Special Lecture II

Molecular mechanism of implantation

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As mammalian fertilized eggs develop by increasing their cell numbers mitotically, they need large amount of nutrients and a device to excrete unnecessary metabolites. The best environment among the maternal reproductive organs for the embryos to work these functions is uterus. By implanting to the uterus, embryos are capable of constructing a placenta, through which they can get nutrients and oxygen from maternal blood and give off their waste into the blood. 'Implantation', defined as the establishment of contact between the trophoblast of the differentiating blastocyst and the uterine tissues, is a complex sequence of events before embryos undergo placentation. Except swine, sheep, cattle and horses, in which no direct contact between trophoblast and endometrial stroma established, the interactions between embryos and uterus occur in two separable phases; adhesion (attachment) and invasion.

In preparation for the adhesion, embryos must hatch from the surrounding zona pellucida, or as seen in ferret and rabbit expanded blastocysts, trophoblastic processes protruded from the embryo penetrate the zona and adhere to the uterine luminal epithelium before shedding. While hatching can take place by either lysis of zona or escape of the blastocysts through a narrow opening in the zona, proteolytic activity is inevitably required for both mechanisms to work. In mice, trypsin-like enzyme (called strypsin) of blastocysts has been suggested to be responsible for the hatching activity (Perona and Wassarman, 1986; Yamazaki et al., 1994) whereas uterine enzymes are also thought to play a role in the event (Rosenfeld and Joshi, 1977; Gonzales and Bavister, 1995).

Apart from the embryonic acquisition for the adhesion, the uterus also needs a kind of differentiation to receive embryos. In human, the uterine receptivity, so called implantation window, is known to exist around cycle day 20 to 24 of an idealized cycle (Bergh and Navot, 1992). Before and after this period implantation does not occur probably due to an absence of the expression of receptive molecules.

Various molecules could possibly participate in the adhesive interaction between trophoblast and uterine luminal epithelium. Included are various integrins, trophinin, CD44, cad-11, the H type 1 and Lewis y oligosaccharides and heparan sulfate proteoglycan (perlecan). Both mammalian embryos and endometrial luminal epithelial cells express many different types of integrins on their cell surface. Adhesion involving the integrins can take place via the interaction of in-

tegrins on the embryonic surface with its receptors on the uterine epithelial cell surface or *vice versa*. Alternatively it can happen via a bifunctional bridging molecules that span between receptors on the embryonic and maternal cell surfaces (Aplin, 1997). Perlecan which binds to $\alpha1\beta1$ and $\alpha v\beta3$ integrins (Carson et al., 1993), thrombospondin (Corless et al., 1992) which binds to $\alpha v\beta3$ and laminin serving as a receptor for $\alpha1\beta1$, $\alpha3\beta1$, $\alpha6\beta1$, $\alpha6\beta4$ and $\alpha7\beta1$ (Carson et al., 1994), are present on the outer surface of mouse trophoblasts. CD44 is present in human blastocysts but absent from first trimester trophoblast (Campbell et al., 1995) and sulfated and sialylated oligosaccharides which can be recognized by CD44 are abundant on the endometrial apical epithelium (Hoadley et al., 1990; Hey and Aplin, 1996). CD44 is also expressed in endometrial epithelium and stromal cells both during the cycle and in pregnancy (Behzad et al., 1994). A complex of trophinin, a cell surface glycoprotein and tastin, a cytoplasmic linking protein, is one of candidates which, via homotypic interaction, mediate adhesion at the time of implantation (Fukuda et al., 1995). They are found in the cell surface of human secretory endometrial epithelium and macaque trophoblast and endometrium. Both blood group H type 1 glycan and Lewis y antigen may be involved in the adhesion as its oligosaccharide (Lindenberg et al., 1988) or antibody (Wang et al., 1998), respectively, inhibited embryo attachment to cultured epithelial monolayers or implantation in vivo. Perlecan present at the surface of mouse blastocysts (Carson et al., 1993) or uterine epithelium (Tang et al., 1987) can mediate embryo attachment by binding to a basic membrane component in a way of heterotypic interaction. Another possible interaction involving heparin-like glycans may happen between heparin-binding epidermal growth factor (HB-EGF) expressed at the uterine epithelial surface at the time of implantation and molecules on the surface of hatched mouse blastocysts (Raab et al., 1996). However, with the exception of $\alpha v\beta3$, no other gene has been identified whose aberrant expression is associated with or results in endometrial infertility (Lessy, 1997).

Following initial adhesion of blastocyst to the uterine luminal epithelium, interdigitation of the microvilli of the trophoblast and uterine epithelium is formed resulting in the firm attachment. After that, the trophoblast invade the uterine epithelium. In various species having invasive implantation, the mode of invasion varies and includes displacement type (mouse, rat), fusion type (rabbit, ruminants) and intrusion type (ferret, carnivoires)(Schlafke and Enders, 1975). Trophoblast bearing displacement type invasion produces a cytolytic activity that leads to mechanical disruption of uterine epithelial cells.

With the aid of that activity, trophoblast processes often intrude, or insinuate themselves between uterine cells. When trophoblast or its process meets uterine basement membrane and extracellular matrix components, again they need enzymatic activities such as the ones targeting these structures. In human, the highly invasive first-trimester trophoblast produces both MMP-9 and MMP-2 (Shimonovitz et al., 1994) and the cultured embryos also secrete MMP-2 (Puistola et al., 1989). However, MMP-2 mRNA was not detected in all cells of the implantation region in

mice, while MMP-9 was strongly expressed in the invading trophoblast (Canete-Soler et al., 1995; Reponen et al., 1995) and MMP-1, -3 and -11 mRNA was also localized to mouse blastocyst or trophoblast (Brenner et al., 1989). Of the tissue inhibitors of metalloproteinases (TIMP), TIMP-2 is expressed in human trophoblast cells of floating villi (Polette et al., 1994) and TIMP-3 in mouse fetal extravillous trophoblast invading maternal decidual tissues (Higuchi et al., 1995). Since TIMP-3 expression is up-regualted by progesterone (Higuchi et al., 1995) and TIMPs completely inhibit cytotrophoblast invasion in vitro (Graham and Lala, 1991; Behrendtsen et al., 1992), TIMP may regulate the invasion process. Recently the presence of another type of MMP, membrane type matrix metalloproteinase-1 (MT1-MMP), was found in mouse blastocysts, trophoblast cells of cultured blastocysts and ectoplacental cones (Tanaka et al., 1998) as well as in human first trimester invasive trophoblastic cells (Nawrocki et al., 1996). Since MT1-MMP possess the ability to cleave not only ECM molecules including collagen and others but pro-MMP-2 resulting in the activation of MMP-2 (Ohuchi et al., 1997), MT-MMPs are also suggested to be involved in the regulation of invasive process. Further studies of MT-MMPs might give access to the key to the regulatory mechanism of implantation.

In short, blastocysts and/or uterus secrete enzymes to dissolve zona pellucida before hatching. Hatched blastocysts adhere to the uterine luminal epithelium by interaction of cell surface molecules of both trophoblasts and uterine luminal epithelial cells. After adhesion trophoblasts invade the uterus by secreting proteolytic enzymes and/or expressing the enzymes on their cell surface while other factors will delimitate the invading margin. While many factors have been suggested to play a role in the events of implantation, further studies are needed to understand the precise molecular mechanism of implantation.

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