly by local uterine NK (uNK) cells and also by decidual stromal cells (19, 109). During gestation, exogenous administration of IFN-gamma or infection with pathogens that preferentially induce IFN-gamma is associated with an increase in fetal demise in mice (110). However, mice with an IFN-gamma-null mutation also display increased rates of fetal loss (111). This loss is associated with impaired decidualization and defective remodeling of uterine arteries. Thus, effect of IFN-gamma on pregnancy is complex and seems to depend on the relative balance of production between IFN-gamma and cytokines that exert antagonistic effects, such as interleukin-10 (IL-10).

Interleukin-10 (IL-10) is a cytokine that is exerts a wide variety of effects on most immune cell types. In T cells and NK cells, IL-10 has been shown to inhibit proliferation, cytokine production, and cytotoxic activity. IL-10 also suppresses macrophage maturation and antigen presentation (112).

Serum levels and utero-placental levels of IL-10 increase significantly during pregnancy (22, 26, 109, 113-115). At the fetal-maternal interface, it is expressed by placental trophoblast, decidual stromal cells, uNK cells, T cells, and macrophages (116-120). Local expression of IL-10 begins soon after implantation and persists through the second trimester, particularly in humans.

IL-10 is thought to contribute to the success of pregnancy through action on many different tissues and cell types. Consistent with its well-studied immunoregulatory effects during immune responses to pathogens, tumors, and in transplantation, IL-10 appears to influence immunity at the fetal-maternal interface. In the placenta, IL-10 induces the downregulation of classical class I major histocompatibility complex (MHC) expression as well as the upregulation of the non-classical class I MHC molecule human leukocyte antigen (HLA-G) (121-123). This would be expected to have an immunoregulatory effect on the immunogenic potential of the placenta as classical class I MHC serves as a positive ligand for cytotoxic T lymphocytes. Moreover, HLA-G expression has been shown to reduce target cell susceptibility to NK cell-mediated cytotoxicity (124-126).

In addition to potential immunoregulatory roles, IL-10 affects placental development and function. Proper placental function is crucially dependent on the activities of matrix metalloproteinases (MMPs), which serve to degrade the extracellular matrix and facilitate trophoblast invasion of the uterus (127). IL-10 both downregulates the expression of MMPs and induces the expression of the MMP inhibitor, tissue inhibitor of metalloproteinase (TIMP) (128-130). IL-10 also appears to influence the survival of trophoblast cells through the protection from apoptosis (Sharma S, Unpublished data).

Corroboration for a critical role for IL-10 in pregnancy can be found in studies of IL-10 expression patterns in various diseases of human pregnancy. Both circulating and local levels of IL-10 appear to be diminished in human pregnancy complications such as recurrent spontaneous abortion (RSA), preterm birth, and pre-eclampisa (131-138). Furthermore, fetal demise in an abortion-prone mouse mating model has been associated with low IL-10 production (139). However, mating of IL-$10^{-/-}$ showed no defect in either syngeneic or allogeneic pregnancy as compared to wild type controls (140). Although pregnancy is successful, IL-$10^{-/-}$ mice exhibit larger and more deeply penetrating placentas (141). This finding is consistent with an abrogation of the regulatory activity that IL-10 exerts on MMPs, which would favor deeper trophoblast invasion into the decidua. While pregnancy appears largely unaffected in IL-$10^{-/-}$ mice, initially-studied mating took place under pathogen-free conditions. However, when pregnant IL-$10^{-/-}$ mice are challenged with very low doses of inflammatory agents, they exhibit multitude of pregnancy-associated complications such as fetal loss, preterm birth, and IUGR. These abnormalities in IL-$10^{-/-}$ mice appear to be largely due to the inability to suppress anti-fetal uNK cell activity (Murphy *et al.*, J Immunol (in press), 2005).

4.2. Cyclooxygenase-2

The involvement of prostaglandins in a wide variety of processes crucial for reproduction has been well documented (142-145). These range from implantation, decidualization, and regulation of cell growth and differentiation. Two enzymes are crucial for prostaglandin synthesis, the constitutively expressed cyclooxygenase-1 (Cox-1) and the inducible cyclooxygnease-2 (Cox-2). These enzymes carry out the rate-limiting reaction in prostaglandin synthesis, the conversion of arachidonic acid into the intermediate endoperoxidases (PGH2), which are subsequently converted into prostaglandins by specific prostaglandin synthases (146, 147). Cox-2 expression, in particular, seems to be important for successful reproduction. It is expressed in the uterus of both human and mouse, and is associated with blastocyst attachment (148, 149).

Certain strains of Cox-$2^{-/-}$ mice display multiple reproductive difficulties including defects in ovulation, fertilization, implantation, and decidualization (150). However, Cox-1 expression can partially compensate for the lack of Cox-2 expression. Elevated Cox-2 expression is associated with LPS-induced preterm birth and fetal resorption in mouse models (151, 152). In humans, differential Cox-2 expression patterns have been associated with several pregnancy-associated diseases. While elevated Cox-2 expression has been associated with endometriosis and preterm birth, decreased Cox-2 expression is implicated in some cases of pre-eclampsia (153-158).

4.3. Insulin like growth factor system

The insulin like growth factors (IGF) system appears to play a central role in most reproductive processes studied in both laboratory animals and humans. IGFs are mitogens that regulate cellular growth and metabolism throughout the body, but appear to play an especially crucial role in reproduction (159-161). The members of the IGF system include two soluble peptides, IGF-I and IGF-II, six IGF binding proteins (IGFBP-1 through IGFBP-6), as well as two transmembrane receptors (IGFR type I and type II). Both IGF I and II are highly homologous peptides, structurally related to insulin. Both IGFs can signal through both IGF receptors, but the major signaling receptor appears to be IGFR type I. IGFR type II preferentially binds IGF-II but appears to only be responsible for the internalization and turnover of IGF-II (162, 163). The six IGFBPs bind IGFs with a much higher affinity than either of the IGF receptors, with their affinity for IGFs increased with phosphorylation (164).

The expression of the members of the IGF system is highly regulated during pregnancy. In humans, mRNA for both IGF-I and IGF-II are expressed in the mid to late proliferative and early secretory phase of the menstrual cycle (165). In mice, there is also a cyclical pattern of IGF expression during the estrous cycle (167). The type I IGF receptor is constitutively expressed in the endometrium of both mice and humans, although human endometrium also expresses the type II receptor (166-168). All six IGFBPs are expressed in humans with IGFBP-1 being predominant in the late secretory phase endometirum (33, 169). Expression of IGFBP-3, -4, and -5 is found within the mouse decidua with IGFBP-4 expression being highly localized to implantation site 24 hours after blastocyst implantation (170, 171). During gestation, mRNAs from all members of the IGF system are expressed in the human placenta (161, 171).

The temporal and spatial expression of members of the IGF system suggests a role for their paracrine regulation of fetal and maternal tissues during gestation, especially the processes of implantation and decidualization. Further insights into the functions of the IGF system during pregnancy have been gleaned from knockout mouse models. IGF-I$^{-/-}$ animals display reduced growth during embryonic and postnatal stages (172). Mating of male IGF-II$^{-/-}$ mice to IGF-II$^{+/+}$ females resulted in embryonic growth restriction but normal growth rates, although the absolute size of heterozygous progeny was reduced as compared to wild type (173). Mice functionally null for the type I IGF receptor exhibit severe embryonic growth restriction, a result to be expected considering the loss of both IGF-I and IGF-II signaling (172). Interestingly, while neither IGF-I$^{-/-}$ nor type I IGFR$^{-/-}$ animals display abnormalities in placental growth and development, IGF-II$^{+/-}$ embryos exhibit decreased placental growth, suggesting an important role of IGF-II in placental development (174).

4.4. Matrix Metalloproteinase System

Matrix metalloproteinases (MMPs) are a family of extracellular matrix degrading enzymes which include MMP-1 through MMP-28 (175). Their actions are regulated in the tissues by a group of MMP inhibitors referred to as tissue inhibitors of metalloproteinases (TIMPs). To date, four TIMPs have been discovered, TIMPs1-4, which appear to each have selective activities on specific MMPs (175).

The expression of many MMPs is observed during pregnancy in rodents, although most studies agree that MMP-2 and MMP-9 are likely to be most crucial for reproduction (176). It appears that the most important factors regulating MMP expression and activity during pregnancy are cytokines. Among the most studied is the IL-1 family. IL-1α and IL-1β expression in blastocysts and invasive trophoblasts seems to be important in the upregulation of MMP expression (177). Moreover, IL-1β administration upregulates MMP expression in both maternal and plancental tissues. In addition to enhancing the expression and activity of MMPs, various cytokines can downregulate MMP activity. Most notably, IL-10 is known to both inhibit the expression of MMP-2 and MMP-9 and upregulate the expression of TIMP-1 (178-180).

Several mouse strains have been created with targeted gene knockouts of members of the MMP system. With initial inspection, there appeared to be no overt reproductive phenotypes. This may, in part be due to an inherent redundancy and compensatory nature of the MMP system. Deletion of a single MMP or TIMP gene seems to result in the compensation by other MMPs or TIMPs. For example, while MMP-7 and MMP-3 knockout mice appear to reproduce normally, MMP-3 and MMP-7 expression appears to be upregulated in these mice respectively (181, 182). Moreover, closer investigation revealed subtle reproductive defects in mice with MMP-family gene knockouts. MMP-9 knockout mice appear to have no obvious reproductive phenotype, however, they present smaller litter sizes and reduced pregnancy rates after copulation (183, 184). In addition, TIMP-1 knockout mice are able to reproduce, but display reduced pregnancy rates with fewer pups per litter (185, 186).

4.5. Transcription Factors Involved in Placental Development

The expression of a vast array of transcription factors in placental cells has been shown to be crucial in the differentiation of the various trophoblast-derived cell types. Of these, a select few knockout mouse models are available. Among the best studied are Hand1 and Gcm1. Hand1 is a basic helix-loop-helix transcription factor that serves to induce trophoblast growth arrest and differentiation to giant cells, an invasion trophoblast-derived cell which functions in the establishment of an efficient fetal-maternal vascular exchange (187, 188). Gcm1 is an unrelated transcription factor expressed in the placental labyrinth (189). Mice with a Gcm1-null mutation display defects in chorioallantoic fusion and syncytiotrophoblast differentiation (190).

4.6. Indoleamine-2,3-dioxygenase

Indoleamine-2,3-dioxygenase (IDO) is a trypthophan catabolizing enzyme expressed in a variety of cell types at the fetal-maternal interface (191). Pharmacological inhibition of IDO in pregnant mice from wild type allogeneic matings resulted in increased fetal demise, which was not observed in wild type syngeneic matings (192). Inhibition of IDO in pregnant RAG-2$^{-/-}$ or SCID mice from allogeneic matings had no effect on pregnancy outcome, suggesting that the major regulatory target cell for IDO activity is T cells (193). However, matings between allogeneic IDO knockout mice display no reproductive defect (194). This suggests that in the absence of IDO, the T cell activity could be controlled by compensatory and redundant mechanisms or that pharmacological targeting of IDO in pregnant mice influenced some other pathways.

5. CONCLUSIONS

The use of gene knockout mice has greatly increased understanding of the roles of specific genes involved in the processes of implantation and fetal development (Table 1). While the expression of some genes is of such importance for these processes that their absence prevents reproduction, others appear to exist in a redundant network. This redundancy obscures the interpretation of the physiological significance of the absence of a specific gene. This phenomenon of redundancy, while greatly prominent in the processes involved in reproduction, is a common pitfall of research using gene knockout mice. It is likely that the investigation of the role of gene deficiency in reproduction will be greatly aided by understanding the contribution of secondary, often temporally encountered insults.

Table 1. Gene Knockouts Affecting Reproduction

Gene	Pattern of Expression	Knockout Reproductive Phenotype	References
IL-1RtI	Preimplantation blastocyst and maternal endometrium	Slight reduction in litter sizes	59
LIF	Endometirum , uNK cells, uterine T cells	Complete failure of blastocyst implantation	87-89
IL-11Rα	Uterine endometrium, uNK cells, uterine T cells	Failure of decidualization, early embryonic death	102
CSF-1	Uterine endometrium	Reduced ovulation and implantation rates	107
IFN-γ	Uterine endometirum, uNK cells, uterine T cells	Primiparous reproductive defects, moderate failure of decidualization	112
IL-10	Uterine endometirum, NK cells, uterine T cells, placenta	Exaggerated inflammatory response to mild inflammatory insults leading to uNK cell-mediated fetal loss, prematurity, and IUGR	141, 142
COX-2	Uterine endometrium, placenta	Defects in ovulation, fertilization, implantation, and decidualization	151
IGF-I	Uterine endometirum	Reduced growth during embryonic and postnatal stages	173
IGF-II	Uterine endometirum	Reduced embryonic growth and placentation defects	174
type I IGFR	Uterine endometirum	Severe embryonic growth restriction	175
MMP-3, -7, -9	Uterine endometirum, uterine immune cells, placenta	Smaller litter sizes and reduced pregnancy rates, Compensation by remaining MMP family members	182-185
TIMP-1	Uterine endometirum	Reduced pregnancy rates with fewer pups per litter	186, 187
Hand1	Placental trophoblast	Failure in trophoblast differentiation to Giant cells	188, 189
Gcm1	Placental labyrinth	Defects in chorioallantoic fusion and syncytiotrophoblast differentiation	191
IDO	Uterine endometrium, uterine mascrophages, placenta	None reported. Pharmacological inhibition results in failure of allogeneic pregnancies	195

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Abbreviations: IDO: indoleamie-2,3-dioxygenase, IGF: insulin-like growth factor, IGFBP: insulin-like growth factor binding protein, IUGR: intrauterine growth restriction, LIF: leukemia inhibitory factor, MMP: matrix metalloproteinase, TIMP: Tissue inhibitor of metalloproteinase, uNK cell: uterine natural killer cell

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Genetic modified mice Humanized mice Lymphopoiesis T cell development Bcl11b.Â A most breakthrough advantage of using the mouse to study the immune system and to model human disease is the availability of a range of genetic technologies. In 1981, several groups produced transgenic mice by injecting transgenic DNA into mouse pronuclei [9, 10]. Importantly, DNA introduced into the mouse genome by this method results in establishment of the transgene in the germ line. This technology offers scientists the opportunities to perform gain-of-function studies for specific genes in the mouse model. Currently, DNA fragments can be conveniently inserted into the host cell genome by Genes that are subject to genomic imprinting in mammals are preferentially expressed from a single parental allele. This imprinted expression of a small number of genes is crucial for normal development, as these genes often directly regulate fetal growth. Recent work has also demonstrated intricate roles for imprinted genes in the brain, with important consequences on behavior and neuronal function. Finally, new studies have revealed the importance of proper expression of specific imprinted genes in induced pluripotent stem cells and in adult stem cells.