PROCEEDING

Parotid acinar cells transiently change to duct-like cells during epithelial-mesenchymal transition

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Abstract : Hyposecretion of saliva and consequent dry mouth lead to severe caries and periodontal disease. Therapeutic radiation for head and neck cancer and sialadenitis result in atrophy and fibrosis of salivary glands, but the mechanism is not clear. As a model for dysfunction of salivary glands, we examined the change of gene expression patterns in primary cultured parotid acinar cells. The expression levels of acinar markers such as amylase and aquaporin-5 rapidly decreased during culture. At the same time, ductal markers began to be expressed although their expression was transient. In the late phase of culture, markers of epithelial-mesenchymal transition began to be expressed and increased. Inhibitor for Src or p38 MAP kinase suppressed these changes. These results suggest that parotid acinar cells transiently change to duct-like cells during epithelial-mesenchymal transition and that these changes are induced by signal transduction *via* Src-p38 MAP kinase pathway. There is a possibility that parotid acinar cells retain a plasticity of differentiation. J. Med. Invest. 56 Suppl. : 258-259, December, 2009

Keywords **:** *parotid glands, differentiation, epithelial-mesenchymal transition, claudin*

INTRODUCTION

Cellular stresses such as tissue injury or inflammation cause epithelial-mesenchymal transition (EMT). Although EMT is considered to be necessary for tissue repair, its deregulation may cause fibrosis or tumorigenesis (1). Therapeutic radiation for head and neck cancer and sialadenitis result in atrophy and fibrosis of salivary glands, but the mechanism is not clear. We have established a system for primary culture of parotid acinar cells (2). The cultured cells retained the ability to secrete amylase stimulus-dependently and to generate new secretory granules. In contrast, the expression levels of amylase, which is considered as a differentiation marker of acinar cells, decreased during culture. As a model for dysfunction of salivary glands, we examined the change in gene expression in primary cultured parotid acinar cells.

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MATERIALS AND METHODS

1. Acinar cells were isolated from rat parotid glands by digestion with collagenase and hyaluronidase, and were cultured in the medium containing 10% rat serum for 7 days. The experiment conforms with institutional guidelines for the use of experimental animals and was approved by the Experimental Animal Ethical Committee of Nihon University School of Dentistry at Matsudo.

2. Expression levels of marker proteins for acinar cells, ducts and EMT were examined by real time RT-PCR and immunoblot analysis.

3. Effects of inhibitors for Src kinase (10 μM PP1) and p38 MAP kinase (20 μM SB203580) on change in gene expression pattern were examined.

RESULTS AND DISCUSSION

We found that the expression pattern of claudins, tight junction proteins, changed during the culture. Claudins-4 and -6, which are detected in the developing salivary glands but not in acinar cells of mature glands, began to be expressed. They transiently increased in early phase (for 2-3 days) as reported previously (3), and decreased after 3 days. The expression levels at 7 days were similar to those at 1 day after isolation. Because claudin-4 changes the paracellular permeability of salivary gland cells (4), its expression may affect the secretory function of parotid acinar cells. In contrast, the expression level of ENaC, which is considered as a marker of mature ducts, was low and unchanged during the culture. After 3 days, EMT markers such as vimentin and fibronectin began to be expressed and increased. The pattern of gene expression shows that acinar cells transiently changed to immature duct-like in the early phase and thereafter transformed to fibroblastic cells. By addition of the Src kinase inhibitor PP1 or p38 MAP kinase inhibitor SB203580, the expression levels of immature duct markers were suppressed (5). At the same time, expression of EMT markers was strongly suppressed by Src kinase inhibitor, suggesting that Src kinase activity is necessary for promoting EMT process.

In conclusion, isolated parotid acinar cells transiently changed to immature duct-like cells during EMT process. This change may be the retrograde process of development and differentiation of salivary glands. These results suggest that parotid acinar cells retain a plasticity of differentiation. Inhibition of Src kinase or p38 MAP kinase activity suppressed or delayed a sequence of that dedifferentiation process.

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