PROCEEDING

Involvement of the IL-6/STAT3/Sca-1 system in proliferation of duct cells following duct ligation in the submandibular gland of mice

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Summary : Ligation of the main excretory duct (MED) of the mouse submandibular gland (SMG) induced the expression of Sca-1, a stem cell marker. Sca-1 expression increased prominently in almost all of cells in the duct system, except the acinar cells. Sca-1 induction was accompanied with phosphorylated-STAT3 (Y705) elevation, which was localized in the nuclei of all duct cells. Electrophoretic mobility shift assay (EMSA) confirmed the specific binding of STAT3 to the GAS sequence, a biding site of gamma interferon activating site. Present study suggested one of the initial steps of the tissue regeneration after injury includes STAT3 pathway. J. Med. Invest. 56 Suppl. : 253-254, December, 2009

Keywords **:** *submandibular gland, IL-6, STAT3, duct-ligation*

INTRODUCTION

Previous studies have established that ligation of the main excretory duct (MED) of the submandibular gland (SMG) induces apoptosis of acinar cells and proliferation of the duct cells (1-3). On the other hand, tissue injury induces a complex inflammatory response, after which regeneration process will be turned on (4). In this study, we focused on the proliferation of duct cells in the MED-ligated SMG to elucidate the molecular mechanism of regeneration onset triggered by inflammatory response.

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METHODS

Eight weeks-old C57BL/6 male mice were used in the present study. The MED of SMG was ligated according to the procedure reported previously (3). Western blotting and immunohistochemistry were employed for the analysis of proteins for Sca-1, AQP5, kallikrein mK22, Na^+,K^+-ATPase α-subunit, and STAT3. RT-PCR was used to analyze the mRNA levels of Sca-1, IL-6, IL-1β, and TNF-α. Electrophoretic mobility shift assay (EMSA) was employed to assess the STAT3 binding to the GAS element of the Sca-1 promoter.

RESULTS AND DISCUSSION

Ligation of the MED of the SMG induced the expression of Sca-1, a stem cell marker, in this tissue. In the normal gland, a low level of Sca-1 was

expressed predominantly in the striated duct cells. At 1 day after ligation, Sca-1 expression was increased prominently in almost all of cells in the duct systems, but not in the acinar cells. The level of IL-6 mRNA was increased and reached to a peak level at 6 hour after ligation. Following the cytokine peak, a continuous elevation of Sca-1 mRNA was observed.

Since the IL-6 signaling is elucidated to be mediated *via* the STAT signaling pathway (5), the possible involvement of this transcription factor in the Sca-1 induction was investigated. It was found that the phosphorylation of STAT3 at Tyr705 was increased in the SMG immediately after ligation and it was localized in the nucleus of all duct cells.

By EMSA, the ligation was found to increase the binding activity in the nuclear extract with GAS sequence in the SMG. Competition by excess cold probe and super-shift experiments in EMSA confirmed the specific binding of STAT3 to the GAS sequence.

In conclusion, ligation of MED of SMG increased the expression of IL-6, which consequently phosphorylated STAT3 at Tyr705. STAT3, having been transferred to the nucleus, obviously bound to GAS element in the Sca-1 promoter resulting in mediation the transcriptional activation of Sca-1 gene. Sca-1, having been induced, is supposed to play an important role in the duct cell proliferation.

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