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Cytokeratin Expression in Squamous Cell Carcinomas of the Tongue and Alveolar Mucosa

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Cytokeratins (CK), the intermediate filament markers for epithelial cells were analysed in 23 squamous cell carcinomas (SCC) of the tongue and 11 SCC of the alveolar mucosa (AM) by SDS–PAGE, immunoblotting and two dimensional gel electrophoresis. Normal human adult ventral tongue expresses CK nos 4, 5, 6, 13, 14, 16 (17) while the dorsal tongue expresses CK nos 1, 5, 6, 10, 14, 16 (17). CK 5 and CK 14 were not detected in a majority of samples and CK 18, a marker of simple epithelia, was aberrantly expressed in 18 samples. Normal human adult AM expresses CK nos 4, 5, 6, 13, 14, 16 (17). Among 11 SCC of AM, CK 4 and CK 5 were detected in only two samples each. CK 1 and CK 10 were aberrantly expressed in nine and one samples, respectively. The basic CKs such as CK 4, 5 and 14 were not expressed in SCC at both these sites while others like CK 1 and 18 were aberrantly expressed. Thus, non-expression of basic keratin, CK 5, of the oral lining epithelia and aberrant expression of simple epithelial keratins seem to be the major events in malignant transformation in the oral epithelia. Copyright © 1996 Elsevier Science Ltd

Key words: cytokeratins, squamous cell carcinomas, tongue, alveolar mucosa

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INTRODUCTION

Cytokeratins (CK) are intermediate filament (IF) proteins specifically expressed by epithelial cells in a cell type specific and differentiation dependent manner [1–3]. Differences in CK expression have been shown between some squamous cell carcinomas and their normal counterparts [4–7]. We had earlier reported differentiation dependent alterations in CK expression in squamous cell carcinomas (SCC) of the buccal mucosa (BM) [6]. These alterations were of two types. (1) Aberrant expression of certain CKs not expressed in the normal tissue; and (2) non-expression (or down regulation) of certain CKs which are expressed in the normal tissue. Head and neck cancers in India are very strongly correlated with tobacco habbit. Since tobacco is the common predisposing factor for SCC at different sites in the oral cavity, we have studied CK expression in SCC of the tongue and the alveolar mucosa (AM) to see if they show patterns of alterations in CK expression similar to those seen in the SCC of the buccal mucosa.

MATERIALS AND METHODS

Chemicals were obtained from the following sources: goat anti-rabbit gamma globulin–horseradish peroxidase conjugated from Lupin Laboratories, India; Ampholines pH 5.0–8.0 and pH 3.0–10.0, phenylmethyl sulphonyl fluoride, antipain, pepstatin, Triton-X-100, ethylene glycol bis-*N,N,N,N'*,*N'*-tetraacetic acid and 3,3'diaminobenzidine, molecular weight markers 14,000–60,000 were obtained from Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Tumour tissues were obtained from patients during surgery at the Tata Memorial Hospital. These were immediately frozen at −80°C until used for keratin extraction. A small piece of this tissue was fixed immediately after collection for histopathology.

Keratins were isolated by a method modified from that described by Eichner *et al.* [8] as described previously [6].

GEL ELECTROPHORESIS

One dimensional separation by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was carried out with a 10% separating gel and a 3.9% stacking gel. Two dimensional gel electrophoresis was performed using isoelectric focusing (IEF) as the first dimension, the second being SDS–PAGE. The gels were visualised either by staining with 0.1% Coomassie Blue or silver [6].

IMMUNOBLOTTING

Polyclonal antibodies were raised in rabbits against human CK [6]. Their cross reactivity was checked against major cytoskeletal proteins, namely actin, tubulin, vimentin and desmin. The antiserum did not cross react with any of these proteins. The reactivity of the polyclonal rabbit anti-

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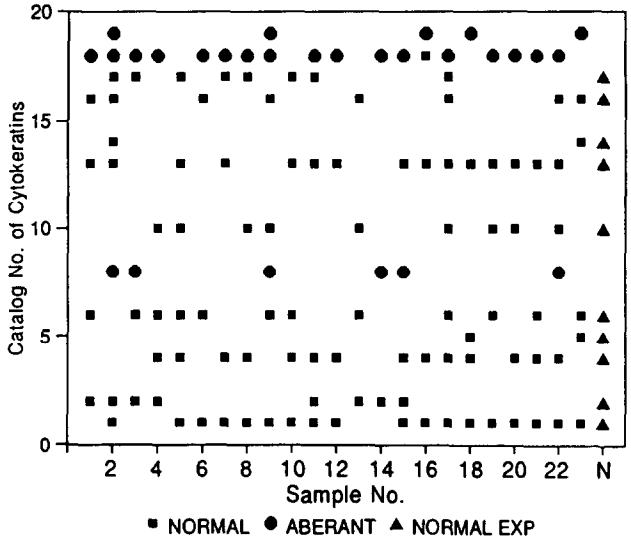

Fig. 1. Scattergram showing CK expression in 23 SCC of the tongue. Squares denote normal CK expression in SCC, circles denote aberrant CK expression in SCC and triangles denote CK expression by normal adult human tongue.

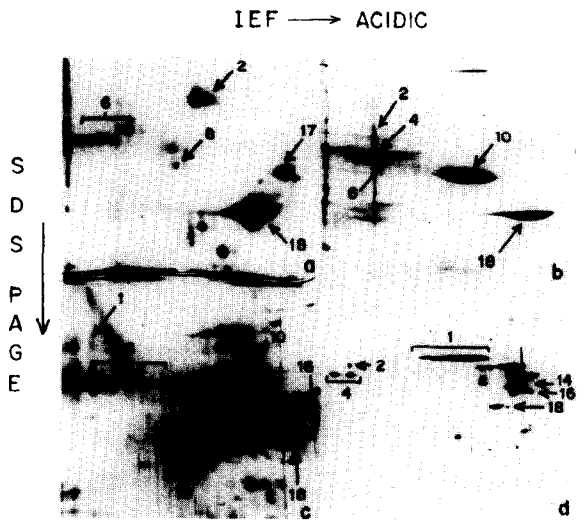

Fig. 3. 2-D gel electrophoresis of CK in SCC of the tongue. (a) Dorsal tongue, (b) and (c) ventral tongue, (d) site undecided. CK indicated by numbers.

serum was checked by immunoblotting against CK isolated from normal human epidermis, buccal mucosa, tongue, gingiva, alveolar mucosa, palate and also from HeLa cells which expressed keratin nos 7, 8, 17, 18, 19. The antiserum thus reacted with all the CKs.

RESULTS

Normal human ventral tongue expresses CK nos 4, 5, 6, 13, 14 and 16/17 and the dorsal tongue expresses CK nos 1, 5, 6, 10, 14 and 16/17. Figure 1 shows CK expression in 23 SCC of the tongue of which seven samples were from the dorsal, eight from the ventral tongue and the exact locations were not available for the remaining eight. CK 1 was expressed by 18 samples (78.2%), while CK 2 was expressed in eight samples (34.8%). CK 1 and CK 2 were simultaneously seen in three samples. Their normal counterpart no. 10 was seen only in nine cases (39%). Representative pictures of SDS–PAGE and immunoblots are shown in Fig. 2. CK 4, was detected in 13 samples (56.5%) and its normal counterpart no. 13 was seen in 15 samples (65.2%). CK 4 and CK 13 were co-expressed in 12 samples (52%) simultaneously. While CK 5 and CK 14 were individually expressed in the tumours, co-expression of these was detected in one sample. CK 6 was seen in 12 tumours (52%). CK 16 and CK 17 were found in eight samples each. CK 18 was aberrantly expressed in 18 samples (78%), while its normal counterpart CK 8 was detected in only six cases (26%). CK 19 was seen in only five samples. Representative 2-D gel photographs are shown in Fig. 3

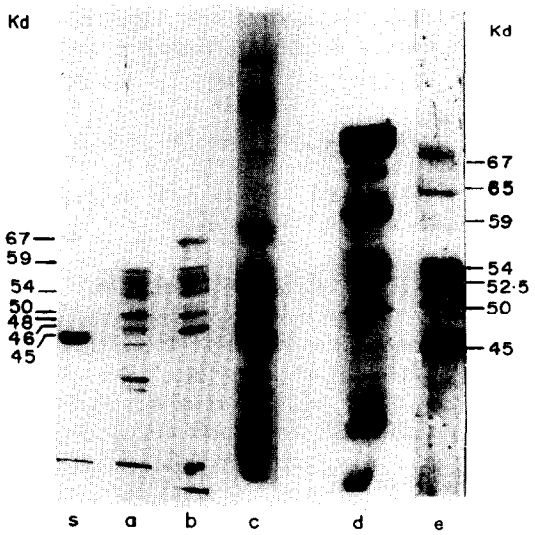

Fig. 2. SDS–PAGE and immunoblot of CK in SCC of the tongue. Lanes: s, molecular weight standards; SDS–PAGE: lanes a and b dorsal tongue; c, site undecided; d and e, immunoblot: site undecided: d, fast green staining; e, DAB staining.

Normal human AM expresses CK nos 4, 5, 6, 13, 14 and 16/17. Figure 4 shows CK expression in 11 SCC of AM and representative photographs of one and two dimensional gels are shown in Figs 5 and 6, respectively. Out of 11 SCC, CK 4 and CK 5 were detected in only two samples each. Their normal counterparts CK 13 and CK 14 were detected in six cases (55.5%) and nine cases (82.8%), respectively. CK 6 was found in five cases (45.5%). CK 16 and CK 17 were seen in 10 cases (90.9%) and four cases (55%), respectively. CK 1 was aberrantly expressed in nine SCCs (81.8%), while its normal counterpart no. 10 was seen in only one sample.

DISCUSSION

Cytokeratins are IF proteins specifically expressed by epithelial cells. CK expression although cell type specific can be modulated by changing the proliferation or differentiation state of the cells [9]. CK expression may or may not be altered after malignant transformation. Head and neck cancers form the single largest group of cancers among the

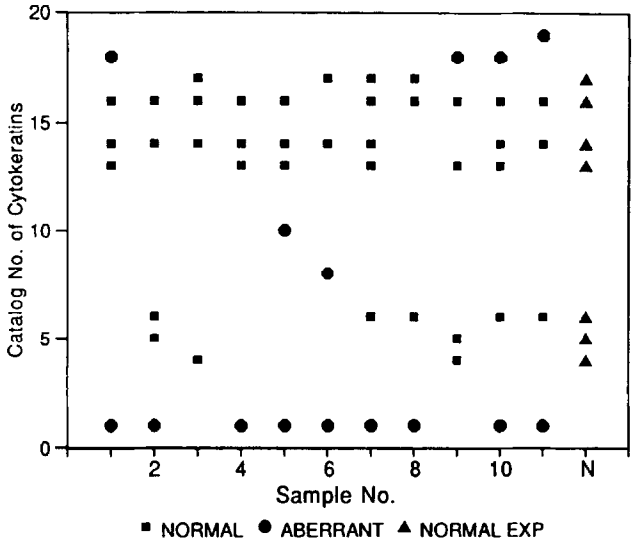

Fig. 4. Scattergram showing CK expression in 11 SCC of AM. Squares denote normal CK expression in SCC, circles denote aberrant CK expression in SCC and triangles denote CK expression by normal AM.

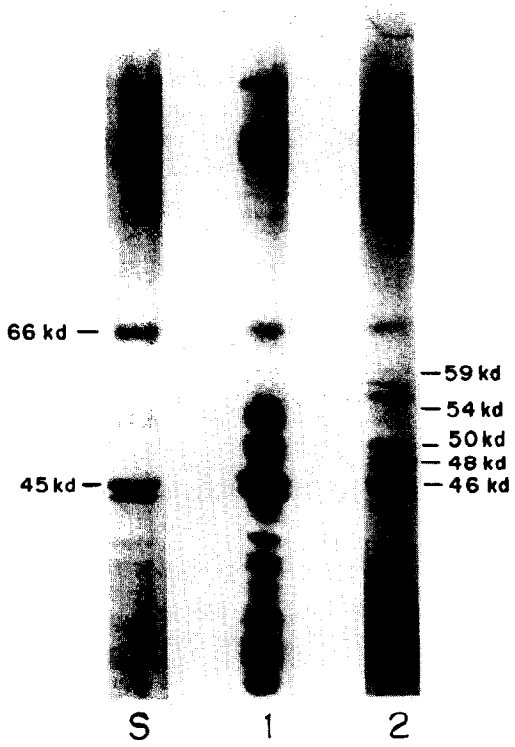

Fig. 5. SDS–PAGE of CK in SCC of AM. Lanes: s, molecular weight standards; a and b, CK.

total cancer patients in India and a large majority of these are associated with tobacco habit. It would, therefore be interesting to see if a common carcinogenic factor induces similar changes in different lining epithelia in the oral cavity during malignant transformation. In addition to the basic pair CK 5 and CK 14 seen in all the oral cavity lining epithelia, other CK are expressed differentially at other sites [10–12]. In SCC of the buccal mucosa, we had observed non-expression of CK 5 and CK 13 which did not seem to correlate with the differentiation of the tumours. However, the aberrant expression of CK 1, 16, 17 and 18 appeared to be differentiation dependent [6]. Aberrant expression of CK 1, 8, 18, 19 has also been reported by others in oral tumours and cell lines derived from them [13–17].

In the present study, we analysed CK expression in 23 SCC of the tongue and 11 SCC of AM. Eighty-eight per cent of the SCC of the tongue and AM together did not express CK 5. Thus, non-expression of CK5 seems to be a common feature in tumours of the oral cavity studied by us and may perhaps be associated with the risk factors, namely tobacco. Non-expression of basic CK pair of 5 and 14 or their down regulation has also been shown by others [17–19].

We observed expression of simple epithelial keratin CK 18 in 78% of SCC of the tongue. However, its normal counterpart CK 8 was detected in only 26% of SCC. CK 8 and 18 but not CK 7 have been shown to be expressed by well-, moderately- and poorly-differentiated SCC of the oral cavity [13–17] and has been suggested as one of the major alterations during malignant transformation. Vigneswaran *et al.* [18] failed to detect simple epithelial CK in formalin fixed paraffin sections of oral SCC which may be due to non-reactivity of these monoclonal antibodies (MAb) in fixed tissues. Several MAb do not react with keratins after fixation and paraffin embedding [20]. Since we have used 2-D gel electrophoresis, our results may not be totally comparable with those obtained by immunohistochemistry.

In the present studies, as well as in the SCC of BM [6], we have observed expression of CK 1 but an absence of coordinate expression of CK 10. Kartasova *et al.* [21] in transfection studies with human and mouse CK 1 and CK 10 genes found CK 1 expression prior to CK 10 expression and occurrence of a proliferative block in CK 10 transfected cell lines but not after CK 1 transfection. Loss of regulation

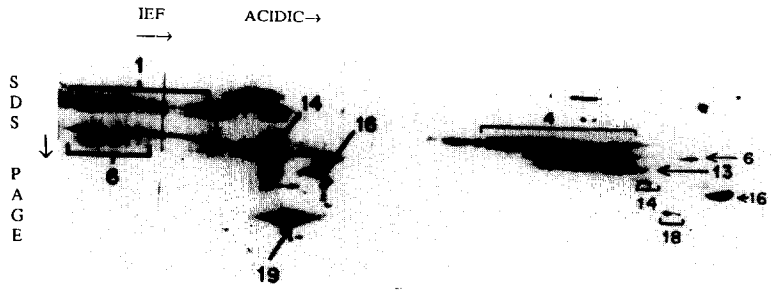

Fig. 6. 2-D gel electrophoresis of CK in SCC of AM. CK indicated by numbers.

of co-ordinate expression of keratin gene pairs may be a common lesion in oral tumours.

It thus appears that the loss of basic keratins CK 5 and 14 and aberrant expression of simple epithelial keratins occurs during malignant transformation of the human oral mucosas. It is not possible, at this stage, to conclude on the role played by risk factors such as tobacco in the differential loss of CK 5 in our samples and that of CK 13, 14 in other reports.

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