**Abstract**

Niban is well expressed in Eker rat renal carcinogenesis and as well as human renal carcinoma. The expression of Niban was up-regulated in early pre-neoplastic lesions in the renal carcinogenesis models. Thus Niban is a candidate as a marker for renal carcinoma. In addition other human carcinomas were stained for Niban. However the role of Niban is still unknown. Head and neck squamous cell carcinoma (HNSCC) represents the sixth most common cancer worldwide. We examined Niban expression in HNSCC by immunohistochemical staining using polyclonal rabbit anti-human Niban antibody. Normal head and neck epithelium was not stained for Niban, but all 43 HNSCC cases were positively stained for Niban and dysplasia in 14/22 (63%) were weakly stained for Niban. RT-PCR verified the Niban expression in HNSCC at the messenger RNA level. Niban may not only play an important role in the human head and neck carcinogenesis but also is a candidate as a good molecular marker of the HNSCC.

**Introduction**

Head and neck cancer represents the sixth most common cancer worldwide. More than 90% of head and neck cancers are squamous cell carcinomas (HNSCC). Dispite advances in diagnosis and therapy, long-term survival of HNSCC patients has only moderately improved during the past 20 years. At the present time few molecular markers have been identified. Niban was initially investigated by our institution and it was well expressed in Eker rat renal carcinogenesis as well as human renal carcinoma. The expression of Niban was up-regulated in early pre-neoplastic lesions in the renal carcinogenesis models, but Niban is not expressed in the normal rat or human kidney. Thus, Niban is a candidate as a marker for renal carcinoma. In addition, other human carcinomas were stained for Niban. The functional information of Niban has not yet been reported, but comparison of the sequence of Niban with the genetic database revealed a dnaJ motif, which was found in members of the heat shock protein family. The most current report for the function of Niban suggests that Niban is involved in the endoplasmic reticulum stress response, and that Niban can modulate cell death signaling by regulating translation. In the present study, we investigated Niban expression in HNSCC, normal epithelium and the other organ tissue in SCC by immunohistochemical methods. Our purpose of the present study was to identify to be new molecular marker Niban for HNSCC.

**Methods and Materials**

This study included 43 HNSCC specimens and 22 head and neck dysplasia specimens which had undergone surgical resection and biopsy in the Department of otolaryngology in Juntendo University School of Medicine.

**Antibody**

Polyclonal rabbit anti-human Niban used for immunization corresponded to the C-terminal portion of Niban protein encoded by exon14. Immunohistochemistry

The sections were treated with EnVision+ system horseradish peroxidase (HRP) --labeled polymer antirabbit immunoglobulins (DakoCytomation, Glostrup, Denmark).

**Reverse transcriptase-polymerase chain reaction for Niban mRNA expression**

Dulplex reverse transcriptase-polymerase chain reaction (RT-PCR) amplification was performed using inter-exon primers for Niban (PCR product size of 191 base pairs [bp]) and internal control primers for the housekeeping gene GAPDH (PCR product size of 226 bp).

**Results**

**Immunohistochemical staining**

Normal head and neck epithelium was not stained for Niban (Fig. 1), but all 43 HNSCC cases were scored as Niban-positive (Table 1) and dysplasia in 14/22 (63%) were positive for Niban (table 2). HNSCC was stained strongly for Niban in 16 cases (Fig. 3), moderately in 19 cases, and weakly in 8 cases. Dysplasia was stained moderately for Niban in 2 cases, and weakly in 13 cases (Fig. 2). HNSCC were strongly stained for Niban as compared with dysplastic epithelium.

**Reverse transcriptase-polymerase chain reaction**

Dulplex RT-PCR analysis for Niban together with the internal control GAPDH gene showed bands of Niban and GAPDH in heart, normal lymph nodes, and 3 cases of HNSCC. These 3 cases were immunostained for Niban. Three cases of normal head and neck tissue only showed signals for GAPDH, but not for Niban. The results verified the Niban expression in HNSCC at the messenger RNA level. (Table 3).

**Conclusions**

Niban expression was not detected in normal mucosa, but we found that Niban protein is expressed in dysplasia lesion and HNSCC. A significant fraction of HNSCC were positively stained. It is suggested that dysplastic epithelium is pre-cancer state. Dysplastic epithelium was stained for Niban in comparison with HNSCC. Therefore, Niban expression appears to be up-regulated from the early stage of head and neck carcinogenesis and to remain up-regulated throughout neoplastic progression. Although the function of Niban is unknown, the presence of a dnaJ motif in the sequence suggests that Niban may be a heat shock-related protein, and can be up-regulated in a variety of cellular stress condition. It is well known that smoking and drinking are most important risk factors of the HNSCC patients. Therefore, there became a stress for the cells in head and neck epitheliums. According to our preliminary data, overexpression of Niban itself dose not accelerate cell proliferation. Niban’s function in tumor cells may be to protect cells from death-inducing stress by modulating protein translation.

**References**