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Leptin receptors are down-regulated in uterine implantation sites compared to interimplantation sites

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Abstract

Leptin is a circulating hormone that plays an important role in the regulation of metabolism, obesity, and reproduction. Leptin binds to its receptors on the cell membrane and is involved in the activation of STAT3. Recently, endometrium was suggested to be a novel target for leptin recently. We, therefore, examined the expression of leptin, leptin receptors, and STAT3 in the mouse uterus (implantation and interimplantation sites) to investigate the role of the leptin system during the early implantation period. Leptin mRNA was not detected in mouse uterine tissues or blastocysts, although adipose tissue, the positive control, showed a strong signal. Both of the receptor splice variants were expressed in the uterus and blastocysts, but the mRNA level was much lower in implantation sites compared to interimplantation sites. The mRNA expression of leptin receptors was determined to be higher in stromal cells than in the luminal epithelium using laser capture microdissection (LCM) analysis. Using immunohistochemistry, leptin was detected as a strong signal in the luminal epithelium and embryo, whereas the receptor was detected in subepithelial stromal cells rather than the luminal epithelium. As leptin itself was not detected by RT-PCR, the immunohistologically detected leptin may originate elsewhere, such as in adipose tissue. The differential expression of leptin receptors in implantation sites compared to interimplantation sites suggests that the leptin/leptin receptor system may be a delicate regulator of the implantation process.

Keywords: Leptin; Leptin receptor; Long variant; STAT3; Implantation site; Interimplantation site; Laser capture microdissection

1. Introduction

Embryonic implantation is a crucial event in the establishment of pregnancy. Implantation starts when the blastocyst makes the first physiological contact with the maternal uterine luminal epithelium and involves a complex series of interactions between the uterus and blastocyst. Cytokines and paracrine molecules have been proposed as the putative local regulators of this process (Giudice, 1994). The uterus and blastocysts generate various factors during implantation, but it is likely that the molecular mechanism of the interaction involves many more factors that are not yet known.

Around day 4.5 to day 5.5 of pregnancy in mice, when apposition and attachment of the embryo to the uterus occurs, uterine tissues undergo dramatic changes along with cellular differentiation. These processes require the regulated expression of specific sets of genes (Nie et al., 2000; Reese et al., 2001; Yoon et al., 2004). Several factors, including epithelial integrins, trophinin, tastin, HB-EGF, LIF, and IL-1β, have been reported to have roles in implantation and uterine receptivity (Giudice, 1994). It has been suggested that leptin is an autocrine and paracrine regulator of the human implantation process; a comparison of the different potentials of arrested and competent blastocysts to secrete leptin during cross-talk between endometrial epithelial cells and preimplantation embryos implicated leptin in this process (Gonzalez et al., 2000).

Leptin, a 16 kDa helical cytokine, is a product of the obese gene (Zhang et al., 1994) and represents an interesting target for studying the molecular interactions between the immune, neuronal, digestive, and reproductive systems (Chehab et al., 1996, 1997; Conway and Jacobs, 1997). Leptin binding to

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target cells results in the formation of a receptor complex, leading to the cross-phosphorylation and activation of the JAKs (Zabeau et al., 2003). These activated JAKs then rapidly phosphorylate tyrosine residues in the cytosolic domain of the receptor, and such phosphorylated residues provide binding sites for signaling molecules, including members of the STAT family. Leptin and STAT3 proteins are immunolocalized in human and mouse oocytes, and preimplantation embryos (Antczak and Van Blerkom, 1997). In addition, leptin receptor mRNA and protein have been detected in mouse metaphase II stage oocytes (Matsuoka et al., 1999).

To date, six splice variants of the leptin receptor, OB-Ra-e (Chen et al., 1996; Lee et al., 1996) and muB219 (Cioffi et al., 1997) have been identified. Among these splice variants, only the long form, OB-Rb, is capable of mediating signal transduction through STAT3, while the short form receptors (called common receptors, OB-R) are not (Tartaglia et al., 1995). Although the endometrium has been suggested as a novel target for leptin (Alfer et al., 2000), the precise role of the leptin system in implantation is poorly understood. To better understand the role of the leptin system during early implantation, we compared the mRNA expression of leptin, leptin receptors, and STAT3 in implantation and interimplantation sites of mouse uterus on pregnancy day 4.5 and day 5.5. In addition, to examine the interaction between the endometrium and the blastocysts, we compared the expression patterns of the leptin system and STAT3 in blastocysts that developed in vivo and in vitro.

2. Materials and methods

2.1. Animals

All procedures described within were reviewed and approved by the Institutional Review Animal Care Committee. Adult virgin ICR female mice (6–8 weeks old) were mated with fertile males of the same strain to induce pregnancy. Implantation sites were visualized by injection of Chicago Blue dye solution (1% in saline, 0.1 ml per mouse; Sigma Chemical Co., St. Louis, MO) into the tail vein 5 min prior to sacrifice. The implantation sites appear as blue bands, and the regions between the blue bands are defined as interimplantation sites. Implantation and interimplantation sites from pregnant mice on day 4.5 and day 5.5 (day 0.5 = vaginal plug) were separated by sharp dissection; only uteri with uniformly distinct blue bands were included, and the regions with crowded embryos were discarded. Both implantation and interimplantation sites were immediately snap-frozen for RNA isolation or frozen with OCT compound for laser capture microdissection (LCM) analysis.

2.2. Laser capture microdissection

Since the uterus comprises several different cell types, we used LCM to examine the cellular distribution of leptin receptor mRNAs in the pregnant mouse uterus. Freshly frozen tissues were cut into 5 μm sections, defrosted, fixed in 70% ethanol for 30 s, stained with hematoxylin and eosin, and dehydrated in an alcohol series followed by xylene. Sections were air-dried and stored in a plastic container with desiccant. LCM was performed with a Pixcell II Laser Capture Microscope (Arcturus, Mountain View, CA). Sections were covered with LCM transfer film (CapSure LCM0201; Arcturus), and specific cells of interest were affixed to the capture film by brief laser pulses. Using a laser beam, intermingled luminal epithelium and subepithelial stromal cells were separated by melting the film, and target cells were removed from the surrounding area. Total RNA was then extracted from epithelial or stromal cells captured by the LCM transfer film using a PicoPure RNA Isolation Kit (Arcturus). Captured cells were incubated with 50 μl of extraction buffer at 42 °C for 30 min, mixed with 50 μl of 70% of ethanol, and pipetted into a Purification Column. After the Purification Column was centrifuged and washed, the total RNA was eluted for use in reverse transcription.

2.3. Reverse transcription—polymerase chain reaction

Total RNA was extracted with Trizol reagent (Gibco, Grand Island, NY) from pools of implantation or interimplantation sites of uterus on pregnancy day 4.5 and day 5.5 ($n = 10$ mice). Adipose tissue was used as a positive control for leptin, and kidney and placenta were used as positive controls for leptin receptors (Hoggard et al., 1997a, 1997b, 2000; Glasow et al., 1998). For LCM samples, cDNA was synthesized from 10 μl of total RNA isolated using a PicoPure Isolation Kit. For embryo samples, mRNA was isolated from equal numbers of blastocysts developed in vivo and in vitro using a Dynabeads mRNA DIRECT kit (Dynal, Oslo, Norway). Complementary DNA was synthesized using a SuperScript Preamplification system (Gibco) according to the manufacturer's protocol. Briefly, after incubation of total RNA with 0.5 μg oligo$(dT)_{12–18}$ primer at 70 °C for 10 min, the reaction was carried out in 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, and 10 mM DTT containing 0.5 mM dNTP mix in a final volume of 20 μl. The tubes were incubated at 42 °C for 5 min and 200 U of reverse transcriptase was added. Reverse transcription was conducted at 42 °C for 50 min, the reaction was terminated by incubation at 70 °C for 15 min, and the cDNA was then used as a template for PCR with the gene-specific primers listed in Table 1. The cDNA primers for the common leptin receptor sequence recognize the splice variants OB-Ra, OB-Rb, OB-Rc, OB-Rd, OB-Re, and muB219 (Hoggard et al., 1997b). PCR was carried out for 35 cycles in 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 25 pmol of each primer, and 2.5 U of *Taq* DNA polymerase (Promega, Madison, WI) in a final volume of 50 μl. The PCR products were analyzed by 1.5% agarose gel electrophoresis with ethidium bromide staining. The gel was scanned and densit-