PROCEEDING

Role of α6 integrin subunit in branching morphogenesis of fetal mouse submandibular gland : investigation by mesenchyme-free epithelial culture system

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Abstract : The submandibular gland (SMG) of the fetal mouse is a well-studied model for the epithelial-mesenchymal interactions required for branching morphogenesis (BrM), which involves cleft formation and stalk elongation. In a previous report, we showed that α6 integrin subunit is involved in BrM of this gland rudiment, since the neutralizing antibody against α6 integrin subunit, GoH3, strongly inhibited branching of cultured intact E13 SMG. In this study, we investigated whether GoH3 inhibits cleft formation and/or stalk elongation during BrM of cultured mesenchyme-free epithelial rudiments of fetal SMG and also analyzed by Western blotting the levels of phosphorylation of ERK1/2, which is a signaling molecule known to regulate BrM of the fetal mouse SMG. J. Med. Invest. 56 Suppl. : 247-249, December, 2009

Keywords **:** *α6 integrin, Branching morphogenesis, Cleft formation, Stalk elongation, Submandibular gland*

INTRODUCTION

Epithelial-mesenchymal interaction regulates the developmental process of branching morphogenesis (BrM) of fetal organs, such as tooth, lung, kidney, liver and all exocrine glands including salivary glands (1). Members of the integrin family of cell adhesion molecules, as well as multiple growth factors, have critical roles in this developmental process (2, 3). Integrins are heterodimeric molecules consisting of α and β subunits, and form at least 20 different dimers (4). The α6 integrin subunit dimerizes with β1 or β4 subunit, and binds to laminin in the basal lamina. The α6 integrin is expressed on the entire cell surface of epithelial cells of developing mouse SMG (5) ; GoH3, a neutralizing antibody against α6 integrin subunit, strongly inhibits branching of cultured intact E13 submandibular gland (SMG) (6). Although α6 integrin subunit plays critical roles in BrM of mouse SMG, its precise role is still unknown.

To further elucidate the mechanism of regulation and function of the α6 integrin subunit, we used salivary epithelium separated from mesenchyme to define distinct morphogenetic roles for α6 integrin subunit.

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MATERIALS AND METHODS

Organ culture

Pregnant mice (ICR strain) were purchased from Japan SLC Inc. (Hamamatsu, Japan). The day of discovery of the vaginal plug was taken as embryonic day 0 (E0). E13 SMG rudiments were removed and placed on a membrane filter (Nuclepore membrane) floating on DMEM/F-12 medium containing 100 units/ml penicillin, 100 μg/ml streptomycin,

150 μg/ml vitamin C, and 50 μg/ml transferrin. In some experiments, the epithelium of E13 SMG rudiments was separated from the mesenchyme. Briefly, isolated E13 SMG rudiments were incubated with 500 units/ml dispase (Godo Shusei, Co., Tokyo, Japan) for 12 min at 37°C, and then the epithelium was separated from the mesenchyme with fine needles in Hank's balanced salt solution containing 10% BSA. The epithelium was placed on a Nucleopore membrane floating on DMEM/F-12 medium as described above with 0.1% bovine serum albumin and covered with growth factor-reduced Matrigel (1 : 1=Matrigel : medium, BD Biosciences, Bedford, MA). The growth factor(s) (EGF : 50 ng/ml or FGF10 : 500 ng/ml) was added to the medium and the epithelium cultured. For inhibition of α6 integrin, monoclonal antibody GoH3 (Beckman Coulter, California, USA) that is neutralizing antibody against α6 integrin subunit (40 μg/ml) was added. All procedures of animal handling were conducted in accordance with the Guideline for Experimental Animals of Asahi University.

Western blotting for signaling proteins

The cultured SMG explants were pooled, homogenized, and centrifuged (12,000 x g, 20 min) at 4°C, and the resulting supernatants were collected. The protein concentrations of the supernatants were measured by using a Protein Assay Kit (BioRad Lab., Inc. Hercules, CA, USA). The extracted protein was analyzed by Western blotting. Briefly, aliquots of protein samples were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. Specific proteins on the membranes were detected by probing with primary antibodies against ERK1/2, phospho-ERK1/2, or β-actin, followed by specific secondary antibodies conjugated to horseradish peroxidase and staining by use of ECL Plus Western Blotting Detection Reagents (GH Healthcare Bio-Sciences Corp., Piscataway, NJ).

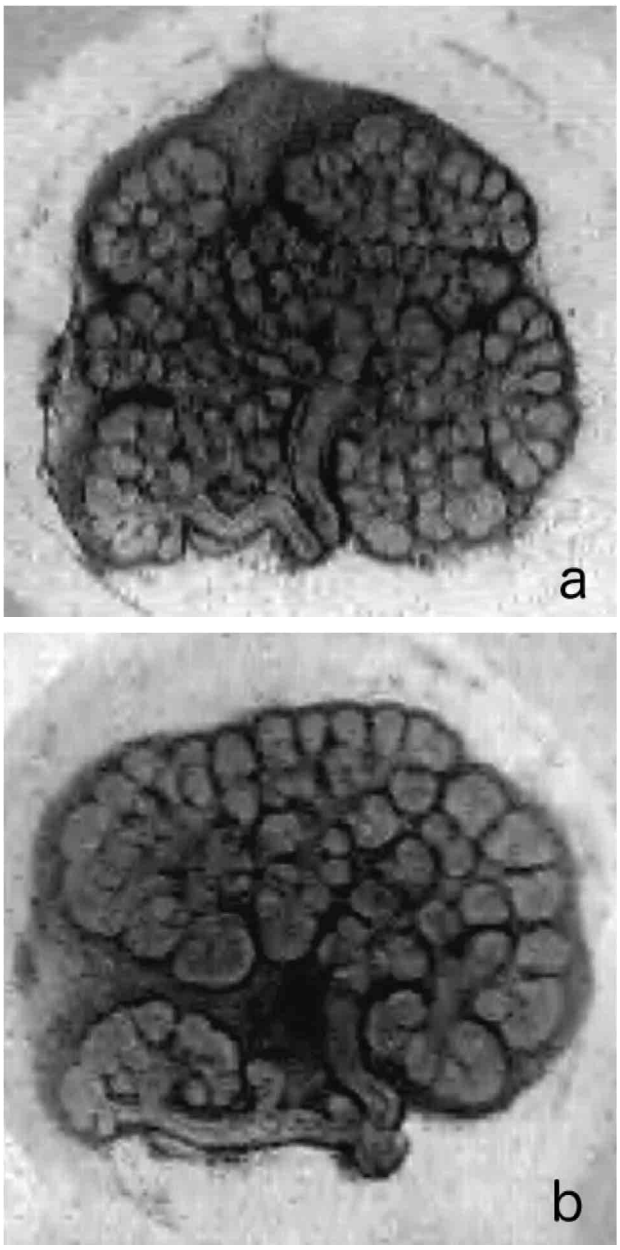

Figure 1. Inhibition of branching morphogenesis of cultured E13 SMG rudiments by α6 integrin monoclonal antibody, GoH3. E13 SMGs were incubated at 37°C, 5% CO_2 and 95% humidity in DMEM-F/12 medium without (a) or with (b) 40 μg/ml GoH3 for 48 h. GoH3 inhibited branching morphogenesis of SMG rudiments, as evident in fewer and larger epithelial endpieces (b).

RESULTS AND DISCUSSION

When cultured with the neutralizing antibody against α6 integrin subunit, GoH3, the number of epithelial branches decrease compared with control SMG (Figure 1). To determine the inhibition effects of GoH3 for the processes of both cleft formation and the elongation of cultured E13 SMG rudiment, we also examined its effect on mesenchyme-free salivary epithelium. Consistent with intact rudiment, GoH3 inhibited cleft formation of the SMG epithelial rudiment. However, GoH3 also strongly inhibited stalk elongation of cultured mesenchyme-free epithelial rudiment.

Our previous work demonstrated that the ERK1/2 signaling pathway is involved in BrM, in particular, cleft formation (7, 8). We next examined whether the ERK1/2 pathway is also involved in integrin signaling in E13 SMGs, by investigating the effect of GoH3 on ERK phosphorylation. Treatment with

GoH3 caused a slight increase over basal levels of ERK1/2 phosphorylation, but it had no discernible effect on the level of phosphosrylation induced by EGF.

Taken together these results confirm that α6 integrin subunit has a critical role in branching morphogenesis of fetal mouse SMG not only for cleft formation but also for stalk elongation, and establish that the action of the α6 subunit is not principally dependent on signaling through the ERK1/2 pathway.

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