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[Short Communication]

Cell-Cell Contact Down-Regulates Expression of Membrane Type Metalloproteinase-1 (MT1-MMP) in a Mouse Mammary Gland Epithelial Cell Line

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ABSTRACT—Membrane type matrix metalloproteinase (MT-MMP), which possesses a C-terminal transmembrane domain, is expressed on the cell membrane (Sato *et al.*, 1994, *Nature* 370: 61-65). It was suspected, therefore, that the expression of MT-MMPs might be regulated by cell-cell interactions. We examined the patterns of MT1-MMP expression in a mouse mammary gland epithelial cell line, HC11, which is capable of responding to prolactin *in vitro*. HC11 cells form well-differentiated monolayer of cuboidal epithelium at confluence. During the log growth phase, cells which are well dispersed and seemingly migrating actively, or located at the periphery of small colonies, reacted strongly with an anti-MT1-MMP antibody, whereas no MT1-MMP immunoreactivity was detected in the cells which established cell-cell contact with adjacent cells. At confluence, the HC11 cells lost MT1-MMP immunoreactivity completely. Northern blot analysis revealed that MT1-MMP mRNA is present at a high level in HC11 cells during the log phase of growth. Although MT1-MMP immunoreactivity disappeared by the 1st day confluence was reached, the decline of MT1-MMP mRNA levels started only a few days later. The discrepancy in the timing of decrease of MT1-MMP protein and that of the transcripts suggests the presence of translational control mechanisms for MT1-MMP expression during cell-cell interaction.

INTRODUCTION

Membrane type matrix metalloproteinase (MT-MMP) recently discovered by Sato *et al.* (1994), is a unique new member of matrix metalloproteinase (MMP) family and expressed on the cell membrane. MT-MMP possesses a C-terminal transmembrane domain which is essential for anchoring MT-MMP to the cell membrane and for the expression of its enzymatic activities (Sato *et al.*, 1994; Cao *et al.*, 1995). More recently, it was found that there are at least 3 species of MT-MMP (Takino *et al.*, 1995; Will and Hinzmann, 1995). Hence, the originally discovered MT-MMP is now

designated MT1-MMP (previously, MT-MMP-1) (Takino *et al.*, 1995; Will and Hinzmann, 1995). Because of its localization on the cell membrane, we wondered if the expression of MT-MMPs might be regulated by cell-cell interactions. We examined, therefore, the patterns of MT1-MMP expression in the cultured mouse mammary gland epithelial cell line, HC11, during the log phase of growth and at confluence.

HC11 is a prolactin-sensitive epithelial cell line, originally derived from a mammary gland of pregnant BALB/c mouse (Ball *et al.*, 1988). HC11 cells can be induced, when cultured *in vitro* on a collagen substratum, to express genes coding for proteins specific to the mammary gland, i.e., β -casein (Ball *et al.*, 1988) and whey acidic protein (WAP) (Kawamata *et al.*, 1995) in response to prolactin. HC11 cells were chosen, therefore, as an *in vitro* model of functional epithelial cells.

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Our results strongly suggest that the expression of MT1-MMP in epithelial cells is down-regulated by cell-cell contact.

MATERIALS AND METHODS

Culture of HC11 cells

HC11 cells (kind gift from Prof. R. K. Ball, Friedrich Miescher Institute, Switzerland) were cultured on collagen-coated plastic dishes (Iwaki Glass) in modified RPMI1640 media (Gibco) under an atmosphere of 5% CO₂ in air (Ball *et al.*, 1988). During the growth phase, the cells were cultured in RPMI1640 supplemented with heat-inactivated fetal bovine serum (FBS; 10% v/v; Cell Culture Laboratories), insulin (5 µg/ml; Sigma), EGF (10 ng/ml; Collaborative Biomedical Products) and gentamicin (50 µg/ml; Wako) (HC11-1640-G medium in our laboratory). After confluence was reached, the medium was shifted to RPMI1640 containing FBS (2% v/v), insulin (5 µg/ml) and gentamicin (50 µg/ml) (HC11-1640-I medium in our laboratory). For passage, trypsinized cells suspended in the growth medium were seeded onto the culture dishes at a density of 1-2.5 × 10⁵ cells/dish.

Electron microscopic observation

HC11 cells were fixed 2 days after confluence was reached (Fig. 1; Day C2), with 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate (pH 7.4). After dehydration in an ethanol series, the specimens were embedded in Araldite resin (Ciba-Geigy). Thin sections were cut using an ultramicrotome (Ivan Sorvall), and stained with uranyl acetate and lead citrate. Observations were made using a Hitachi electron microscope (model 7000).

Immunocytochemical staining

Fixation was done with 4% para-formaldehyde in phosphate buffer (pH 7.4) overnight at 4°C. After the incubation with the blocking solution containing 5% skim milk (Wako) and 5% normal goat serum (Dako), the specimens were treated for 1 hr at room temperature with anti-MT1-MMP mouse IgG₃/κ monoclonal antibody (5.0 µg/ml) raised against the MT1-MMP oligopeptide sequence, CDGNFDTVAMLRGEM, that is conserved between human and mouse MT1-MMP (Sato *et al.*, 1994). After washing with PBS, the specimens were incubated with goat anti-mouse IgG₃ antibody conjugated with rhodamine (2.5 µg/ml; Southern Biotechnology Associates) for 30 min at room temperature.

Northern blot analysis

Poly(A)⁺ RNA from HC11 cells, collected on different days of culture, was purified using Quick Prep Micro mRNA Purification Kit (Pharmacia). Three micrograms of poly(A)⁺ RNA were electrophoresed on agarose gels and transferred to a Hybond-N⁺ membrane (Amersham). For hybridization, the membrane was incubated with ³²P-labelled human MT1-MMP probe containing the open reading frame, in 50% formamide hybridization buffer at 42°C for 16 hr. After rinsing with 1 × SSC containing 0.1% SDS at room temperature for 20 min, the membrane was washed in 0.2 × SSC containing 0.1% SDS at 60°C for 20 min twice. For the autoradiography, the membrane was exposed to an X-ray film (type RX, Fuji Film) for 24 hr. Densitometry was carried out using a Color Image Scanner (CS-7151, Seiko Instrument) and NIH Image (ver. 1.44) software (NIH Research Services Branch).

RESULTS AND DISCUSSION

During the log phase of growth, HC11 cells were elongated and irregular in shape, extending numerous pseudopodia and filopodia, suggesting a high degree of motility. At confluence which was reached about 6 days after

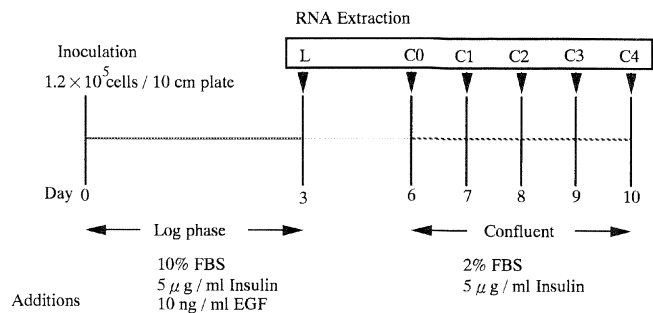


Fig. 1. Culture schedules for HC11 cells.

the initiation of culture (Fig. 1), the cells assumed a polygonally-shaped, paving stone like appearance. On electron microscopic examination, HC11 cells at confluence formed a simple cuboidal epithelium (Fig. 2a, b); they extended numerous microvilli on their apical surface (Fig. 2b), and formed a well developed basement membrane (Fig. 2a). The cells contained numerous mitochondria, lysosomes and protein vesicles similar in ultrastructural appearance to those found in normal mammary gland epithelial cells *in vivo* (Bargman and Knoop, 1959). Close to the apical surface of the cells, well-developed intermediate junctions were formed between adjacent cells (Fig. 2b). Altogether, HC11 cells at confluence retained characteristics of well differentiated mammary gland epithelial cells.

We examined the presence of MT1-MMP in HC11 cells by immunofluorescence methods using the anti-MT1-MMP monoclonal antibody, as described in Materials and Methods. Many HC11 cells during the log growth phase were stained strongly with the anti-MT1-MMP antibody (Fig. 3a-d). The cells which were well dispersed and seemingly migrating actively, or those located at the periphery of small colonies, were stained particularly strongly (Fig. 3b, d). Some cells during the log growth phase established cell-cell contact and no MT1-MMP immunoreactivity was detected in these cells (Fig. 3 b, d). Essentially the same patterns of expression of MT1-MMP immunoreactivity were observed when HC11 cells were cultured in HC11-1640-I medium during the log growth phase (data not shown).

At confluence, HC11 cells lost almost totally immunoreactivity to the anti-MT1-MMP antibody (Fig. 3e, f); the absence of staining in the confluent cells was quite remarkable. Culture of HC11 cells at confluence in HC11-1640-G medium, instead of HC11-1640-I medium, caused no change in the expression of MT1-MMP immunoreactivity.

Since the results of immunofluorescence staining strongly indicated that MT1-MMP expression is down-regulated by cell-cell contact, we carried out Northern blot analysis of MT1-MMP mRNA in HC11 cells during the different stages of culture. The transcripts of expected size, i.e., 4.2 kb, were detected at varying levels in all of the samples examined, i.e., those collected during the log growth phase and those at the different stages of confluence (Day C0, C1, C2, C3 and C4; Fig. 4A, B). The highest level of MT1-MMP transcripts was

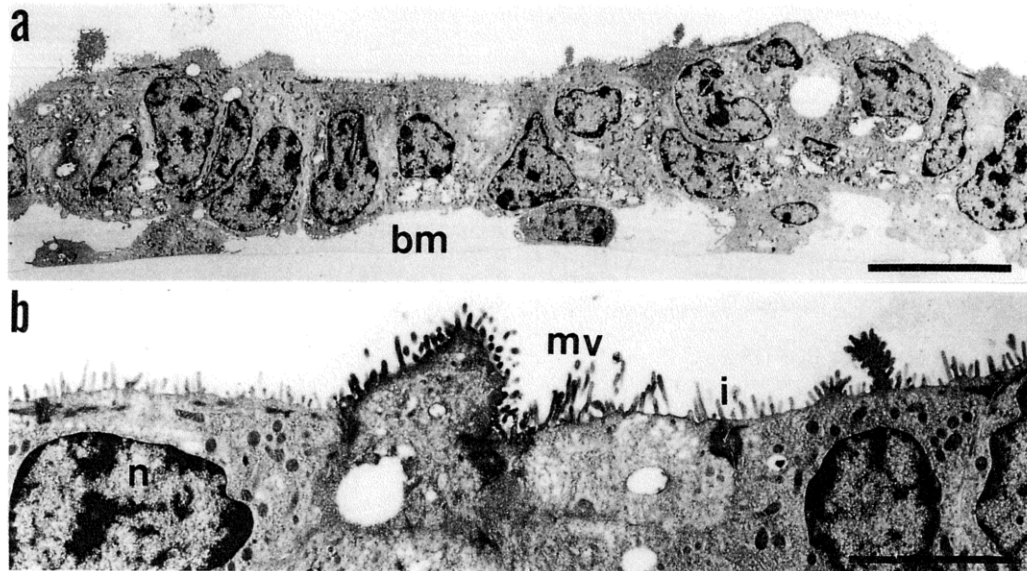


Fig. 2. Electron micrographs showing HC11 cells at confluence. (a) A well-differentiated monolayer of HC11 cells exhibiting typical morphology of a cuboidal epithelium. Scale bar, 10 μ m. (b) Apical region of the monolayer of HC11 cells. Scale bar, 5 μ m. bm, basement membrane; i, intermediate junction; mv, microvilli; n, nucleus.

observed on Day 3 of culture (the log growth phase) and the lowest level on Day C4 (Fig. 4A, B), once the confluence was reached.

In the mammary gland *in vivo*, the expression of MMPs is temporally and developmentally regulated and probably involved in the remodelling of the extracellular matrix during the different stages of mammary development (Talhok *et al.*, 1991). Coordinated expression of MMPs is also required for the involution of the mammary gland (Talhok *et al.*, 1992; Lund *et al.*, 1996). In female mice where the involution of the mammary gland was inhibited by the administration of hydrocortisone at the onset of weaning, the level of MT1-MMP mRNA decreased concomitantly with the inhibition of activation of latent gelatinase A, indicating the central role played by MT1-MMP in mammary gland involution (Lund *et al.*, 1996). However, the precise mechanisms controlling the expression of MT1-MMP or its tissue distribution remain to be clarified.

The presence of MT-MMPs or MT1-MMP mRNA in epithelial cells including those in the mammary gland, has not been reported. The results of our preliminary survey of different tissues in fetal and/or adult mice, indicated that few epithelial cell populations *in vivo* express MT1-MMP (unpublished data). We demonstrated, as described here in HC11 cells, that MT1-MMP immunoreactivity is manifested during the log phase of growth, while it disappeared almost totally in cells at confluence. Our data showed, furthermore, that while MT1-MMP immunoreactivity disappeared on the 1st day when confluence was reached (Day C0), the decline of MT1-MMP mRNA level started only a few days later (Day C3-C4). The discrepancy in the timing of the decrease in MT1-MMP protein and the transcripts might indicate the presence of a translational control mechanism for MT1-MMP expression in HC11 cells.

MT1-MMP is highly conserved at both protein and DNA levels among different species of mammals (Mariko *et al.*, unpublished data) and thought to play important roles in a variety of normal and pathological processes. Although the precise biological functions of MT-MMPs are yet to be worked out, it has been proposed that MT1-MMP links the pro-gelatinase A (pro-MMP-2) production by the stromal cells of host tissues and the invasion of tumor cells, by converting pro-gelatinase A to active gelatinase A (MMP-2) at the invasive edge of tumor-cell nests (Sato *et al.*, 1994; Vaselli and Pepper, 1994).

The present findings may have important implications in solving the classical problems of contact inhibition of cellular movement (Abercrombie, 1970; Guelstein *et al.*, 1973; Turbitt and Curtis, 1974; Curtis and Rooney, 1979; Abercrombie, 1979), possibly leading to a new insight into the mechanisms of not only placentation but also histogenesis during development and metastasis of cancer cells.

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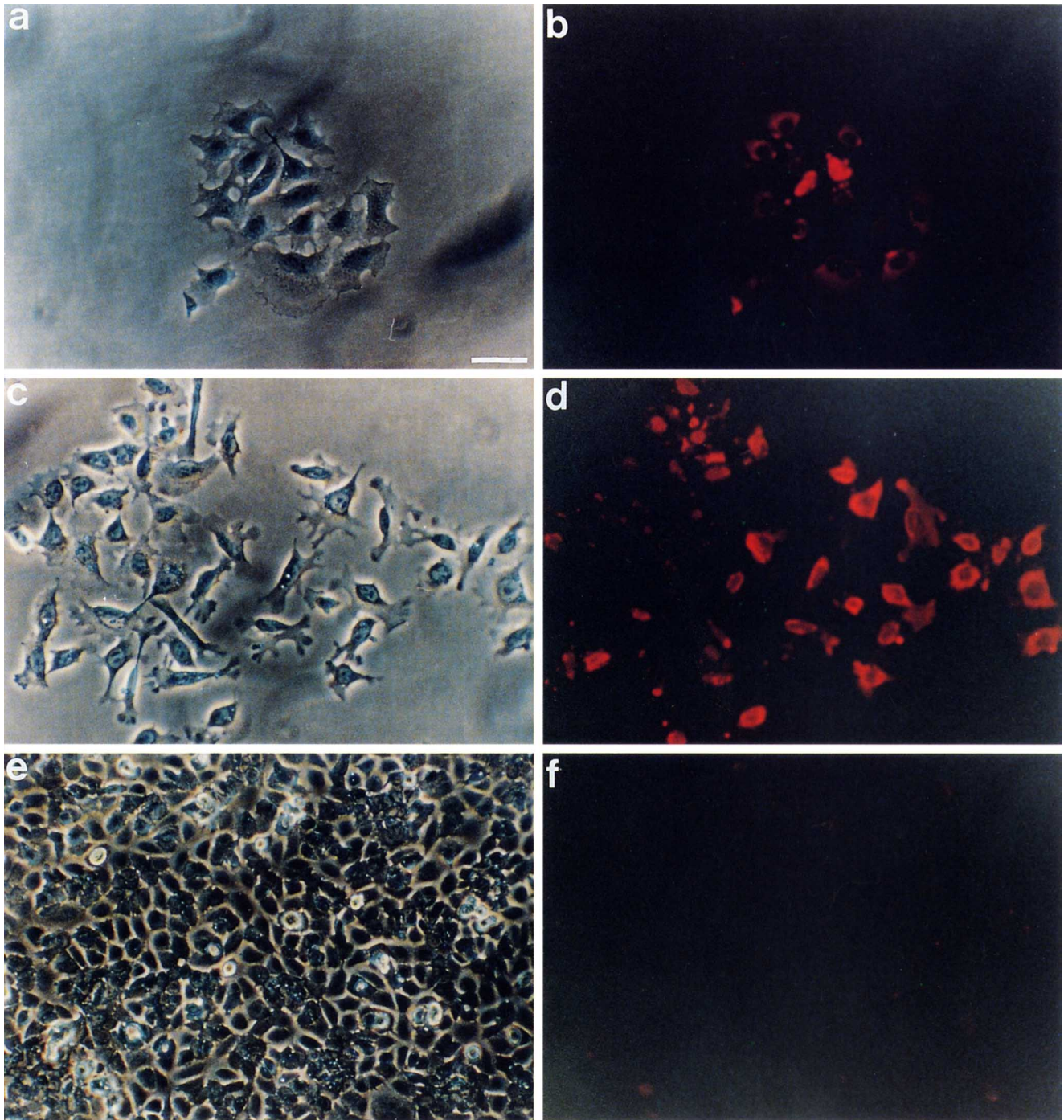


Fig. 3. Immunofluorescence microscopy of HC11 cells during different stages of culture treated with anti-MT1-MMP antibody. **(a-d)** During the log phase of growth. **(e, f)** At confluence. **(a, c, e)** Cells observed with phase contrast microscope. **(b, d, f)** Cells observed with fluorescence microscope. Note that two cells located within a colony observed during the log growth phase **(b)** are stained strongly with the anti-MT1-MMP antibody while the cells nearby are not stained or only faintly stained. In the phase contrast photomicrograph of the corresponding field **(a)**, the two cells are clearly seen extending pseudopodia and probably in active movement. Scale bar, 10 μm . Aldehyde-fixed HC11 cells were treated with anti-MT1-MMP mouse IgG₃/ κ monoclonal antibody as the first antibody and with goat anti-mouse IgG₃ antibody conjugated with rhodamine as the secondary antibody.

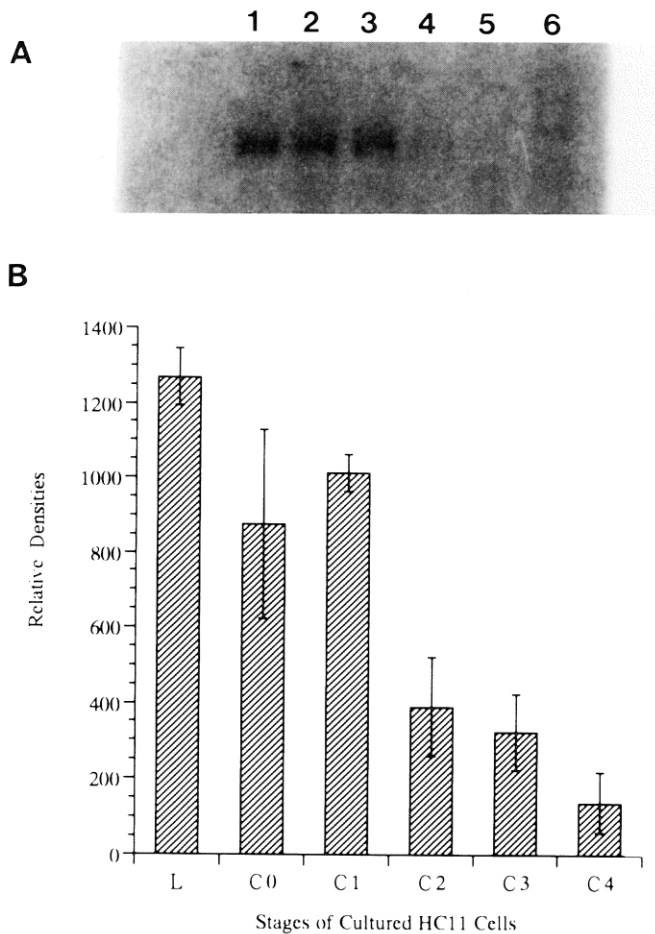


Fig. 4. Expression levels of MT1-MMP mRNA in HC11 cells during different stages of culture. (A) Northern blots. Lane 1, the log growth phase; lane 2, C0; lane 3, C1; lane 4, C2; lane 5, C3; lane 6, C4. (B) Relative MT1-MMP expression levels plotted as relative densities of the blots. At least three independent measurements were done for each stage. Vertical bars indicate S. D.

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