Note

Induction of Osteopontin Gene Expression during Mammary Gland Involution and Effects of Glucocorticoid on its Expression in Mammary Epithelial Cells

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To understand the molecular mechanisms of mammary gland involution, an involution-induced clone was identified from a cDNA library of mouse mammary gland by differential screening. Characterization of a clone by sequencing and northern analysis showed that expression of the osteopontin gene was induced during involution of mouse mammary gland. But induction of the osteopontin gene was not observed in apoptotic HC11 mammary epithelial cells under serum starvation. In HC11 cells, dexamethasone treatment from the seeding stage showed five-fold induction of osteopontin gene expression, but the expression was not changed when dexamethasone was added to confluent cells.

Key words: osteopontin gene; involution; mouse mammary gland; dexamethasone

The mammary gland is a unique tissue with a developmental potential after birth, since following the onset of pregnancy, mammary epithelial cells proliferate and differentiate into milk-secreting cells during lactation. After completion of lactation, the mammary gland undergoes involution, regressing to a state resembling that of a virgin animal, and the gland prepares for another round of pregnancy and lactation.1) The involution phase of mammary gland is characterized by dramatic epithelial cell death by apoptosis and tissue remodeling.2,3) Previously, we used differential hybridization of mRNA from mammary gland of mice in an attempt to find genes expressed differentially during such involution, and reported the induction of several genes: those that code for sulfated glycoprotein-2, WDNM1, lactoferrin, and ferritin heavy chain.4,5) In this paper, we found that the osteopontin gene was induced during involution of mouse mammary gland by further differential screening. Dexamethasone treatment increased expression of the osteopontin gene at the growing stage, but not in the confluent or the apoptotic mammary epithelial HC11 cells.

To find involution-induced genes, we used an involution-specific cDNA library of mouse mammary gland constructed earlier,4) identifying clones by differential screening as described there. A partial sequence of a clone showed 100% identity to the cDNA sequence of osteopontin gene.6)

Northern analysis was used to examine expression of the osteopontin gene at various reproductive stages of mouse mammary gland. The care and treatment of the experimental animals conformed with The Chonnam National University guidelines for the ethical treatment of laboratory animals. Tissues obtained from ICR mice were used in all experiments. For the induction of involution, the young were removed 10 days after parturition, and the mammary tissues were obtained at the indicated time after weaning. Total RNA was extracted by the acid guanidinium phenol-chloroform method from the tissues.7) The membrane was hybridized with a labeled insert of the cDNA clone. Northern analysis produced a 1.6-kb osteopontin transcript and expression of the osteopontin gene was very low or not detected in virgin, pregnant, or lactating mammary gland, but it was induced at involution day 2 and peaked at involution day 4 (Fig. 1). In tissue-specific northern analysis, the osteopontin gene was highly expressed in the involuted mammary tissues, medium levels were detected in kidney tissues, but the expression was barely detectable or not detected in other tissues such as thymus, spleen, uterus, ovary, liver, brain, heart, lung, and testis (Fig. 1). Osteopontin mRNA was also abundant in rat kidney, while it was very low or not detected in spleen, liver, brain, heart, lung, and testis of rat tissues.8) The highest levels of osteopontin mRNA in the involuted mammary gland suggest that osteopontin has an important function in the process of mammary gland involution.

A combination of high density (confluency) and...
serum starvation has induced apoptosis of HC11 mouse mammary epithelial cells and DNA fragmentation was observed under these conditions. To examine expression of the osteopontin gene in apoptotic conditions, HC11 cells, derived from the BALB/c mouse mammary epithelial cell line COMMA-1D, were cultured in RPMI1640 medium containing 10% heat-inactivated fetal bovine serum, insulin, EGF, and 1% gentamycin as described. Medium was changed every 2 days. For serum starvation, confluent cells were cultured for 2 days in serum-free medium without insulin and EGF. We confirmed DNA fragmentation in these conditions, but we could not find induction of the osteopontin gene during serum starvation (data not shown).

There are two phases of mammary involution. The initial involution events occurred 1 to 3 days after weaning and were characterized by induction of the apoptosis-associated genes and apoptosis of fully differentiated mammary epithelial cells without visible degradation of the extracellular matrix. The second phase begins at day 4 of involution in BALB/c mice and is characterized by upregulation of mRNA and activity for proteolytic enzymes, including gelatinase A, stromelysin-1, and uPA, resulting in active tissue remodeling, including destruction of basement membranes and alveolar structures and irreversible loss of the differentiated function of the mammary gland. It has been suggested that hydrocortisone abolished membrane-type matrix metalloproteinase mRNA and it has a specific action in inhibiting the remodeling phase. Dex induces expression of the osteopontin gene in cardiac myocytes and rat bone marrow stromal cells, while Dex decreases expression of the osteopontin gene in osteoblastic osteosarcoma cells. We examined the Dex effect on the expression of the osteopontin gene in HC11 cells. Induction of the osteopontin gene was cell-density dependent; Dex treatment from the seeding stage showed five-fold induction of osteopontin gene expression (Fig. 2). But Dex treatment of confluent cells did not affect expression of the osteopontin gene. We also could not find induction of osteopontin gene in serum-starved apoptotic cells (data}

Fig. 1. Expression of Osteopontin Gene in Mouse Tissues.

Top panel. The 20 μg of total RNA isolated at virgin (V), pregnant day 10 (P), lactating day 10 (L), and involution 0.5, 1, 2, 3, 4, and 7 days of ICR mouse mammary gland were separated on a 1% formaldehyde/agarose gel, and transferred onto the membrane by capillary reaction. The blot was hybridized with the [32P] labeled cDNA clone. The cDNA containing a cDNA insert was converted into the pBluescript plasmid by a Lambda ZAP II Automatic Excision Process (Stratagene, La Jolla, CA). The plasmid was digested with EcoRI and XhoI, the digest was separated by electrophoresis on a low melting agarose gel, and the insert was obtained. The insert of cDNA clone was labeled with a Prime-It Random Primer Labeling Kit (Stratagene). That equal amounts of RNA were present in each lane was checked by the intensities of 28S and 18S bands as shown, and the efficiency of transfer was monitored by ethidium bromide staining. Bottom panel. The 20 μg of total RNA isolated from thymus (1), spleen (2), uterus (3), ovary (4), liver (5), brain (6), heart (7), lung (8), kidney (9), mammary gland at involution days 2 (10) of the female mouse and testis (11) and mammary gland (12) of male mouse were analyzed by the northern method.

Fig. 2. Effects of Dexamethasone Treatment on the Expression of Osteopontin Gene in HC11 Mammary Epithelial Cells.

A. Cells were seeded and cultured in RPMI1640 medium containing 10 ng/ml EGF, 5 μg/ml insulin, and 10% fetal bovine serum. The 0.1 μM of dexamethasone (Dex) was added at either seeding or confluent stage and cells were cultured for 2 days. The 20 μg of total RNA prepared from each treatment were analyzed by the northern method. B. The mRNA levels were measured by scanning densitometry and relative values were normalized to 1 for the mRNA levels of cells cultured with no Dex from seeding stage. Bars indicate SEM (n = 3).
not shown).

Osteopontin is a secreted phosphoprotein that was originally isolated from bone.\textsuperscript{19} Osteopontin supports cell adhesion through its arginine-glycine-aspartic acid (RGD) integrin recognition motif and integrin $\alpha_\beta_1$ is the established receptor for osteopontin.\textsuperscript{16,17} Several studies have suggested that osteopontin has an important function in development and differentiation of mammary gland. Targeted inhibition of osteopontin expression by antisense RNA in the mammary gland caused abnormal morphogenesis and lactation deficiency and they have suggested that osteopontin has an essential role of osteopontin in mammary gland development and differentiation.\textsuperscript{18} Our results indicate that expression of osteopontin was induced during involution of mammary gland but the expression was not induced in apoptotic HC11 mammary epithelial cells under serum starvation. These suggest that the function of osteopontin may not be related to apoptosis of mammary epithelial cells. Osteopontin may help to protect specific groups of epithelial cells from the widespread apoptosis going on in the involuting mammary gland rather than that related to apoptosis of epithelial cells as suggested by Ritting and Novick.\textsuperscript{19} After weaning of the pups, the mammary gland involutes and proceeds through a rapid remodeling process to resembling that of the mature virgin gland and the gland prepares another round of pregnancy and lactation. Induction of osteopontin expression during involution may promote cell adhesion for the specific groups of epithelial cells, which remain for the proliferation during the next round of reproduction upon pregnancy.

Glucocorticoid hormones are essential for lobular and alveolar development during pregnancy and milk protein synthesis during lactation. During involution, glucocorticoids and other systemic lactogenic hormone levels drop and the gland is dismantled.\textsuperscript{20} Our study has demonstrated that Dex increases expression of the osteopontin gene at the growing stage, but not at the confluent and the serum-starved apoptotic mammary epithelial cells. Dex-induced osteopontin gene expression observed at the growing stage may function to promote cell adhesion during proliferation of mammary epithelial cells.

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References


