imc-415 Gene Expression in the Proliferation and Cell Death Phases of Mammary Epithelial Cells

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ABSTRACT : We examined expression patterns of imc-415 gene in mammary gland and in HC11 mammary epithelial cells in culture. mRNA levels of imc-415 gene were higher at pregnancy and involution stages of mouse mammary gland compared with lactation period. Expression of imc-415 gene was induced with serum starvation or treatment with Fas monoclonal antibody in HC11 mammary epithelial cells in culture. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 9 : 1201-1204*)

Key Words : Suppression Subtractive Hybridization, imc-415, Mammary Involution, Programmed Cell Death, Serum Starvation, Fas Monoclonal Antibody

INTRODUCTION

The mammary gland is an excellent system for investigating the factors involved in epithelial growth, differentiation, morphogenesis, and involution. The female mammary gland undergoes repeated modifications in response to hormonal and mechanical stimuli (Rillema, 1994; Topper and Freeman, 1980). During the involution, the mammary gland of mouse and several other species is characterized by extensive tissue degeneration and loss of the majority of luminal epithelial cells by programmed cell death (PCD) or apoptosis (Strange et al., 1992; Walker et al., 1989). Involution of mammary gland undergoes through two distinct stages. The first stage is reversible and controlled by local mammary derived signals associated with milk accumulation (Marti et al., 1997), and the second stage is characterized by the complete loss of survival factors due to decreased levels of systemic lactogenic hormones and activation of proteinase-dependent pathway (Lund et al., 1996).

Previously, we carried out suppression subtractive hybridization (Diatechenko et al., 1996) and identified several clones induced during the involution stage. By the analysis of nucleotide sequences, a clone was identified as one encoding inducible murine mast cell (imc)-415 gene (Cho et al., 1998). In this study, we examined expression patterns of imc-415 gene in mammary gland at various reproductive stages and in HC11 mammary epithelial cells under serum starved- and Fas induced-apoptotic conditions.

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Received December 8, 1999; Accepted March 2, 2000

MATERIALS AND METHODS

Samples and isolation of total RNA

Mouse mammary gland samples were prepared from the ICR mouse at day 18 of pregnancy, day 7 of lactation and day 1 to 4 of involution after weaning. Involution was induced by removing the pups from the mother at day 7 after parturition. Mammary gland was aseptically removed, immediately frozen in liquid nitrogen, and then stored at -70℃ until RNA isolation. Total RNA was isolated by the guanidine isothiocyanate method (Chomczynski and Sacchi, 1987).

Cell culture and isolation of total RNA

HC11 cells (Ball et al., 1988) were cultured in a growth medium containing RPMI1640 (Gibco BRL), 50 μg/ml gentamicine (Sigma), 10% fetal bovine serum (FBS, Gibco BRL), 5 μg/ml insulin and 10 ng/ml EGF (Sigma). Confluent cells were cultured in a differentiation medium containing RPMI1640, 50 μg/ml gentamicine, 10% fetal bovine serum, 5 μg/ml insulin, 5 μg/ml prolactin, and 0.1 μM dexamethasone. Fas monoclonal antibody treatment was performed: HC11 cells were incubated in the differentiation media containing 100 ng/ml mouse Fas monoclonal antibody (Clontech) after 10 days of the differentiation. Medium was changed every 2 days. Total RNA was isolated with Trizol (GibcoBRL) as described by the manual.

Northern blot analysis

Twenty μg of total RNA isolated from mouse and 5 μg of total RNA from HC11 cells were denatured by adding 50% formamide, 6.2% formaldehyde, 20 mM MOPS and incubating at 70℃ for 5 min. Electrophoresis was carried out at a 5 V/cm of gel length. After transferring to a nylon membrane by

capillary transfer method, RNA was cross-linked by using cross-linker (UVP). cDNA was ^{32}P-labeled as described by Laddrman labelling kit manual (Takara). Labeled cDNAs were purified with Qiagen oligo-nucleotide removal kit by manufacture's protocol.

The nylon membrane was immersed in 2× SSC/0.1% SDS. Hybridization bottle was filled with 2 ×SSC/0.1% SDS and the nylon membrane was placed into the hybridization bottle. 2×SSC/0.1% SDS solution in the bottle was discarded and formamide pre-hybridization solution was added. After incubating at 42℃ for 2 h, pre-hybridization solution was discarded and hybridization solution and ^{32}P-labeled imc-415 or G3PDH probes were added. Membrane was subjected to a hybridization at 42℃ overnight and then washed with 2×SSC/0.1% SDS and 1×SSC/ 0.1% SDS at 60℃ for 20 min. Membrane was briefly air-dried and exposed to a X-ray film for 3 days.

RESULTS AND DISCUSSION

The imc-415 protein is highly conserved among the species and had a 97.5% amino acid sequence homology to a human β4 integrin-binding protein (p27^{BBP})/a human translation initiation factor 6 (eIF6). Also, it is an essential protein for cell maintenance.

In this study, we examined the expression of imc-415 gene in mammary gland at various reproductive stages by northern blot analysis. mRNA levels of imc-415 gene were higher at involuted tissues compared with lactating tissues (figure 1). However, the induction of mRNA expression by involution was attenuated after day 3 of involution (figure 1). High levels of imc-415 mRNA were also observed at pregnant tissues (figure 1). Biffo et al. (1997) reported that mRNA level of p27^{BBP} in epithelial cells was higher in subconfluent growing cells than in confluent cells and the mRNA was highly expressed in proliferating epithelia, such as in the embryonic skin. Our results also showed that imc-415 gene is highly expressed in the proliferation stage (during pregnancy) of the mammary gland.

We examined expression patterns of imc-415 gene in HC11 mammary epithelial cells in culture at growing and differentiation stages by northern blot analysis. Cells were cultured to the confluency in the growth medium and then cells were cultured in the differentiation medium. The imc-415 gene was highly expressed at the growing stage and in the cells cultured in the differentiation medium for 4 days, and

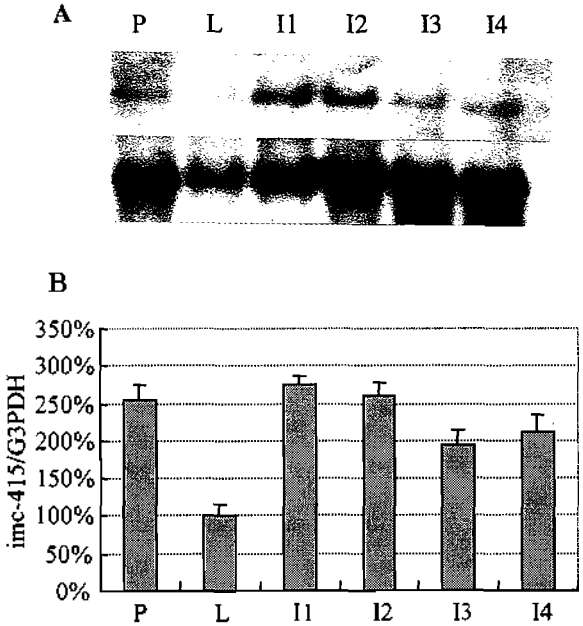

Figure 1. Expression of imc-415 mRNA in mouse mammary gland. Twenty μg of total RNA isolated from mouse mammary gland at pregnancy day 18 (P), lactation days 7 (L), involution day 1 (I1), days 2 (I2), days 3 (I3), and days 4 (I4) were loaded. (B) The relative levels of imc-415 mRNA were normalized by the level of G3PDH mRNA. mRNA level at lactation stage was expressed as 100% and bar indicates standard deviation of three experiments.

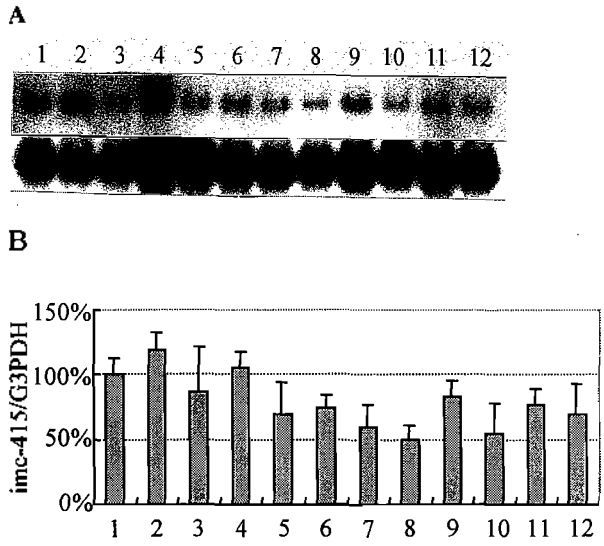

Figure 2. Expression of imc-415 mRNA in HC11 cells cultured in the growth medium and the differentiation medium. (A) Five μg of total RNA from the cells at growing stage (lane 1), day 0 (lane 2), day 2 (lane 3), day 4 (lane 4), day 6 (lane 5), day 8 (lane 6), day 10 (lane 7), day 12 (lane 8), day 14 (lane 9), day 16 (lane 10), day 18 (lane 11), and day 20 (lane 12) of differentiation were loaded. (B) The relative levels of imc-415 mRNA were normalized by the level of G3PDH mRNA. mRNA level at lane 1 was expressed as 100% and bar indicates standard deviation of three experiments.

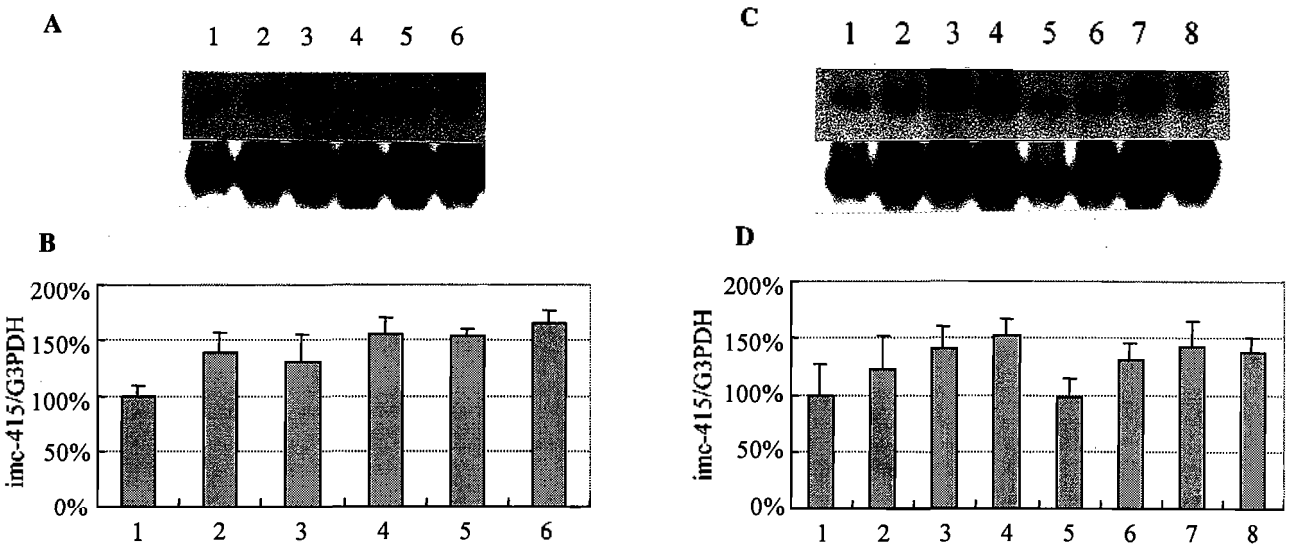

Figure 3. Expression of imc-415 mRNA in HC11 cells under serum starvation or mouse Fas monoclonal antibody treatment. (A) Five μg of total RNA isolated from serum starvation-0 h (lane 1), -1/2 h (lane 2), -1 h (lane 3), -2 h (lane 4), -4 h (lane 5), and 8 h (lane 6) were loaded. (C) Total RNA isolated after Fas antibody treatment at 0 h (lane 1), 1/2 h (lane 2), 1 h (lane 3), 2 h (lane 4), 4 h (lane 5), 8 h (lane 6), 16 h (lane 7), and 24 h (lane 8) were loaded. The relative imc-415 mRNA levels were normalized by the level of G3PDH mRNA in figure 3B and D. mRNA level at lane 1 was expressed as 100% and bar indicates standard deviation of three experiments.

mRNA levels were gradually decreased in the cells grown in the differentiation medium over 6 days (figure 2).

In this study, high mRNA levels were also observed in the involution stage of the mammary gland. In the involuting mammary gland, an elimination of a majority of secretory epithelial cells is regulated by PCD which is characterized by the requirement for new gene expression. HC11 mammary epithelial cells undergo PCD when deprived of serum at high cell density but not at low density (Merio et al., 1997). Fas is a well-characterized involution specific gene and known to function as external cell death signal (Baik et al., 1995; Keane et al., 1996). We examined if imc-415 mRNA is induced under serum starvation and after treatment with mouse Fas monoclonal antibody. Cells were cultured under serum starvation containing only RPMI1640 medium or treatment with mouse Fas monoclonal antibody after 10 days of differentiation. Both serum starvation and treatment with mouse Fas monoclonal antibody treatments increased expression of imc-415 mRNA (figure 3).

These results suggest that imc-415 may have a function in the mammary gland involution such as in the up-regulation of protein synthesis related to mammary gland involution and remodeling.

In summary, we demonstrated that imc-415 gene is highly expressed in mammary tissues and mammary epithelial cells both during proliferation stage (at pregnant tissues and growing HC11 cells) and cell death stage (at involuted tissues and serum starved- and Fas antibody treated-apoptotic cells). The imc-415 mRNA has been induced in inflamed lung tissues (Cho et al., 1998) and imc-415 protein binds to 60S ribosomal subunit after termination of translation and binds to cytodomain part of β4-integrin (Biffo et al., 1997; Si et al., 1997). These findings suggest that imc-415 may play several roles in the proliferation and cell death processes of the mammary gland. Therefore the exact role of imc-415 in mammary gland should be investigated.

ACKNOWLEDGEMENT

This work was supported by a grant from Ministry of Agriculture, Korea.

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