The Regulation of Development and Lactation of the Mammary Gland by Leptin

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Abstract: The development and lactation of mammary gland is a complex process regulated by various growth factors and one of which is leptin. Besides in circulation, in many species, leptin is also produced and secreted by the mammary fat pad and the mammary epithelial cells. Leptin can influence the branches of ducts and the proliferation of the mammary epithelial cell in pregnancy. In lactation, it influences the expression of milk protein gene and delays the involution after the removing of the pups. Leptin act all those function through JAK/STAT or JAK/MAPK pathway. However, it is not very clear about these pathways in mammary epithelial cell and it remains to be tested. [The Journal of American Science. 2005;1(1):63-67].

Key Words: lactation; leptin; mammary gland

1. Introduction

Leptin is mainly an adipocyte-secreted protein. It is involved in the regulation of food intake and energy balance (Zhang, 1994). Besides that it is also involved in normal sexual maturation and reproduction (Wauters, 2000). Recent studies have shown that leptin induces development of reproductive organs and allows fertility in both female (Chehab, 1996) and male (Mounzih, 1997) ob/ob mice and that serum leptin concentration rise during gestation in mice (Tomimatsu, 1997). In addition, the identification of leptin in human, rat, mouse, bovine, and porcine milk suggests that this hormone could also be involved in the physiology of the neonate. The presence of lepitn in milk opens the question of the mechanisms by which the mammary gland transfers leptin from the blood and/or synthesizes it. It is well known that mammary gland development and function are controlled by hormones acting in synergy with growth factors produced by the mammary epithelium itself or by the adjacent stroma, which consists of a matrix of adipose and fibrous connective tissue called the "mammary fat pad". The mammary fat pad is considered as an inert matrix, but more recent evidence emphasizes the influence of adipocyte epithelial interactions as being critical for mammary gland physiology (Zangani, 1999). Locally produced growth factors can mediate the adipocyte-epithelial interaction and can alter the activity of several steroid and peptide hormones at this level (Hovey, 1999; Baratta, 2000). Thus, leptin may exert autocrine and paracrine actions, as well as a general influence, when secreted by the adipose tissue of the mammary gland. The objective of this review is to show the function of leptin to the development and lactation of mammary gland, as well as its signaling transduction pathway in the mammary gland.

2. The expression and localization of leptin in mammary gland

Earlier in 1997, Houseknecht et al found that human leptin was produced in mammary gland (Houseknecht, 1997). To examine whether mouse leptin was also expressed in mammary gland, Aoki et al excised the mammary gland from virgin, pregnant, lactating, and post-lactating female mice. RT-PCR and Southern blotting revealed that in the mammary gland of virgin mice, the expression of leptin was detected obviously. On day 16 of gestation, the expression of leptin was decreased to about 50% of the virgin level and kept significantly lower during the lactation period. Eight days after weaning on the pups, when the mammary gland was involuting, the expression of leptin was up-regulated and restored to the level of virgin mice (Aoki, 1999). During the lactation period, more than 20 ng/ml immunoreactive leptin was detected in whole colostrums which concentration was much higher than that in serum on the same day. Furthermore, leptin concentration in whole milk was significantly higher than that in skim milk at all days examined, suggesting that leptin was present in breast

milk as a part of milk fat, associated with milk fat globule membrane, as reported for human milk leptin (Smith-Kirwin, 1998). As expected, the mammary fat pad is the main position to produce and secrete leptin. Except that, leptin is also produced and secreted by mammary epithelial cells. The mouse mammary epithelial cell line, COMMA-1D, which is maintaining expression and secretion of major milk proteins, produced leptin when were cultured with lactogenic hormones. When the cells are treated with prolactin, the expression of leptin decreased to 70% as compared with untreated cells. A similar reduction in leptin expression was observed, though it was not statistically significant, when the cells were treated with hydrocortisone. When the cells were treated with both hydrocortisone and prolactin, the leptin expression decreased to about 55%. These results clearly indicated that leptin was expressed by mammary epithelial cells and that the expression was suppressed by the lactogenic hormones (Aoki, 1999). In another mouse mammary gland cell line, HC11, leptin and its long-form receptor mRNA was expressed during both proliferation and differentiation phases. Protein determination showed that leptin was secreted by these mouse mammary epithelial cells (Baratta, 2003).

In 2002, Bonnet et al studied the expression of leptin in mammary gland of ovine. They found that leptin mRNA level varied significantly depending on the pregnancy or lactation stage. During pregnancy, the leptin mRNA level decreased strongly between day 80 and day 112, before increasing slightly at day 141 to levels similar to those assayed at day 15 and 80. Throughout lactation, leptin mRNA levels did not vary significantly but were lower than the level assay at day 80 of pregnancy. Moreover, at day 3 of lactation, the level of leptin mRNA was significantly lower than those assayed at day 15, 80, or 142 of pregnancy (Bonnet, 2002).

Bonnet et al also studied the localization of leptin. Immunofluorescence performed with the anti-leptin antiserum showed a labeling located in adipose, epithelial, or myoepithelial cells depending on the pregnancy or lactation stage. During early pregnancy, leptin immunostaining was located in adipocytes, mainly in their cytoplasm. At the end of pregnancy, fluorescent labeling was detected on the apical membrane of the epithelial cells. Just after parturition, leptin labeling was observed as a continuous fringe surrounding the acini and as a discontinuous fringe until day 70 of lactation. Just after parturition, the leptin labeling was colocalized with the F-actin labeling, indicating that leptin protein was mainly located in myoepithelial cells (Bonnet, 2002).

Ovine mammary gland also expresses the long and short forms of the leptin receptor mRNA in each stage of pregnancy and lactation. Expression of ovine leptin receptors were greater during mid-pregnancy than at any other time that tissues were sampled and the short form of the ovine leptin receptor was predominantly expressed. These observations were consistent with the reports that the long form of the leptin receptor was expressed at low levels in peripheral tissues and at high levels in the brain. *In situ* hybridization analysis provided a confirmation of the temporal variation in leptin receptor mRNA expression throughout pregnancy and lactation. Receptor mRNA expression was greatest at day 70 of pregnancy, decreased to day 112 of pregnancy and remained detectable during lactation (Laud, 1999).

In 2004, using RT-PCR, Sayed-Ahmed et al demonstrated that leptin and its short and long form receptor isoforms were expressed both in the dry and the lactating mammary gland tissue. Using *in situ* hybridization, the morphological data suggested that the epithelial leptin mRNA expression of the lactating gland was higher than that of the dry gland. To compare the leptin mRNA levels between dry and lactating udders competitive PCR was used, which showed no difference in leptin expression for the whole mammary tissues. The lack of difference in total leptin mRNA levels was explained by the high adipose tissue content of the dry mammary gland. Leptin and its receptor transcripts were expressed mainly in the epithelial cells of lactating cows, while in dry mammary tissue the signal was found in the stromal tissues as well. The results provided additional evidence that locally produced leptin took part in the regulation and maintenance of mammary epithelial cell activity (Sayed-Ahmed, 2004).

3. The effect of leptin in mammary gland

It is crucial that leptin transcripts are detected in the adipose tissue of the mammary gland because the stromal-epithelial interactions are fundamental for mammary gland growth and development. Each stage of mammary glandular development, from ductal branching through terminal end bud development,

alveolar formation and milk production, is dependent on the presence of the mammary fat pad. Leptin might be a local growth factor, which acts as a functional link between adipocytes and epithelial cells of the mammary gland. By acting as an autocrine or paracrine factor in the epithelial cell, leptin may play an important role in the modulation of the mammary gland development, lactation or involution.

Leptin can influence the development and lactation of mammary gland. Repeated administrations of the recombinant human ob protein, leptin, to homozygous ob/ob female mice have been reported to correct their sterility, exiting in ovulation, pregnancy and parturition. Furthermore, ob/ob mothers were effective in feeding newborns as a result of the increased milk production induced by a continuous leptin treatment (Chehab, 1996). In leptin-deficient and leptin receptor-deficient mice, there was a notable lack of mammary duct formation, compared with the control (Hu, 2002).

Like other cytokines, leptin is effective in influencing the growth or apoptosis of various cell types (Fantuzzi, 2000). Only higher concentration is able to inhibit HC11 cell growth. On the other hand, leptin can also influence the expression of β-casein gene. When HC11 cells are in the terminally differentiated state, leptin is effective in influencing the induction of β-casein gene promoter. Usually, these cells are able to produce milk protein after the synergistic action of prolactin and glucocorticoids, in the presence of insulin. The effect of leptin on the induction of the β-casein gene promoter appears to be potentiated when prolactin is present, but leptin is also able to exert a positive influence on gene expression in the absence of this hormone (Baratta, 2003).

In bovine mammary epithelial cell line, MAC-T, DNA synthesis increases linearly with increasing concentrations of IGF-I in the media. But addition of recombinant ovine leptin to media containing 5 ng/ml of IGF-I decreases DNA synthesis linearly within the range of doses tested. The minimal dose of ovine leptin that decreases DNA synthesis is 64 ng/ml (20% decrease). With 16 ng/ml of ovine leptin there is already a tendency for reduced DNA synthesis. The highest dose of ovine leptin decreases IGF-I-stimulated DNA synthesis by 30%. Without the addition of IGF-I, ovine leptin at 100 ng/ml has no effect on the basal rate of DNA synthesis (Silva, 2002). This result is in agreement with other models where leptin also inhibits proliferation of a mouse pituitary cell line and antagonizes IGF-I-augmentation of steroidogenesis in ovarian cells (Agarwal, 1999).

4. The signaling transduction pathway of leptin in mammary gland

It has been demonstrated that the leptin long form receptor mRNA is expressed in mammary epithelial cells in different species (i.e. humans, rodents, ovine and bovine). This long form isoform is commonly believed to be functional while the shorter cytoplasmic domain isoform, mainly expressed in peripheral tissues (Laud, 1999), is generally considered ineffective in exerting a functional activity, in particular in activating the STAT pathway (Fantuzzi, 2000). The presence of the long form receptor in mammary gland suggests that leptin could act directly through receptor activation to inhibit proliferation and reinforces the evidence for a regulatory role of leptin in parenchymal growth (Silva, 2002). The leptin receptor belongs to the class I cytokine receptor family and is strongly related to the gp130 signal-transducing component of the interleukin-6 (IL-6) receptor (Baumann, 1996), to the granulocyte colony-stimulating factor receptor and to the leukemia inhibitory factor receptor (Sone, 2001). So they have very similar signaling capabilities.

IL-6 inhibits both human (Chiu, 1996) and bovine mammary epithelial cell proliferation (Okada, 1999). IL-6 at 50 ng/ml decreases mammary cell proliferation 30%. It is possible that cytokines, leptin, and IL-6 act through similar post-receptor pathways to inhibit proliferation of mammary epithelial cells. One possible pathway for leptin-induced inhibition of mammary epithelial cell proliferation is activation of signal transducers and activators of transcription (STAT). It has been demonstrated that in mouse myeloid progenitor cells, the stimulation of the long form receptor resulted in the activation of the associated JAK2 tyrosine kinase and the subsequent regulation of the downstream -dependent signals, in particular STAT (Banks, 2000). Maybe in mammary epithelial cells, the long form receptor is activated and transduces the downstream signals in the same way. Phosphorylated STATs can dimerize and migrate to the nucleus and function as transcription factors (Bjorbaek, 1999). Activation of STAT3 can induce G_0 growth arrest in mouse mammary epithelial cells (Hutt,

2000). At the time of mammary involution, STAT3 is strongly activated. In some cell types, activated STAT3 induces the expression of cytokine signaling, SOCS-3, which in turn down-regulates the JAK/STAT pathway (Banks, 2000). Furthermore, tissues continuously exposed to leptin have been reported to accumulate excessive amounts of SOCS-3 (Emilsson, 1999). The important of the role of this factor on cellular inhibitory feedback in mammary gland development and involution after lactation has been recently suggested, particularly in mice after pup withdrawal, when the mammary gland is rendered unresponsive to PRL by increased levels of SOCS-3 (Tam, 2001). So it is possible that the negative effect on mammary cell proliferation is mediated through an enhancement of SOCS-3 protein.

STAT5 can regulate the milk protein synthesis. The milk protein genes are target genes of STAT5. At least one STAT5-binding site is found in the promoters of the milk protein genes, β-casein, αs1-casein, whey acidic protein, and β-lactoglobulin. In HC11, which was transfected with BLG/STAT5 variants, higher levels of β-casein were detected. STAT5 also plays an essential role in the development and differentiation of mammary epithelial cells and decreases proliferation rate. In pregnancy, the lobuloalveolar structures in line BLG/STAT5 were expanded more fat pad compared to that in the mammary gland of the wild-type mice. The maturation of fully functional secretory alveoli in BLG/STAT5 mice occurred earlier than in wild-type mice. After removal pups from the mother mice, the substantial regression of the alveoli was detected in three days. In contrast, the compaction of the alveoli and the loss of epithelial cells were not found in tissue of BLG/STAT5 mice in which the involution process was markedly delayed. To confirm the higher proliferation rate of mammary cells in STAT5 mice, explants were prepared from the glands of wild-type and transgenic mice at day 14 of pregnancy and labeled *in vitro* with [^{3}H]thymidine for 90 min. A 2-fold higher incorporation of labeled thymidine was found in explants of BLG/STAT5 mice when compared to wild-type mice (Iavnilovitch, 2002).

In other cell types, leptin can also activate JAK/MAPK pathway. It activates Ras/Raf, MAPK/ERK. The MAPK/ERK migrates into the nucleus and activates c-fos/c-Jun/Brf or Egr-1. It has been demonstrated that c-fos/c-Jun/Brf or Egr-1 can influence the branches of duct of mammary gland. So it is perhaps that leptin influences the development of duct through this pathway. However, this hypothesis remains to be tested.

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