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Expression of Leptin Receptor at Implantation Sites Compared to Interimplantation Sites in the Mouse Uterus

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INTRODUCTION

The process of embryo implantation involves complex interactions between the blastocyst and the uterus. It has been well established that for successful implantation, not only is the growth of the blastocyst critical, but a synchronous change in the uterus from a nonreceptive to receptive state must also occur (Nie et al., 2000).

Leptin, the *Obese* (*ob*) gene product, is synthesized and secreted by adipocytes (Zhang et al., 1994) and interacts with hypothalamic leptin receptors (OB-R). The leptin receptor gene has been shown to have at least six splice variants, OB-R (a-e) (Chen et al., 1996) and muB219 (Cioffi et al., 1996). The OB-Rb variant encodes a receptor with a long intracellular domain that is thought to be essential for intracellular signal transduction (Tartaglia et al., 1995). Leptin is involved in the stimulation of reproductive functions and that local expression of leptin and OB-R in the ovary, oocyte, embryo, and placenta plays a role in early development (Kitawaki et al., 2000).

In the mouse, the embryos are starting to attach to the endometrium on day 4.5 of pregnancy. At about this time, the uterus undergoes dramatic morphological changes along with cellular differentiation, processes requiring regulated expression of a specific set of genes. Uterine remodeling at this time is marked by an increase in vascular permeability at the implantation sites, and the injection of Chicago blue dye into the tail vein permits the identification of the implantation sites.

The overall aim of this study was to identify differentially regulated uterine leptin and leptin receptor varients at the implantation sites around the time of implantation compared to interimplantation sites in the mouse uterus.

MATERIALS & METHODS

Adult female ICR mice (6-8 weeks old) were mated, and a vaginal plug was designated as day 0 of pregnancy on the next morning. Uterine tissue was collected from nonpregnant mice and pregnant mice. For nonpregnant and 3.5 day pregnant mice, the entire uterus was collected. For 4.5 and 5.5 day pregnant mice, implantation sites were visualized by intravenous injections of a Chicago Blue dye solution (1% in saline, 0.1 mℓ/mouse) into the tail vein 5 min before killing the animals, and implantation sites were separated from interimplantation sites and both were collected.

Total RNA was extracted from tissues of each site tissues using TRIzol (Gibco BRL) and cDNA was synthesized from 2 μg of total RNA using 0.5 μg oligo(dT) primer following the protocol of SuperScript Preamplification system (Gibco BRL). The cDNA primers to the leptin gene (244-bp product) were used as described by Jin et al. (2000). The cDNA primers to the common leptin receptor sequence (which recognizes the splice variants Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re, and muB219; 473-bp product) and to the long form of the receptor, Ob-Rb (533-bp product) were used as described by Hoggard et al. (1997). The quantity of each gene product was estimated

by normalization to the amount of β-actin mRNA expression.

RESULTS & DISCUSSION

In the present study, we investigated the expression of leptin and its receptors in the mouse uterus to identify the role in implantation. Using RT-PCR, we identified the mRNA expression of leptin, the extracellular domain of the leptin receptor, Ob-R, and the long splice variant, Ob-Rb in the mouse uterus, adipose tissue, kidney, and placenta. There was no signal for leptin mRNA in the mouse uterine tissue, whereas white adipose tissue, serving as positive control, showed a strong signal. The receptor splice variants, OB-R (common form) and OB-Rb (long form), were stronly expressed in the kidney and placenta which were served as positive controls. The expression pattern of common and long form was similar in the uterine tissue. RT-PCR revealed no signal for leptin receptor mRNA in the nonpregnant uterus and weak signal in 3.5 day pregnant uterus. The mRNA level was much lower in implantation sites compared to interimplantation sites in both day 4.5 and 5.5 day of pregnancy. Results of the present study suggest that these genes for receptor are expressed during the attachment of embryos, but tend to be down-regulated in implantation sites compared to interimplantation sites.

SUMMARY

1. Leptin itself was not expressed in mouse uterine tissues.

2. Leptin receptors were not expressed in nonpregnant and llittle expressed in 3.5 day of pregnant uterine tissues. However, there was a signal in 4.5 and 5.5 day of tissues.

3. The expression level of leptin receptor variants in the implantation sites at around the time of initial embryo attachment (day 4.5 of pregnancy) and during the actual implantation period (day 5.5 of pregnancy) was much lower than that in the interimplantation sites.

4. Finding of the differential expression of leptin receptors in implantation sites compared to interimplantation sites suggests that leptin - leptin receptor system may be one of the delicate regulators in the molecular mechanism of the implantation process.

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