Overexpression and Clinicopathological Significance of Homeobox Gene Quox-1 in Oral Squamous Cell Carcinoma

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The expression and clinicopathological significance of Quox-1 gene was studied in oral squamous cell carcinoma (OSCC). Immunocytochemistry and western blot analysis were used to examine the different expressions of Quox-1 protein in 114 OSCC specimens, 34 oral epithelial dysplasia specimens, and 16 normal oral mucosa specimens. RT-PCR and virtual Northern Blot were also used to examine the expression of Quox-1 mRNA. It was found that Quox-1 was not expressed in normal epithelium. However, as dysplastic lesions progressed Quox-1 expression increased (p < 0.01), and Quox-1 expression was not significantly different between severe dysplasia and highly differentiated OSCCs (p > 0.05). As the degree of differentiation decreased, Quox-1 positivity increased in OSCC (p < 0.01), and the rate of Quox-1 (81.58%) positivity in OSCC was higher than that in normal oral mucosa (p < 0.01). Our findings imply that the positive expression of Quox-1 is correlated with the histological classification of OSCCs. Thus, the expression of Quox-1 in OSCC may serve as a significant predicting factor of proliferative status and malignant degree, and it may also be a biological detection marker of oral mucosas initial cancer and of OSCC.

Keywords: Homeobox gene, Oral aquamous cell carcinoma, Quox-1

Introduction

The homeobox genes are a network of genes that are highly conserved throughout evolution and development. They encode DNA-binding proteins that commit cells to specific developmental pathways, and have the potential to regulate the expressions of certain genes during embryogenesis (Gehring et al., 1994; Kappen, 2000; Pederson et al., 2000). In mammals the homeobox genes have attracted the considerable attention from biologists because mutations within developmental genes could lead to tumorigenesis (Hatano, 1991; Stuart and Gruss, 1995; Johnson et al., 1996; Chariot and Castronovo, 1996; Kawabe et al., 1997; Ford, 1998a). Thus, in-depth investigations of homeobox genes should not only reveal the molecular mechanisms of development, but also lead to the discovery of the relationship between developmental genes and cancer.

Quox-1 is the only gene in the hox family that has been found to be expressed in the prosencephalon and mesencephalon (Xue et al., 1991), and is also involved in central and peripheral nerve cell differentiation (Xue et al., 1993).

In this paper, immunocytochemistry, western blot, RT-PCR, and virtual Northern Blot were used to study the expression and clinicopathological significance of the Quox-1 gene in oral squamous cell carcinoma (OSCC). We believed that such a study could reveal the relation between Quox-1 gene expression and oral squamous cell carcinoma (OSCC), and provide an insight into our understanding of the relationship between the expression of the Quox-1 gene, tumorigenesis, and the stimulation of malignancy.

Materials and Methods

Patients and tissue specimens From September 1998 to August 2001, 148 fresh surgical tumour biopsy samples and 16 matching
parallel normal oral mucosa tissue samples were obtained from consecutive patients with previously untreated OSCCs who were surgically at the Department of Oral Surgery at the Oral Hospital, Wuhan University. Tissue samples were immediately submerged in liquid nitrogen and then dispatched to the College of Life Science, Wuhan University.

**Western blot** Ten micrograms of protein lysate, prepared from tissue using M-PER reagent (Pierce, Rockford, USA), was separated by 10% SDS-PAGE, and then electrophoretically transferred to a nitrocellulose membrane. The membrane was then incubated with Quox-1 anti-serum (1:500). Western blot was carried as described by Titeux (Titeux et al., 2001).

**Probes for hybridization** The cDNA of Quox-1 was labeled with 32P-dCTP using the Prime-a Gene Labeling System (Promega, Madison, USA).

**RNA-extraction/cDNA synthesis** Total RNA was isolated using TRIzol® Reagent (Gibco/BRL, Gaithersburg, USA). Contaminating DNA was removed by treating with 30 units of RNase-free DNase 1 (Roche Molecular Biochemicals) for 15 min at 37°C (Martel et al., 2001). 20 mM EDTA was then added and the mixture extracted with phenol: chloroform-isooamylic alcohol (1:1); RNA was subsequently precipitated with ethanol, and pelleted RNA was reconstituted in RNase-free water. Samples were confirmed to be free of DNA by the absence of intron-containing c-myc DNA by nested PCR (Dhellin et al., 1997).

For each tissue specimen, first-strand cDNA was synthesized using 5-10 µg of total RNA. cDNA was then synthesized and amplified using a Clontech SMART PCR cDNA library construction kit (Clontech, Palo Alto, USA).

**Virtual northern blot** Virtual Northern blot was carried out under high-stringency conditions, as described by Xie et al. (2001).

**Detection of the Quox-1 gene in specimens by RT-PCR** The homeobox region Quox-1 (forward primer H1: 5’-AAACTCGAG GTGCTCCTTCTTCCACTTCA TCCG-3’ and reverse primer H2: 5’-TTAGA TA TCAGGAAGCGAGGGCGCCAGACCTACA-3’) was amplified to confirm Quox-1 mRNA expression.

**Immunocytochemistry** Immunohistochemical procedures were carried as described previously (Xue et al., 1993), except that antibodies to Quox-1 (Xue et al., 1993) were diluted 1:150 in phosphate-buffered saline.

**Statistical analysis** Data was analyzed using the Statistical Package for Social sciences (SPSS) software (Version 10.0). \( p < 0.05 \) was considered statistically significant.

**Results**

**Over expression of Quox-1 protein in OSCC** The expression of Quox-1 protein was detected by western blot in OSCC and in normal oral mucosa (Fig. 1). However, in the lane of total proteins obtained from normal human oral mucosa, we could find no Quox-1 hybridization signal (Fig. 1, Lane 2), whereas in the lane of total proteins from oral squamous cell carcinoma, the 40kD form of Quox-1 protein was detected (Fig. 1, Lane 4). Moreover, in the lane of total proteins from Quox-1 embryo, the 40kD form of Quox-1 protein was also observed.

Immunocytochemistry analysis was used to confirm the above results (Fig. 2, Table 1). Our data indicate that Quox-1 protein was overexpressed in OSCC (Fig. 2a), but not in normal human oral mucosa (Fig. 2b), which corresponds well with the virtual Northern blot result.

**Expression of the Quox-1 gene in OSCC** Transcripts of Quox-1 were electrophoresed by RT-PCR and detected by virtual Northern blot in OSCC tissues and normal oral mucosa (Fig. 3b). No hybridization signal was obtained in the cDNA of normal oral mucosa (Fig. 3b, Lane 1); whereas in the cDNA of OSCC tissue, a hybridization fragment of nearly 1.0 kb was detected by virtual Northern blotting (Fig. 3, Lane 3). In order to analyze the expression array of the Quox-1 gene in OSCC, we used RT-PCR and statistical analysis (Fig. 4, Table 2). Immunocytochemistry was also used to confirm the above results (Table 1). It was found that the expression of Quox-1 was negative in normal epithelium. In OSCC, as lesions progressed positive expression of Quox-1 increased \( (p < 0.01) \). The expression of Quox-1 was not significantly different in severe dysplasia and highly differentiated OSCC.
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As the degree of carcinoma differentiation reduced, the positive rate of Quox-1 increased in OSCC ($p < 0.01$), but the positivity of Quox-1 (87.57%) in OSCC was higher than in normal oral mucosa ($p < 0.01$).

**Discussion**

The targets of mammalian homeobox proteins are not known. It has been postulated that the homeobox genes are a family of regulatory genes that encode transcription factors. Today, many scientists have found an association between congenital anomalies, which may result from homeobox gene mutations, and cancer (Anbazhagan and Raman, 1997; Cillo et al., 1999). Thus, it is not surprising that many homeobox genes have been implicated in carcinogenesis (Cillo et al., 1999).

Evidence from our laboratory and others suggest that certain homeobox genes may play an important role in oncogenesis (Ford et al., 1998b; Zhu et al., 2002). Our results show that Quox-1 is not expressed in normal epithelium, but that in dysplasia its positivity increases with disease progression ($p < 0.01$). However, the expression of Quox-1 was not significantly different in severe dysplasia and highly differentiated OSCC ($p > 0.05$). As the degree of carcinoma differentiated was reduced, the positive rate of Quox-1 increased in OSCC ($p < 0.01$). However, positivity for Quox-1 (81.58%) in OSCC was higher than in normal oral mucosa ($p < 0.01$). Other scientists have also found that many cancers exhibit modulated homeobox gene expressions (Lawrence et al., 1996; Ford et al., 1998b).

A model proposed by Sager (Sager, 1997) suggested that tumorigenesis is the result not only of genetic mutations, but
also of wild-type overexpression. Moreover, our analysis showed that Quox-1 also expressed in ALL, M1 (Zhu et al., 2002) and tumor of the stomach (unpublished data). It suggest that Quox-1 may be expressed in tumors other than OSCC. Thus, our data suggests that Quox-1 has an important function in the development of different types of tumors.

Summarizing, Quox-1 is a homeobox gene that is overexpressed in a range of tumors, i.e., ALL, M1, tumor of stomach, and OSCC, which lends credence to the hypothesis that the overexpression of Quox-1 is involved in tumorigenesis/tumor progression. Indeed, Quox-1 is overexpressed in the large majority of OSCCs, and preliminary data suggests that it also is overexpressed in a variety of other cancers. In the present study, the positive expression of Quox-1 was found to be correlated with histological classification of OSCC. The significance of these findings for patients and for future research in this area is that Quox-1 may provide a potential diagnostic/prognostic marker for OSCC and present a potential target for therapeutic intervention.

Table 2. Expression of Quox-1 mRNA in oral squamous cell carcinoma and oral epithelial dysplasia by RT-PCR

|                        | Numbers of patients | +  | -  | %   |
|------------------------|---------------------|----|----|-----|
| poorly differentiated OSCC | 19                  | 18 | 1  | 94.74 |
| moderately differentiated OSCC | 62              | 55 | 7  | 88.71 |
| highly differentiated OSCC    | 33               | 24 | 9  | 72.73 |
| mild epithelial dysplasia    | 13               | 2  | 11 | 15.38 |
| moderate epithelial dysplasia | 12               | 3  | 9  | 25   |
| severe epithelial dysplasia  | 9                | 7  | 2  | 77.78 |
| normal oral mucosa          | 16               | 0  | 16 | 0    |

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