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Ekspresija citokeratina 18 i 19 u normalnoj sluznici, lichen planusu i karcinomu pločastih stanica

Cytokeratin 18 & 19 Expression in Normal Mucosa, Lichen Planus and Squamous Cell Carcinoma

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Sažetak
Citokeratini (CK) strukturalni su proteini u epitelnim stanicama. Zapažene su promjene u njihovoj ekspresiji tijekom neoplastične transformacije. Svrha: procjena ekspresije citokeratina 18 i 19 u normalnoj sluznici, lichen planusu i karcinomu pločastih stanica. Trideset slučajeva svake od navedenih - normalne sluznice, lichen planusa i karcinomi pločastih stanica - uzeti su iz arhiva Zavoda za oralnu dijagnozu Sveučilišta Khona Kaena. Preparati tkiva obojeni su protutijelima na citokeratin 18 i 19. Obavljena je i evaluacija imunoreaktivnosti prebrojavanjem stanica. Oznaka za obilježavanje CK-a izračunate su dijeljenjem broja pozitivno obojenih stanica x 100 s ukupnim zbrojem izbrojenih stanica. Devet od 30 (30,0%) normalnih sluznica, 5 od 30 (16,7%) lichen planusa i 4 od 30 (13,3%) karcinoma pločastih stanica pokazivali su pozitivno bojenje na CK18. Karcinom pločastih stanica pokazivao je najvišu srednju vrijednost indeksa označavanja CK18 te su zatim u silaznom redoslijedu slijedile normalna sluznica i lichen planus. Dvadeset i tri od 30 (76,7%) normalnih sluznica, 9 od 30 (30,0%) lichen planusa i 1 od 30 (3,33%) karcinoma pločastih stanica postigli su pozitivno bojenje na CK19. Normalna je sluznica pokazala najvišu srednju vrijednost indeksa označavanja sa CK19 te su u silaznom redoslijedu slijedili lichen planus i karcinom pločastih stanica.Ekspresija CK18 u normalnoj sluznici, lichen planusu i karcinomu pločastih stanica niska je i nema statističkih razlika među trima vrstama lezija. Ekspresija CK19 u normalnoj sluznici statistički je viša od one u lichen planusu te karcinomu pločastih stanica. CK19 mogao bi biti dobar za uključivanje u skupinu protutijela za detekciju malignih transformacija oralne sluznice u karcinom pločastih stanica.

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Ključne riječi
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Uvod

Citokeratini su članovi multigene obitelji 10 nm posrednih filamenata. Može ih se razvrstati u dvije skupine prema biokemijskim svojstvima i izoelektričnim točkama. Tip I, ili kiseli citokeratini, sastoje se od citokeratina 9 do 20 s molekularnom težinom između 40 i 64 kD, a tip II su neutralno-bazični citokeratini koji se sastoje iz citokeratina 1 do 8 s molekularnom težinom između 52 i 68 kD (1-4). Kako bi oblikovali posredni filament, barem jedan član iz skupine citokeratina tipa I ili tipa II polimerizira te nastaju heteropolimeri (1,5). Citokeratini su strukturalni proteini epitelnih stanica (1). Osim što pridonose fizičkoj snazi epitelnih stanica, imaju važnu zadaću u transdukciji signala, regulaciji stanične migracije i invazije (1,6). Citokeratinska ekspresija razvojno je regulirana, specifična lokalitetu i pokazuje stadij diferencije epitelnih stanica (4,7-9). Nekoliko epitelnih tumora još zadržava određene citokeratine primarnoga tkiva (4), a promjene u određenim citokeratinskim ekspresijama zapažene su tijekom neoplastične transformacije (3,6,10,11).

Svrha istraživanja jest evaluacija ekspresije citokeratina 18 i 19 u normalnoj sluznici, lichen planusu te u karcinomu pločastih stanica.

Materijali i metode

Trideset slučajeva normalne sluznice, lichen planusa i karcinoma pločastih stanica pronađeni su u arhivima Zavoda za oralnu dijagnozu Sveučilišta Khona Kaena. Skupinu ispitanika s normalnom sluznicom činilo je 8 muškaraca i 22 žene u dobi između 16 i 23 godine i prosječnom vrijednosti dobi ± SD = 20,50 ± 1,68 godina. U skupini ispitanika s oralnim lichen planusom bilo je 10 muškaraca i 20 žena u dobi između 21 i 64 godine s prosječnom vrijednosti dobi ± SD = 46,10 ± 11,60 godina. Skupina ispitanika s karcinomom pločastih stanica sadržavala je 12 muškaraca i 18 žena u dobi između 29 i 97 godina s prosječnom vrijednosti dobi ± SD = 66,10 ± 14,71 godina. U skupini s karcinomom pločastih stanica 10 je slučajeva pokazivalo tumor veći od 5 cm, a 6 je imalo tumore manje od 5 cm. Prema histološkoj klasifikaciji, 21 je slučaj uvršten u skupinu dobro diferenciranih karcinoma pločastih stanica, a 9 među srednje diferencirane karcinome pločastih stanica. Preparati tkiva izrezani su iz parafinskih blokova debljine 5 μm, te su deparafinizirani. Antigen je pronađen pedesetominutnom inkubacijom tkivnih preparata u citratnom puferu 0,01M u vodenoj kupelji (95ºC), a zatim je slijedila tripsinska digestija jed-

Introduction

Cytokeratins are members of the 10 nm intermediate filament multigene family. They can be divided into 2 groups based upon biochemical properties and isoelectric points. Type I or acidic cytokeratin consists of cytokeratin 9-20 with a molecular weight ranging from 40-64 kD, whereas type II or neutral-basic cytokeratin consists of cytokeratin 1-8 with a molecular weight ranging from 52-68 kD (1-4). In order to form an intermediate filament, at least one member from type I and type II cytokeratins polymerize to form heteropolymers (1,5). Cytokeratins are structural proteins of epithelial cells (1). Besides giving physical strength to epithelial cells, cytokeratins also play an important role in signal transduction, regulation of cell migration and invasion (1,6). Cytokeratin expression is developmentally regulated, site-specific and reflects the stage of epithelial cell differentiation (4,7-9). Several epithelial tumors still retain certain cytokeratins of the primary tissue (4), while changes in certain cytokeratin expression have been observed during neoplastic transformation (3,6,10,11). The objective of this study was to evaluate cytokeratin 18 and 19 expression in normal mucosa, lichen planus and squamous cell carcinoma.

Materials and Methods

Thirty cases each of normal mucosa, lichen planus and squamous cell carcinoma were retrieved from the archives of the department of Oral Diagnosis, Khon Kaen University. The normal mucosa subjects consisted of 8 males and 22 females and the age ranged from 16 to 23 years with the mean age ± SD = 20.50 ± 1.68 years. Oral lichen planus patients consisted of 10 males and 20 females and the age ranged from 21 to 64 years with the mean age ± SD = 46.10 ± 11.60 years. Oral squamous cell carcinoma patients consisted of 12 males and 18 females and the age ranged from 29 to 97 years with the mean age ± SD = 66.10 ± 14.71 years. In the squamous cell carcinoma group, 10 cases had tumors of sizes greater than 5 cm and 6 cases had tumors sized less than 5 cm. According to histological grading, 21 cases were classified as well differentiated squamous cell carcinoma and 9 cases were classified as moderately differentiated squamous cell carcinoma. Tissue sections were cut from paraffin blocks at the thickness of 5 μm and deparaffinized. Antigen retrieval was carried out by incubating the tissue sections in 0.01M citrate buffer in the waterbath (95ºC) for 50 min followed by trypsin digestion for

nu minutu na temperaturi od 37 stupnjeva kod pH 7,8 za CK18 te tridesetominutna tripsinska digestiija na temperaturi od 37 stupnjeva na pH 7,8 za CK19. Preparati tkiva nakon toga su 10 minuta imerzirani u 3%-ni vodikov peroksid, kako bi se blokirala endogena peroksidaza, a zatim je slijedila inkubacija s mišjim protu-humanim monoklonskim protutijelom na CK18 (Novocastra, Velika Britanija 1:50) i mišjim protu-humanim monoklonskim protutijelom na CK19 (Novocastra, Velika Britanija 1:100) tijekom noći na temperaturi od 4 stupnja. HRP-označeno kozje protu-mišje protutijelo (DAKO, Danska 1:100) dodano je tijekom jednoga sata tkivnim preparatima na sobnoj temperaturi. Produkt reakcije razvijen je dodavanjem tkivnim preparatima 3, 3' diaminobenzidin-tetrahidroklorida. Tkivni su preparati zatim protu-obojeni hematoksilinom. Reakcija imunohistokemijskog bojenja na CK18 i 19 u tkivnim je preparatima evaluirana prebrojavanjem stanica kod svakog slučaja na fotografijama dvoje promatrača. Kriteriji za selekciju područja za fotografiranje na preparatima bili su sljedeći (Slike 1, 2):

1. tri su područja odabrana za fotografiranje na svakom preparatu - jedno na krajnjem lijevom te na krajnjem desnom dijelu, te još jedno na sredini preparata;
2. fotografije su snimljene lećom objektiva 40x s finalnim izvornim povećanjem od 200x.

Svaki je promatrač neovisno prebrojavao i pozitivno i negativno obojene stanice. Broj pozitivno obojenih stanica izračunan je na temelju prosjeka brojeva oboje promatrača. Prosječna vrijednost CK-a izračunana je dijeljenjem broja pozitivno obojenih stanica x 100 s ukupnim zbrojem izbrojenih stanica. Odnosi između spola, veličine tumora, histološke klasifikacije i prosjeka indeksa CK-obilježavanja, analizirani su Mann-Whitneyevim U-testom. Odnosi između dobi i prosjeka indeksa CK-obilježavanja analizirani su Pearsonovom korekcijom. Kruskal-Wallisov H-test koristio se u određivanju statističke razlike u prosjeku indeksa CK-obilježavanja između normalne sluznice, lichen planusa te karcinoma pločastih stanica. Post hoc analiza obavljena je Mann-Whitneyevim U-testom. Vrijednost p manja od 0,05 smatrala se statistički znatnom.

Rezultati

Od 90 proučavanih primjera, 18 slučajeva (20,0%) pokazivalo je pozitivno CK 18 bojenje. Ako se svaka skupina razmatra zasebno, 9 od 30 (30,0%) normalnih sluznica pokazivalo je pozitivno bojenje na CK18. Pet od 30 (16,7%) lichen planusa

1 min at 37 ºC pH 7.8 for CK18 and by trypsin digestion for 30 min at 37 ºC pH 7.8 for CK19. Tissue sections were immersed in 3% hydrogen peroxide for 10 min to block endogenous peroxidase and subsequently incubated with mouse anti-human monoclonal antibody to CK18 (Novocastra, UK 1:50) and mouse anti-human monoclonal antibody to CK19 (Novocastra,UK 1:100) overnight at 4 ºC. HRP-labeled goat anti-mouse antibody (DAKO, Denmark 1:100) was added to the tissue sections at room temperature for 1 hour. Reaction product was developed by adding 3, 3' diaminobenzidine tetrahydrochloride to the tissue sections. Tissue sections were then counterstained with hematoxylin. Immunohistochemical staining reaction for CK18 and 19 in tissue sections was evaluated by counting cells in each case in the photographs by 2 observers. The criteria for selecting areas for photography on the slides were as follows (Figures 1, 2):

1. Three areas were selected for photography in each slide: one area each on the far left and the far right, the other in the middle of the slides.
2. Photographs were taken by the 40x objective lens with the final original magnification of 200x.

Each observer independently counted both the positively stained and negatively stained cells. The number of positively stained cells was calculated by averaging figures from the 2 observers. The mean CK labeling index was calculated by dividing the number of positively stained cells x 100 by the total number of cells counted. Relationships between gender, tumor size, histological grading and mean CK labeling index were analyzed by Mann-Whitney U test. Relationships between age and mean CK labeling index were analyzed by Pearson correlation. Kruskal-Wallis H test was used to determine whether there was a statistical difference in mean CK labeling index among normal mucosa, lichen planus and squamous cell carcinoma. Post hoc analysis was carried out by Mann-Whitney U test. A p-value less than 0.05 was considered statistically significant.

Results

Out of the 90 specimens studied, 18 cases (20.0%) showed positive CK 18 staining. If each category was considered separately, 9 out of 30 (30.0%) normal mucosa demonstrated positive staining for CK18. Five out of 30 (16.7%) lichen

pokazivalo je pozitivno bojenje na CK18. Četiri od 30 (13,3%) karcinoma pločastih stanica bilo je pozitivno obojeno na CK18. Karcinom pločastih stanica pokazivao je najvišu prosječnu vrijednost indeksa CK18-označivanja (1,21), zatim slijede u silaznom redoslijedu normalna sluznica (0,59) te oralni lichen planus (0,58). No, Kruskal-Wallisov H-test nije pokazivao nikakve statistički znatne razlike u prosječnom indeksu CK-označavanja između normalne sluznice, lichen planusa te karcinoma pločastih stanica. Rezultati bojenja na CK18 sažeti su u Tablici 1. Nije bilo povezanosti između dobi, spola, veličine tumora i histološke klasifikacije te prosječnog indeksa CK18-označavanja.

Od svih 90 uzoraka, 33 slučaja (36,70%) bila su pozitivno obojena na CK19. Ako se svaka skupina razmatra zasebno, 23 od 30 (76,67%) normalnih sluznica pokazalo je pozitivno bojenje na CK19. Devet od 30 (30,0%) lichen planusa pokazalo je pozitivno bojenje na CK19. Jedan od 30 (3,33%) karcinoma pločastih stanica pokazao je pozitivno bojenje na CK19. Normalna je sluznica imala statistički viši prosjek indeksa CK19-obilježavanja od lichen planusa i karcinoma pločastih stanica (p<0,005). Isto tako, lichen planus je pokazao statistički viši prosjek indeksa CK19- obilježavanja od karcinoma pločastih stanica (p=0,005). Rezultati bojenja na CK19 sažeti su u Tablici 1. Nije bilo povezanosti između dobi, spola, veličine tumora i histološke klasifikacije te prosječnog indeksa CK19-označavanja.

planus showed positive staining for CK18. Four out of 30 (13.3%) squamous cell carcinoma elicited positive staining for CK18. Squamous cell carcinoma showed the highest mean CK18 labeling index (1.21) followed in descending order by normal mucosa (0.59) and lichen planus (0.58), respectively. However, Kruskal-Wallis H test demonstrated no statistical difference in the mean CK 18 labeling index among normal mucosa, lichen planus and squamous cell carcinoma. The results of CK18 staining were summarized in Table 1. There was no association between age, gender, tumor size and histological grading and mean CK 18 labeling index.

Out of all the 90 specimens, 33 cases (36.70%) showed positive CK19 staining. If each category was considered separately, 23 out of 30 (76.67%) normal mucosa demonstrated positive staining for CK19. Nine out of 30 (30.0%) lichen planus showed positive staining for CK19. One out of 30 (3.33%) squamous cell carcinoma elicited positive staining for CK19. Normal mucosa elicited statistically higher mean CK19 labeling index than lichen planus and squamous cell carcinoma (p<0.005). Likewise, lichen planus showed statistically higher mean CK19 labeling index than squamous cell carcinoma (p=0.005). The results of CK19 staining were summarized in Table 1. There was no association between age, gender, tumor size and histological grading and mean CK19 labeling index.

Tablica 1. Rezultati imunohistokemijskog bojenja na CK18 i 19 na normalnoj sluznici, lichen planusu i karcinomu pločastih stanica

Table 1 The results of immunohistochemical staining for CK18 and 19 in normal mucosa, lichen planus and squamous cell carcinoma.

Lezija • Lesion	Broj CK 18 pozitivno obojenih slučajeva • Number of CK 18 positively stained cases	Prosječni indeks CK 18 obilježavanja • Mean CK 18 labeling index	Broj CK 19 pozitivno obojenih slučajeva • Number of CK 19 positively stained cases	Prosječni indeks CK 19 obilježavanja • Mean CK 19 labeling index
NS • NM	9	0,59	23	24,37*
LP	5	0,58	9	6,32 * †
KPS • SCC	4	1,21	1	0,20

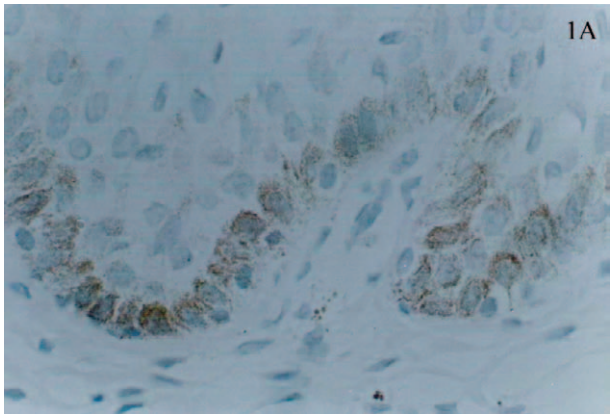
Legenda • Legend:
NS - normalna sluznica, LP - lichen planus, KPS - karcinom pločastih stanica, * p < 0,005, † p = 0,005
NM - Normal mucosa, LP - Lichen planus, SCC - Squamous cell carcinoma

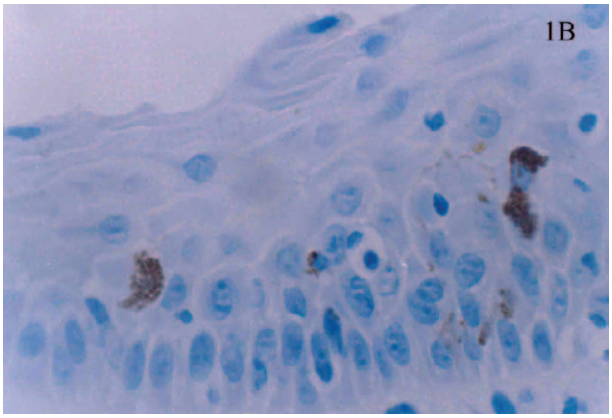
Rasprava

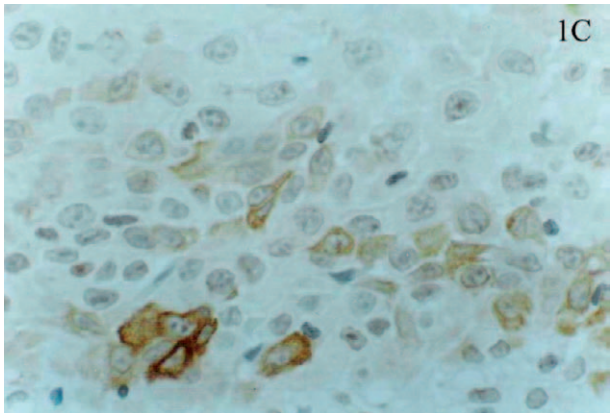
U ovom istraživanju 30,0% normalnih sluznica i 16,7% lichen planusa pokazivali su pozitivnu imunoreaktivnost protiv CK18 i imali su vrlo nizak prosjek indeksa CK18-obilježavanja. Ti se rezultati slažu s ispitivanjem Luomanena i suradnika

Discussion

In the present study, 30.0% of normal mucosa and 16.7% of lichen planus elicited positive immunoreactivity against CK18, both of which showed a very low mean CK18 labeling index. These results are in agreement with the study of Luomanen et al[12]

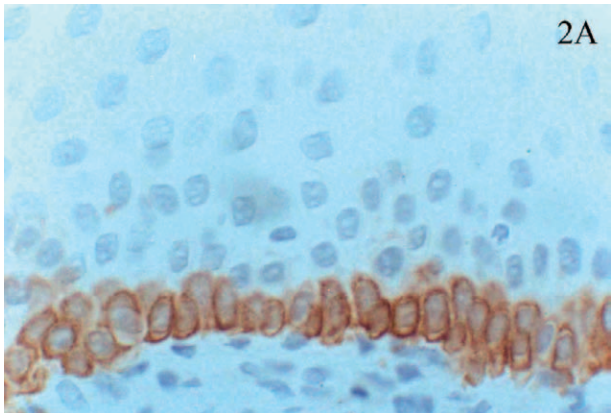


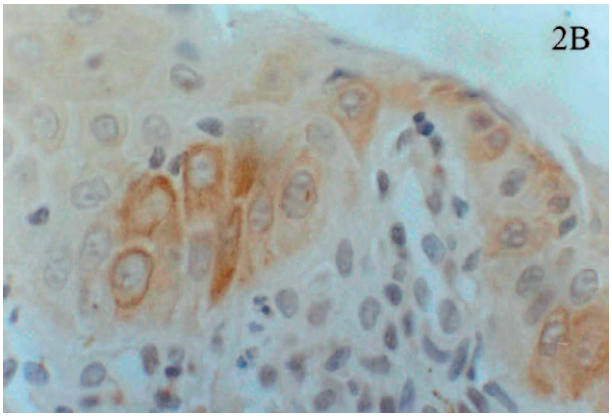


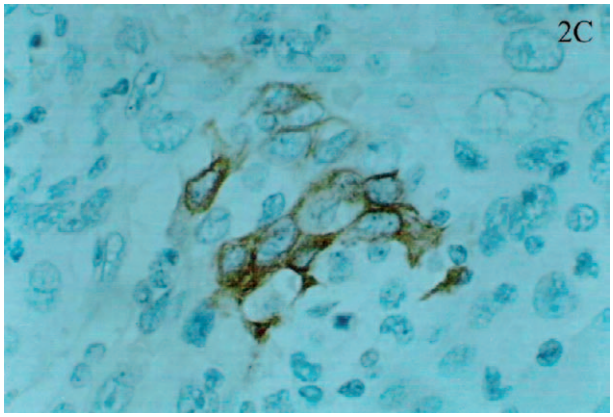


Slika1. Fotomikrografi koji pokazuju imunohistokemijsko bojenje na CK18 u normalnoj sluznici (1A), lichen planusu (1B) i karcinomu pločastih stanica (1C). (Imunohistokemijsko bojenje kontra-obojeno hematoksilinom, izvorno povećanje 200x)

Figure 1 Photomicrographs showing immunohistochemical staining for CK18 in normal mucosa (1A), lichen planus (1B) and squamous cell carcinoma (1C). (Immunohistochemical staining counterstained with hematoxylin, original magnification 200x)







Slika 2. Fotomikrografi koji pokazuju imunohistokemijsko bojenje na CK19 u normalnoj sluznici (2A), lichen planusu (2B) i karcinomu pločastih stanica (2C). (Imunohistokemijsko bojenje kontra-obojeno hematoksilinom, izvorno povećanje 200x)

Figure 2 Photomicrographs showing immunohistochemical staining for CK19 in normal mucosa (2A), lichen planus (2B) and squamous cell carcinoma (2C). (Immunohistochemical staining counterstained with hematoxylin, original magnification 200x)

(12) u kojemu nije pronađena nikakva imunoreaktivnost protiv CK18 u normalnoj ili u snuff-afektiranoj oralnoj sluznici te s ispitivanjem koje su obavili Chaiyarit i suradnici (13) u kojemu također nije pronađeno vidljivo bojenje na CK18 u lichen planusu. Samo 13,3% karcinoma pločastih stanica u ovom je ispitivanju pokazivalo pozitivno bojenje na CK18. To je također u skladu s ispitivanjem Suoa i suradnika (14), a u kojem je pronađeno nekoliko slučajeva pozitivne CK18- imunoreaktivnosti u karcinomima pločastih stanica, unatoč gotovo stopostotnoj pozitivnosti u karcinomima pločastih stanica larinksa i hipofarinksa. No, prosječna vrijednost indeksa CK18-obilježavanja kod karcinoma pločastih stanica u ovom istraživanju niska je i ne razlikuje se statistički od one u normalnoj sluznici i lichen- planusu. Zato se CK18 ne može koristiti kao biljeg za detekciju maligne transformacije u oralnoj sluznici.

U ovom istraživanju imunoreaktivnost na CK19 zapažena je dominantno u bazalnim stanicama normalne sluznice i u nekoliko ispitivanja (3,15,16). Lindberg i suradnici (17) također ističu da je CK19 najizraženiji u bazalnom sloju neorožnjene oralne sluznice, ali ne i u orožnjenoj sluznici. Su i suradnici (18) pronašli su i CK19 mRNA i njegovu bjelančevinu u neorožnjenom oralnom epitelu, ali samo CK19 mRNA u orožnjenom oralnom epitelu. Oni su, dakle, zaključili da ekspresija CK19 ovisi o regulaciji genske ekspresije u neorožnjenom oralnom epitelu, a regulirana je posttranskripcijski u orožnjenom epitelu. Maed i suradnici (19) detektirali su CK19- pozitivnost u malim lokaliziranim područjima u sloju bazalnih stanica u 23,53% svojih slučajeva. Naši su rezultati CK19-bojenja kod lichen planusa slični su onima Maeda i suradnika (19) - kako u postotku tako i u distribuciji u malim lokaliziranim područjima u bazalnom staničnom sloju, dok Ichikawa i suradnici (20) ističu negativno bojenje na CK19 u lichen planusu. Te bi se razlike mogle protumačiti različitim anatomskim lokusima i činjenicom da smo se koristili različitim klonovima protutijela i neidentičnim postupcima bojenja.

Alteracije u ekspresiji CK-a uočene su tijekom neoplastične transformacije (10,11)i te bi se promjene mogle pridružiti tumorigenezi, invaziji i metastazi (3). Onesposobljavanje dezmosoma zbog silazne regulacije, moglo bi uzrokovati gubitak adhezije i povećanje migratorne sklonosti (6). U ovom ispitivanju samo je jedan slučaj karcinoma pločastih stanica postigao pozitivno CK19-bojenje. Hansson i suradnici (3) također su došli do istoga rezultata - da samo nekoliko malignih bukalnih stanica u oga-

which found no immunoreactivity against CK18 in either normal or snuff-affected oral mucosa and the study by Chaiyarit et al (13) which also found no demonstrable CK18 staining in lichen planus. Only 13.3% of squamous cell carcinoma in the present study demonstrated positive CK18 staining. This is also in agreement with the study by Suo et al (14) which found few cases of positive CK18 immunoreactivity in oral squamous cell carcinoma despite almost a hundred percent positivity in squamous cell carcinoma of the larynx and hypopharynx. However, the mean CK18 labeling index of squamous cell carcinoma in the present study is low and not statistically different from that of normal mucosa and lichen planus. Hence, CK18 can not be used as a marker for the detection of malignant transformation of oral mucosa.

In the present study, CK19 immunoreactivity was encountered predominantly in the basal cells of normal mucosa as in several studies (3,15,16). Lindberg et al (17) also found that CK19 was mostly expressed in the basal layer of non-keratinized oral mucosa, but not in keratinized oral mucosa. Su et al (18) detected both CK19 mRNA and its protein in non-keratinized oral epithelium, but only CK19 mRNA in keratinized oral epithelium. They, thus, concluded that CK19 expression depended on the regulation of gene expression in non-keratinized oral epithelium and was regulated post-transcriptionally in keratinized epithelium. Maeda et al (19) detected CK19 positivity in small localized areas in the basal cell layer in 23.53% of their cases. Our results of CK19 staining in lichen planus were similar to those of Maeda et al (19) in both the percentage (30.0% vs 23.5%) and the distribution in small localized areas in the basal cell layer, while Ichikawa et al (20) reported negative staining for CK19 in lichen planus. These differences may be accounted for by a different anatomical sites and the fact that we used different antibody clones as well as non-identical staining procedures.

Alterations in CK expression have been detected during neoplastic transformation (10,11) and these alterations may be associated with tumorigenesis, invasion and metastasis (3). Impairment of desmosome due to keratin downregulation may lead to loss of adhesion and increase the migratory propensity (6). In the present study, only one case of squamous cell carcinoma elicited positive CK19 staining. Hansson et al(3) also found that only a few malignant buccal cells in organotypic culture gave positive staining to CK19. Some studies suggest-

notipnoj kulturi daje pozitivno bojenje na CK19. U nekim se ispitivanjima predlagalo da je CK19 silazno reguliran u invazivnom karcinomu pločastih stanica (6,11,17). Paladini i suradnici (21) predložili su da je potrebna silazna regulacija ekspresije CK19 u bazalnim stanicama, prije nego što bi se one mogle udaljiti od bazalne membrane.

Maligna bi transformacija mogla promijeniti ekspresiju CK-a i na razini transkripcije i posttranskripcije te bi se tako CK-ekspresija izgubila ili povećala. CK19 mogao bi biti dobar kandidat u skupini biljega korištenih u detekciji transformacije oralne sluznice u karcinom pločastih stanica. No, treba biti oprezan u interpretaciji rezultata CK19-imunobojenja, jer lezije poput lichen planusa mogu pokazivati smanjenu reaktivnost na CK19 zbog degeneracije bazalnih stanica u kojima se obično događa reaktivnost na CK19.

U zaključku istaknimo - ekspresija CK18 u normalnoj sluznici, lichen planusu i karcinomu pločastih stanica niska je i nema statističkih razlika između tih triju skupina lezija. Ekspresija CK19 visoka je u normalnoj sluznici, ali je niska u karcinomu pločastih stanica. Ekspresija CK19 u normalnoj sluznici statistički je viša od one u lichen planusu i karcinomu pločastih stanica. CK19 mogao bi biti dobar kandidat za uključivanje u skupinu protutijela za detekciju maligne transformacije oralne sluznice u karcinomu pločastih stanica.

Zahvale

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ed that CK19 was downregulated in invasive squamous cell carcinoma (6,11,17). Paladini et al (21) proposed that downregulation of CK19 expression in basal cells was required before basal cells could be able to migrate away from the basement membrane.

Malignant transformation may alter CK expression at both the transcriptional and post-transcriptional levels rendering specific CK expression either lost or increased. CK19 might be a good candidate in the panel of markers used for detecting transformation of oral mucosa into squamous cell carcinoma. However, caution must be exercised when interpreting the result of CK19 immunostaining since lesions such as lichen planus may exhibit reduced reactivity to CK19 because of the degeneration of basal cells in which reactivity to CK19 usually takes place.

In conclusion, CK18 expression in normal mucosa, lichen planus and squamous cell carcinoma is low and there is no statistical difference among the three groups of lesions. CK19 expression is high in normal mucosa, but low in squamous cell carcinoma. CK19 expression in normal mucosa is statistically higher than that of lichen planus and squamous cell carcinoma. CK19 might be a good candidate to be included in a panel of antibodies for detecting malignant transformation of oral mucosa into squamous cell carcinoma.

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Abstract
Cytokeratins (CK) are structural proteins in epithelial cells. Changes in cytokeratin expression have been observed during neoplastic transformation. Objectives: To evaluate cytokeratin 18 and 19 expression in normal mucosa, lichen planus and squamous cell carcinoma. Materials and methods: Thirty cases each of normal mucosa, lichen planus and squamous cell carcinoma were retrieved from the archives of the Department of Oral Diagnosis, Khon Kaen University. Tissue sections were stained with antibodies to cytokeratin 18 and 19. Immunoreactivity was evaluated by counting cells. The CK labeling index was calculated by dividing the number of positively stained cells x 100 by total number of cells counted. Results: 9 out of 30 (30.0%) normal mucosa, 5 out of 30 (16.7%) lichen planus and 4 out of 30 (13.3%) squamous cell carcinoma demonstrated positive staining for CK18. Squamous cell carcinoma showed the highest mean CK18 labeling index, followed in descending order by normal mucosa and lichen planus, respectively. Twenty three out of 30 (76.7%) normal mucosa, 9 out of 30 (30.0%) lichen planus and 1 out of 30 (3.33%) squamous cell carcinoma elicited positive staining for CK19. Normal mucosa elicited the highest mean CK19 labeling index, followed in descending order by lichen planus and squamous cell carcinoma, respectively. Conclusions: CK18 expression in normal mucosa, lichen planus and squamous cell carcinoma is low and there is no statistical difference among the three groups of lesions. CK 19 expression in normal mucosa is statistically higher than that of lichen planus and squamous cell carcinoma. CK19 might be a good candidate to be included in a panel of antibodies for detecting malignant transformation of oral mucosa into squamous cell carcinoma.

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References

1. Koch PJ, Roop DR. The role of keratins in epidermal development and homeostasis--going beyond the obvious. J Invest Dermatol. 2004;123(5):x-xi.
2. Steinert PM, Parry DA, Racoosin EL, Idler WW, Steven AC, Trus BL et al. The complete cDNA and deduced amino acid sequence of a type II mouse epidermal keratin of 60,000 Da: analysis of sequence differences between type I and type II keratins. Proc Natl Acad Sci U S A. 1984;81(18):5709-13.
3. Hansson A, Bloor BK, Haig Y, Morgan PR, Ekstrand J, Grafstrom RC. Expression of keratins in normal, immortalized and malignant oral epithelia in organotypic culture. Oral Oncol. 2001;37(5):419-30.
4. Bloor BK, Tidman N, Leigh IM, Odell E, Dogan B, Wollina U et al. Expression of keratin K2e in cutaneous and oral lesions: association with keratinocyte activation, proliferation, and keratinization. Am J Pathol. 2003;162(3):963-75.
5. Upasani OS, Vaidya MM, Bhisey AN. Database on monoclonal antibodies to cytokeratins. Oral Oncol. 2004;40(3):236-56.
6. Crowe DL, Milo GE, Shuler CF. Keratin 19 downregulation by oral squamous cell carcinoma lines increases invasive potential. J Dent Res. 1999;78(6):1256-63.
7. Clausen H, Moe D, Buschard K, Dabelsteen E. Keratin proteins in human oral mucosa. J Oral Pathol. 1986;15(1):36-42.
8. Chisholm JC, Houliston E. Cytokeratin filament assembly in the preimplantation mouse embryo. Development. 1987;101(3):565-82.
9. Kasper M, Karsten U, Stosiek P, Moll R. Distribution of intermediate-filament proteins in the human enamel organ: unusually complex pattern of coexpression of cytokeratin polypeptides and vimentin. Differentiation. 1989;40(3):207-14.
10. Trask DK, Band V, Zajchowski DA, Yaswen P, Suh T, Sager R. Keratins as markers that distinguish normal and tumor-derived mammary epithelial cells. Proc Natl Acad Sci U S A. 1990;87(6):2319-23.
11. Hu L, Crowe DL, Rheinwald JG, Chambon P, Gudas LJ. Abnormal expression of retinoic acid receptors and keratin 19 by human oral and epidermal squamous cell carcinoma cell lines. Cancer Res. 1991;51(15):3972-81.
12. Luomanen M, Tiitta O, Heikinheimo K, Heinaro I, Happonen RP. Effect of snuff on cytokeratin expression in oral vestibular sulcus epithelium. J Oral Pathol Med. 1997;26(3):110-6.
13. Chaiyarit P, Kafrawy AH, Miles DA, Zunt SL, Van Dis ML, Gregory RL. Oral lichen planus: an immunohistochemical study of heat shock proteins (HSPs) and cytokeratins (CKs) and a unifying hypothesis of pathogenesis. J Oral Pathol Med. 1999;28(5):210-5.
14. Suo Z, Holm R, Nesland JM. Squamous cell carcinomas. An immunohistochemical study of cytokeratins and involucrin in primary and metastatic tumours. Histopathology. 1993;23(1):45-54.
15. Coltrera MD, Zarbo RJ, Sakr WA, Gown AM. Markers for dysplasia of the upper aerodigestive tract. Suprabasal expression of PCNA, p53, and CK19 in alcohol-fixed, embedded tissue. Am J Pathol. 1992;141(4):817-25.
16. Ogden GR, Chisholm DM, Adi M, Lane EB. Cytokeratin expression in oral cancer and its relationship to tumor differentiation. J Oral Pathol Med. 1993;22(2):82-6.
17. Lindberg K, Rheinwald JG. Suprabasal 40 kd keratin (K19) expression as an immunohistologic marker of premalignancy in oral epithelium. Am J Pathol. 1989;134(1):89-98.
18. Su L, Morgan PR, Lane EB. Keratin 14 and 19 expression in normal, dysplastic and malignant oral epithelia. A study using in situ hybridization and immunohistochemistry. J Oral Pathol Med. 1996;25(6):293-301.
19. Maeda H, Reibel J, Holmstrup P. Keratin staining pattern in clinically normal and diseased oral mucosa of lichen planus patients. Scand J Dent Res. 1994;102(4):210-5.
20. Ichikawa E, Watanabe S, Takahashi H. Keratin and involucrin expression in discoid lupus erythematosus and lichen planus. Arch Dermatol Res. 1997;289(9):519-26.
21. Paladini RD, Takahashi K, Bravo NS, Coulombe PA. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. J Cell Biol. 1996;132(3):381-97.