Implantation: molecular basis of embryo-uterine dialogue

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ABSTRACT Implantation is a complex developmental process that involves an intimate “cross-talk” between the embryo and uterus. Synchronized development of the embryo to the blastocyst stage and differentiation of the uterus to the receptive state are essential to this process. Successful execution of the events of implantation involves participation of steroid hormones, locally derived growth factors, cytokines, transcription factors and lipid mediators. Using gene-targeted mice and a delayed implantation model, our laboratory has been exploring potential interactions among steroid hormones, growth factors, cytokines and prostaglandins in this process. This review article highlights some of our recent observations on the roles of estrogen, catecholestrogen, the EGF family of growth factors, leukemia inhibitory factor and cyclooxygenase-2 derived prostaglandins and their interactions in embryo-uterine “cross-talk” during implantation.

KEY WORDS: Embryo, implantation, growth factors, cytokines, prostaglandins.

Procreation, both sexual and asexual, is a fundamental and evolving process to sustain life in this universe. Thus, understanding this process has been the inspiration of investigators for centuries. Sexual procreation in higher eukaryotes, especially in mammals, is inferior to asexual procreation in prokaryotes and in some lower eukaryotes with respect to sheer number of progeny produced. Mammalian reproduction on the other hand, is more complex and highly regulated for the propagation of superior offspring to carry on the task of procreation. In sexual reproduction, the fate of the union of a sperm with an egg marks the beginning of embryonic development or death. A successful union leads to fertilization and subsequent development, while failure to achieve such a union leads to the demise of the gametes. In most eutherian mammals, the one-cell fertilized egg, here termed the embryo, undergoes mitotic cell division ultimately forming a blastocyst with two distinct cell populations, the inner cell mass (ICM) and a layer of trophoderm cells surrounding the ICM (Gardner and Papaioannou, 1975). The ICM forms the embryo proper and trophoderm cells first make the physical and physiological contact with the maternal uterine luminal epithelium to initiate the process of implantation, leading to the establishment of pregnancy (reviewed in Dey, 1996). A substantial embryo loss due to preimplantation death is common to many mammals, and it is considered a selection process that leads to the survival of superior embryos for implantation. Also, the failure of the events during and immediately following implantation often adds to the poor pregnancy rate in eutherian mammals (reviewed in Cross et al., 1994). Therefore, understanding and unraveling the secrets of preimplantation embryo development and implantation in the maternal uterus has been a challenge to the investigators in the field aiming to alleviate the problems of infertility, ensuring the birth of quality offspring, or developing novel contraception to restrict world population.

The current state of the knowledge of preimplantation and implantation physiology is the result of accumulation of scientific observations gathered by many pioneers through the years. One of the pioneers in this field is Ann McLaren whose seminal observations have formed the basis of further investigation in the field. This review article on implantation is dedicated to Ann McLaren to show our tribute for her contribution in the field of preimplantation and implantation biology.

Embryonic development to the blastocyst stage and uterine differentiation to the receptive environment are essential to the establishment of the embryo-uterine “cross-talk” that leads to the initiation and progression of successful implantation. A complex series of interactive molecular events is associated with this process. However, the precise sequence and details of these molecular events have not yet been defined. Since the process of implantation is complex and varies across species, a unified theme has not yet been formulated, resulting in an accumulation of literature often with contradictory interpretations. Thus, it is an arduous task to write an overview on the molecular basis of embryo-uterine interactions during implantation that could be

Abbreviations used in this paper: COX, cyclooxygenase-2; LIF, leukemia inhibitory factor.

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relevant to mammals in general. This review focuses on the molecular basis of implantation based on the information generated with mice primarily from our laboratory. However, a few of the distinct differences with other species are highlighted.

**Preimplantation embryo development**

Preimplantation embryo development to the blastocyst stage, which requires activation of the embryonic genome, is essential to the process of implantation. This maternal-zygotic transition occurs at the 2-cell stage in mice and other rodents, between the 8- and 16-cell stage in cows and sheep, and between 4- and 8-cell stage in humans (reviewed in Artley et al., 1992). Once the embryonic genome is activated, the embryo grows rapidly to form a blastocyst. At this stage, embryos mature and escape from their zona pellucida to achieve implantation competency. The fully expanded blastocyst is composed of three cell types: (i) the outer polarized epithelial trophectoderm, (ii) the primitive endoderm, and (iii) the pluripotent ICM. The ICM provides the future cell lineages for the embryo proper (Hogan et al., 1994a; McLaren, 1982a). However, no ICM is identifiable in marsupial blastocysts; they appear as a hollow ball of cells with similar morphological characteristics. It is also not known which cells are programmed to form the embryo proper (reviewed in McLaren, 1982a).

In mice and rats, normal embryonic development to the blastocyst stage within the reproductive tract requires the presence of ovarian estrogen and progesterone (McLaren, 1971). There is a decrease in the number of embryos and number of cells per embryo in the absence of these hormones (Bowman and McLaren, 1970); treatment with estrogen and progesterone reverses these defects (Roblero and Garavagno, 1979). Since there is no evidence that estrogen and/or progesterone act directly on the preimplantation embryo, embryonic development is considered to depend on growth-promoting factors originating from the reproductive tract under the influence of these hormones. However, normal development in defined media in cultures suggests that preimplantation embryos themselves produce the growth-promoting factors (reviewed in Paria and Dey, 1990). This is consistent with the expression of several growth factors, cytokines and their receptors in the embryo and uterus, as well as beneficial effects of these factors on embryonic development and functions (reviewed in Caron et al., 1994; Paria, 1990; Paria et al., 1994; Stewart and Cullinan, 1997).

**Uterine preparation for blastocyst implantation**

The molecular mechanism(s) that renders the uterus receptive to blastocyst implantation is not fully understood. The “window” of uterine receptivity is defined as the limited time when the uterine environment is conducive to blastocyst implantation. The synchronized development of the embryo to the blastocyst stage and the differentiation of the uterus to the receptive state are critical to this process (Dey, 1996; Paria et al., 1993; Psychoyos, 1973). Although various aspects of molecules associated with this process are extensively being explored, a comprehensive understanding of the subject is still very limited.

Uterine differentiation to support embryo development and implantation is coordinated by progesterone and estrogen in a spatiotemporal manner (Huet-Hudson et al., 1989). In the rodent, the first conspicuous sign that the implantation process has been initiated is an increased uterine vascular permeability at the sites of blastocyst apposition (Psychoyos, 1973). This is demarcated by discrete blue bands along the uterus after an intravenous injection of a blue dye solution (Paria et al., 1993; Psychoyos, 1973). This heightened, localized vascular permeability coincides with the initial attachment reaction between the luminal epithelium and the blastocyst (Enders and Schlafke, 1967) and is considered one of the earliest prerequisites for implantation (Psychoyos, 1973). In the mouse, these events occur in the evening (2200-2300 hour) of day 4 (day 1 = vaginal plug) of pregnancy (Das et al., 1994). The attachment reaction is followed by localized decidualization of the stroma and apoptosis of the luminal epithelium at the sites of implantation (Part et al., 1987), facilitating invasion of the trophoblast cells through the underlying basement membrane (Schlafke and Enders, 1975). The blastocyst attachment with the uterine luminal epithelium and its subsequent apoptosis is illustrated in Fig. 1. These processes are accompanied by remodeling of the extracellular matrix and angiogenesis in the stromal bed (reviewed in Caron et al., 2000; Dey, 1996). The molecular mechanisms by which increased localized vascular permeability and angiogenesis occur at the implantation site are still poorly understood.

The requirement of ovarian estrogen is species-specific. While estrogen is essential for preparation of the progesterone-primed uterus for implantation in mice and rats, progesterone alone is capable of initiating implantation in species, such as the hamster, guinea pig, pig and rabbit (Deanesly 1960; Kwun and Emmens, 1974; Orsini and Meyer, 1962; Perry et al., 1973). Although progesterone is an absolute requirement for implantation, it is not yet clear whether estrogen is a requirement for implantation in primates (de-Ziegler et al., 1998; Meyer et al., 1969; Ravindranath and Moudgal, 1987; Yoshinaga, 1994). Most of our understanding about the requirement of ovarian steroid hormones for the preparation of the uterus for implantation is based on studies in mice and rats. In the adult mouse, estrogen stimulates proliferation of uterine epithelial cells, and progesterone stimulates stromal cell proliferation. These two steroids together further potentiate stromal cell proliferation (Huet-Hudson et al., 1988). Similar hormonal stimulation of cell-specific proliferation occurs in the preimplantation uterus. Preovulatory ovarian estrogen secretion induces proliferation of the luminal and glandular epithelial cells during the first two days of pregnancy. On the third day, progesterone from newly formed corpora lutea initiates stromal cell proliferation which is further potentiated by preimplantation estrogen secretion on day 4 of pregnancy (the day of implantation) (Huet-Hudson et al., 1989). Uterine sensitivity to implantation in response to ovarian steroids is programmed into three phases: prereceptive, receptive, and nonreceptive. The prereceptive uterus on day 3 of pregnancy becomes receptive on day 4 under the influence of rising progesterone and preimplantation ovarian estrogen secretion (Dey, 1996; Paria et al., 1993). Blastocysts implant only in the receptive uterus, which spontaneously progresses to the nonreceptive phase. The preimplantation estrogen secretion is absolutely necessary for the establishment of uterine receptivity in the rat and mouse (McCormack and Greenwald, 1974; Paria et al., 1993; Psychoyos, 1973). Although the hormonal requirement for uterine preparation and implantation is different in various species, the molecular basis of these differences is not yet known and needs to be addressed. However, the preimplantation embryo as a source of estrogen
influencing implantation in the species not requiring ovarian estrogen can not be ruled out. Indeed, rabbit and pig blastocysts have an estrogen-synthesizing capacity, which is likely to participate in implantation (Hoversland et al., 1982; Perry et al., 1973). On the other hand, preimplantation mouse embryos do not possess the machinery for producing estrogen (Stromstedt et al., 1996). Furthermore, normal implantation of mouse or human blastocysts carrying a nonfunctional aromatase gene suggests that embryonic estrogen is not required for implantation in these species (Fisher et al., 1998; Morishima et al., 1995). It is still unclear whether embryonic estrogen is required for the initiation of implantation in hamsters and primates. Further investigation is warranted to understand the molecular basis of these differential roles of estrogen and progesterone in the preparation of the receptive uterus and implantation among various species. In this respect, the delayed implantation model in certain species provides an opportunity to dissect out the distinctive and overlapping functions of estrogen and progesterone in the uterus as it relates to implantation.

Delayed implantation

As discussed above, in the mouse preimplantation ovarian estrogen secretion on the morning of day 4 of pregnancy is essential for the attachment reaction that occurs in the evening of this day. However, the implantation process is deferred if ovaries are removed on day 4 of pregnancy before the secretion of preimplantation ovarian estrogen. This condition, known as delayed implantation, can be maintained for several days by daily progesterone treatment (Psychoyos, 1973; Yoshinaga and Adams, 1966). Under this condition, the uterus remains in a neutral state (analogous to the prereceptive phase) and blastocysts undergo dormancy. Delayed implantation can be terminated by an injection of estrogen to initiate implantation with blastocyst activation (McLaren, 1971; Paria et al., 1993; Psychoyos, 1973). Delayed implantation can also be induced by other means including hypophysectomy and interference with hypothalamic functions (Bindon, 1969). Lataste in 1891 first described the natural suckling-induced delayed implantation (facultative) in mice and rats that had been mated during the post-partum estrus. This type of naturally-occurring delayed implantation varies between strains of mice and rats and even between individuals within a particular strain (Gidley-Baird, 1981). However, dormant blastocysts become activated and undergo implantation upon termination of the suckling stimulus by removal of pups. In many wild animals including mustelids and marsupials, delayed implantation is obligatory and seasonal (reviewed in McLaren, 1982b). Interestingly, delayed implantation does not occur in hamsters, guinea pigs, rabbits, and pigs. However, it is not known whether this condition ever occurs in primates. Although estrogen is essential to terminate delayed implantation in the progesterone-primed mouse or rat uterus, the mechanism by which estrogen directs this function is not clearly understood.

McLaren’s group using labeled substrates, first showed that dormant mouse blastocysts are metabolically less active during delayed implantation than they are during normal implantation or during reactivation after estrogen treatment (Menke and McLaren, 1970). This was later confirmed by other investigators (Weitlauf, 1974). This observation by McLaren’s laboratory created a tremendous interest in the field and led to the proposal that the lower metabolic rate of dormant blastocysts is the result of the presence of an inhibitor in the delayed uterus (Weitlauf, 1974; 1978). On the other hand, Surani (1975) suggested that the disappearance of a stimulatory factor from the uterus during the delay is the cause for blastocyst dormancy. Although dormant blastocysts gain metabolic competence in vitro (McLaren, 1973) suggesting the pres-
ence of an inhibitor, the subject still remains unresolved. We raised the question of whether metabolic competency is similar to implantation competency of the blastocyst. Our work, as described below, suggests that the blastocyst’s state of activity is also an important factor in the initiation of implantation in the receptive uterus.

**Blastocyst state of activity and uterine receptivity determine the window of implantation**

The uterine receptivity for blastocyst implantation lasts only for a limited period, followed by spontaneous progression to the non-receptive phase when the uterine milieu becomes hostile to blastocyst survival. In rats and mice, uterine receptivity lasts for about 24-36 h (Paria et al., 1993; Psychoyos, 1973), while this window appears to last longer in primates (reviewed in Yoshinaga, 1994). In the past, it was considered that the uterine receptivity was the primary determinant for successful implantation regardless of the blastocyst’s state of activity. However, our recent work, using blastocyst transfer experiments in delayed implanting mice, have demonstrated that the blastocyst’s state of activity is also an important factor in defining the “window” of implantation in the receptive uterus (Paria et al., 1993). For example, dormant blastocysts transferred into uteri of progesterone-treated delayed recipients implanted only if they were transferred within one hour of an estrogen injection to the recipients. In contrast, day 4 normal recipients implanted only if they were transferred within one hour of the estrogen treatment of blastocysts transferred into uteri of progesterone-treated delayed recipients. These results suggested a unique role of estrogen in influencing blastocyst functions. To examine the role of estrogen in blastocyst activation for implantation, we raised the question of whether metabolic competency is similar to implantation competency of the blastocyst. Our work, as described below, suggests that the blastocyst’s state of activity is also an important factor in the initiation of implantation in the receptive uterus.

Furthermore, an antiestrogen fails to block 4-OH-E2 mediated surface activation of dormant blastocysts in vitro, suggesting a unique role of estrogen in influencing blastocyst functions. To examine the site-specific actions of primary estrogen and catecholestrogen in uterine preparation and blastocyst activation for implantation, respectively, 2-fluoroestradiol-17β (2-FI-E2) was used. Although 2-

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**TABLE 1**

**GENES INVOLVED IN PREIMPLANTATION AND IMPLANTATION BIOLOGY: GENE-TARGETED MOUSE MODELS AND THEIR REPRODUCTIVE PHENOTYPES**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Reproductive Phenotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td>Junctional protein</td>
<td>Failure of blastocyst formation</td>
<td>Lanue et al., 1994</td>
</tr>
<tr>
<td>FGF-4</td>
<td>Growth factor</td>
<td>Defective proliferation of inner cell mass</td>
<td>Feldman et al., 1995</td>
</tr>
<tr>
<td>erbB-1</td>
<td>Receptor for EGF-like growth factors</td>
<td>Postimplantation failure depending on the genetic background</td>
<td>Threadgill et al., 1995; Miettinen et al., 1995; Sibilia and Wagner, 1995</td>
</tr>
<tr>
<td>α-catenin</td>
<td>Adhesion molecule</td>
<td>Disruption of blastocyst trophoderm</td>
<td>Torres et al., 1997</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Growth factor</td>
<td>Defective ovarian and uterine functions</td>
<td>Baker et al., 1996</td>
</tr>
<tr>
<td>Wnt7a</td>
<td>Cell signaling</td>
<td>Female sterility</td>
<td>Parr and McMahon, 1995</td>
</tr>
<tr>
<td>COX-2</td>
<td>Prostaglandin synthesis</td>
<td>Ovulation, fertilization and implantation defects</td>
<td>Lim et al., 1997</td>
</tr>
<tr>
<td>ERα</td>
<td>Nuclear estrogen receptor</td>
<td>Female sterility, Various aspects of reproductive failures</td>
<td>Lubahn et al., 1993</td>
</tr>
<tr>
<td>PR</td>
<td>Nuclear progesterone receptor</td>
<td>Female sterility, Various aspects of reproductive failures</td>
<td>Lydon et al., 1995</td>
</tr>
<tr>
<td>LIF</td>
<td>Cytokine</td>
<td>Implantation and decidualization failures</td>
<td>Stewart et al., 1992</td>
</tr>
<tr>
<td>IL-11Rα</td>
<td>IL-11 receptor</td>
<td>Defective decidualization</td>
<td>Robb et al., 1998</td>
</tr>
<tr>
<td>Hmox3</td>
<td>Homeobox transcription factor</td>
<td>Defective implantation</td>
<td>Wang et al., 1998</td>
</tr>
<tr>
<td>Hoxα-10</td>
<td>Homeobox transcription factor</td>
<td>Implantation and decidualization defects</td>
<td>Benson et al., 1996; Lim et al., 1999b</td>
</tr>
<tr>
<td>Hoxα-11</td>
<td>Homeobox transcription factor</td>
<td>Implantation and decidualization defects</td>
<td>Hsieh-Li et al., 1995; Gendron et al., 1997</td>
</tr>
<tr>
<td>cPLA2</td>
<td>Arachidonic acid release</td>
<td>Small litter size. Cause unknown</td>
<td>Uozumi et al., 1997; Bonventre et al., 1997</td>
</tr>
<tr>
<td>CSF-1</td>
<td>cytokine</td>
<td>Reduced female fertility</td>
<td>Pollard et al., 1991</td>
</tr>
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</table>
Fl-E₂ is a poor substrate for and a potent inhibitor of estrogen 2/4-
hydroxylase that converts primary estrogens to catecholestrogens,
it is equipotent to E₂ with respect to cell-specific uterine growth and
gene expression (reviewed in Paria et al., 1998). However, 2-Fl-E₂
even at a dose of 75 ng/mouse failed to induce implantation in
progesterone-primed delayed implanting mice, whereas E₂ at this
dose or even at 10 ng/mouse induced implantation in similarly
treated delayed implanting mice. These results suggested that 2-
Fl-E₂ prepared the uterus but failed to activate the dormant
blastocyst, possibly because of its failure to form catecholestrogens.
This is consistent with the finding that day 4 normal blastocysts,
or dormant blastocysts cultured in the presence of 4-OH-E₂ did
implant when they were transferred into uteri of progesterone-
primed delayed recipients receiving an injection of 2-Fl-E₂. How-
ever, dormant blastocysts cultured in the presence of E₂ failed to
implant in similarly treated mice. These results suggest that dor-
mant blastocysts become implantation competent when they are cultured with 4-OH-E₂, but not with E₂. Collectively,
our results demonstrate that primary estrogen participates in
the preparation of the progesterone-primed uterus to the
receptive state in an endocrine manner, whereas its
metabolite catecholestrogen produced in the uterus medi-
ates blastocyst activation for implantation in a paracrine
manner, suggesting target-specific effects of estrogens dur-
ing implantation. However, the molecular mechanisms by
which these estrogens mediate their effects are not clearly
understood. There is increasing evidence that the steroid
hormones interact with the local factors at the targets to
initiate the process of embryo-uterine interactions during
implantation.

Potential molecular signaling during implantation

Recent progress in genetics and molecular biology has
remarkably increased our knowledge regarding the roles of
locally derived growth factors, cytokines, homeotic gene
products and lipid mediators during implantation and
decidualization. These factors are produced by the uterus or
embryo independently or cooperatively under the influence
of steroid hormones. Due to the space limitation, this review
will discuss only a few of these local factors that are critical
to the processes of implantation and decidualization. How-
ever, roles of various other genes in preimplantation and
implantation phenomena as evident from gene targeting
experiments are presented in Table 1.

The EGF family of growth factors

Among various growth factors, the EGF family of growth
factors is considered to be involved in implantation. This
family includes EGF itself, TGF-α, HB-EGF, amphiregulin,
betacellulin, epiregulin and neuregulins (Das et al., 1997;
Paria et al., 2000). Although TGF-α and amphiregulin, but
not EGF, are expressed in the mouse uterus during the
perimplantation period (Das et al., 1997; Paria et al., 2000),
they do not appear to be essential for implantation since
mice deficient of these genes are fertile (Luetteka et al.,
1993;1999). However, it is possible that the other members
of the EGF family compensate for the loss of these mem-
bers. Both epiregulin and betacellulin are also expressed in the
luminal epithelium and stroma at the sites of blastocyst apop-
sition during implantation (Das et al., 1997). However, their roles
in implantation are not yet clearly understood.

HB-EGF appears to have an expression pattern highly relevant
to the implantation process. In the mouse, it is induced exclusively
in the luminal epithelium at the site of blastocyst apposition 6-7
hours before the initial attachment reaction (Das et al., 1994;
Paria et al., 2000). Since HB-EGF is not expressed in the uterus during
the delayed implantation, it is considered that a signal is transmit-
ted from the active blastocyst to the luminal epithelial cells for the
expression of HB-EGF at the site of subsequent implantation. In
vitro experiments have shown that soluble HB-EGF stimulates
proliferation, zona-hatching, trophoblast outgrowth and ErbB1
phosphorylation of murine blastocysts (Das et al., 1994). Further-
more, cells expressing the transmembrane form of HB-EGF ad-
LIF exists in both soluble and matrix-associated forms (Kishimoto et al., 1994). In the mouse, LIF functions. This family include IL-6, IL-11, CNTF, oncostatin M, and HB-EGF. They are not essential for implantation (Stewart et al., 1992). LIF is a member of the cytokine family that often possesses overlapping functions. This family includes IL-6, IL-11, CNTF, oncostatin M, and cardiotrophin-1 (reviewed in Kishimoto et al., 1994). LIF functions through heterodimerization of LIF receptor (LIF-R) and gp130 (Davis et al., 1998). LIF was shown to be transiently expressed in uterine glands on day 4 of pregnancy in the mouse (Bhatt et al., 1991). Indeed, implantation failures in LIF deficient mice confirm its essentiality in implantation (Stewart et al., 1992). However, our recent studies show that LIF expression is biphasic in the mouse uterus on day 4 of pregnancy. Not only is LIF expressed in the uterine glands, but it is also expressed in stromal cells surrounding the blastocyst at the time of the attachment reaction (Song et al., 2000). This suggests that LIF has dual roles: first in the preparation of the uterus and later in the attachment reaction. Reciprocal embryo transfer experiments suggest that maternal LIF is essential for implantation (Stewart et al., 1992), although a role for this cytokine in embryonic functions cannot be ignored since preimplantation embryos express LIF-R and gp130, and exogenous LIF improves embryos viability and hatching (Nichols et al., 1996; Robertson et al., 1991). Taken together, these data suggest that both the preimplantation embryo and the uterus are potential sites of LIF action. However, embryos lacking either LIF-R or gp130 develop to the blastocyst stage and implant normally, although disruption of these genes leads to embryonic and perinatal lethality (Ware et al., 1995; Yoshida et al., 1996). Uterine LIF also appears to be important for implantation in other species including humans (Arici et al., 1995). Indeed, LIF deficiency is apparent in infertile women and in those with unexplained recurrent abortions (Hambartsoumian, 1998). It appears that LIF expression in the uterus is maximal around the time of implantation in most species examined, although the steroidal hormonal requirements for the preparation of uterine receptivity and implantation are different in different species.

Cytokines

Various cytokines are considered to be involved in various aspects of implantation. Among these cytokines, IL-1, IL-6, TNF-α, and CSF-1 have been extensively studied (reviewed in Carson et al., 2000; Stewart and Cullinan, 1997). However, genetic studies with mice lacking CSF-1, IL-1 or IL-6 indicate that although these factors can affect the frequency of successful implantation (Poli et al., 1994; Pollard et al., 1991; Zheng et al., 1995), unlike LIF they are not essential for implantation (Stewart et al., 1992). LIF is a member of the cytokine family that often possesses overlapping functions. This family includes IL-6, IL-11, CNTF, oncostatin M, and cardiotrophin-1 (reviewed in Kishimoto et al., 1994). In the mouse, LIF exists in both soluble and matrix-associated forms (Kishimoto et al., 1994; Rathjen et al., 1990). LIF functions through heterodimerization of LIF receptor (LIF-R) and gp130 (Davis et al., 1993).

Prostaglandins

It has long been speculated that prostaglandins (PGs) are involved in many aspects of the reproductive functions including implantation and decidualization. Cyclooxygenase (COX) is the rate-limiting enzyme in PG biosynthesis and exists in two isoforms, COX-1 and COX-2 (reviewed in Lim and Dey, 2000; Lim et al., 1997; Lim et al., 1999a; Paria et al., 2000). The expression of COX-1 is constitutive, whereas that of COX-2 is inducible (Smith and deWitt, 1996). COX genes are expressed in a temporal and cell-specific manner in the uterus during the periimplantation period. Expression of COX-2, but not COX-1, in the luminal epithelium and stroma surrounding the blastocyst at the time of the attachment reaction suggests its role in implantation (Chakraborty et al., 1996). Indeed, gene deletion studies have clearly demonstrated that COX-2 derived PGs are essential for implantation and decidualization. The implantation and decidualization defects in COX-2(-/-) mice are not due to any aberrant expression of other implantation-specific genes or reduced responsiveness of the uterus to steroid hormones (Lim et al., 1997). Among various PGs, the levels of prostacyclin (PGI₂) are highest at the implantation sites of wild-type mice and implantation defects are partially restored in COX-2(-/-) mice by administration of a more stable prostacyclin agonist, carbaprostacyclin (Lim et al., 1999a).

PGs can act via dual receptor signaling systems. Receptors for PGE₂, PGF₂α, PGD₂, PGI₂ and thromboxanes have been identified as EP1-EP4, FP, DP, IP and TP, respectively, and they belong to G protein coupled cell surface receptors (reviewed in Lim and...
Dey, 2000; Negishi et al., 1995). Although EP receptors are expressed in the periimplantation mouse uterus, gene targeting experiments have demonstrated that three of the four EP receptor subtypes (EP1-EP3) are not critical for implantation, while EP4 deficiency results in embryonic lethality and thus its role in implantation has not yet been determined (reviewed in Lim and Dey, 2000). Furthermore, mice deficient in FP or IP show normal implantation. PGs can also exert their effects by utilizing peroxisome proliferator-activated receptors (PPARs) which belong to a nuclear hormone receptor superfamily. Three members of the PPAR family are PPARα, PPARγ and PPARδ. PPARs can respond to a wide variety of ligands including natural and synthetic eicosanoids, fatty acids, hypolipidemic and hyperlipidemic drugs, and PGI2 agonists. To act as a transcriptional activator, PPARs must form heterodimer with a member of the retinoid X receptor (RXR) subfamily (reviewed in Lim and Dey, 2000; Lim et al., 1999a). We have recently shown that COX-2 derived PGI2 participates in implantation via activation of PPARδ (Lim et al., 1999a). This is consistent with reversal of implantation defects in COX-2(-/-) mice by a PGI2 agonist or a PPARδ agonist (Lim et al., 1999a). Taken together, the results provide evidence that COX-2-PGI2-PPARδ signaling is important for implantation.

Concluding remarks

Although considerable information regarding the roles of growth factors, cytokines, homeotic genes and lipid mediators in embryo-uterine interactions during implantation has been generated, their hierarchical arrangement in directing uterine and embryonic functions during implantation is not clearly understood. Dysregulation of expression of EGF-like growth factors and COX-2 in the uterus at the site of blastocyst apposition in LIF deficient mice with implantation failure (Song et al., 2000) suggests an interaction between these molecules in this process (Fig. 2). Although COX-2 expression is aberrant in LIF deficient uteri, uterine LIF expression is normal in Hoxa-10 deficient uteri and vice versa (Lim et al., 1997). In contrast, LIF expression is normal in Hoxa-10 deficient uteri and vice versa (Benson et al., 1996; Song et al., 2000). This suggests that regulation of these two genes is independent of each other. However, uterine COX-2 expression is attenuated during decidualization in Hoxa-10(-/-) mice (Lim et al., 1999b). Thus, it is possible that the information sequentially originating from LIF, EGF like growth factors and Hoxa-10 signaling pathways finally converge to the COX-2 pathway for implantation (Fig. 3). Alternatively, each pathway may have its own distinctive functions or may work in parallel with each other. Thus, disruption of one pathway may result in implantation failure, and apparent dysregulation of other pathways could be the consequence of implantation failure. Finally, two of the three pathways may be interwoven and interruption of either pathway will lead to implantation failure.

Cellular and physiological aspects of preimplantation and implantation biology that had been pioneered through the years have provided the basis of our current molecular and genetic understanding of the subject. To further advance our knowledge with respect to the precise hierarchical arrangements for the functions of the genes in implantation, uterine-specific conditional gene deletion and DNA microarray approaches are of paramount importance.

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References


