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S100A8 calcium-binding expression in radicular and dentigerous cysts and in keratocystic odontogenic tumors

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Ekspresija kalcijum-vezujućeg S100A8 u radikularnim i dentogenim cistama keratocističnim odontogenim tumorima

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SUMMARY

Introduction: *Recently the term Keratocystic Odontogenic Tumor (KCOT) has been recommended for Odontogenic Keratocysts (OKC) to address the neoplastic nature of the lesion compared to radicular and dentigerous cysts. S100 are calcium-binding proteins involved in cell differentiation and inflammation, with a potential role in neoplastic transformation.*
Aim: *The aim of this study was to evaluate whether S100A8 protein expression is different in KCOT compared to radicular cysts (RC) and dentigerous cysts (DC).*
Methods: *A total of 84 consecutive odontogenic cysts, 34 RC, 25 DC, and 25 KCOT, were analyzed in this study.*
Results: *Epithelial cells in KCOT cases were not immunoreactive for S100A8 except focally in cases associated with inflammation, while RC cases showed a variable positivity of all the epithelial layers from the basal to the superficial in 19/34 cases and DC cases showed a weak positivity of the intermediate and superficial layers in 7/25 cases.*
Conclusion: *The lack of S100A8 protein expression seems to be observed more frequently in KCOT compared to RC and DC. This difference might be related to their neoplastic nature and a potential aggressive biological behavior for odontogenic cystic lesions.*

Key words: cell cycle proteins, S100A8/9, odontogenic tumours

KRATAK SADRŽAJ

Uvod: *Nedavno je izraz "Keratocistični odontogeni tumor (KCOT)" preporučen za odontogene keratociste (OKC) da bi se istakla neoplastična priroda ovih lezija u poređenju sa radikularnim i dentogenim cistama.*
Cilj: *Cilj ovog rada je bio da se ispita ekspresija S100A8 proteina u KCOT u poređenju sa radikularnim cistama (RC) i dentogenim cistama (DC).*
Materijal i metod: *Ukupno 84 odontogene ciste, 34 RC, 25 DC i 25 KCOT, su bile uključene u studiju.*
Rezultati: *Epitelne ćelije u slučajevima KCOT nisu pokazivale imunoreaktivnost na S100A8 osim u izvesnim slučajevima povezanim sa inflamacijom, dok su u slučajevima RC pokazivale varijabilnu pozitivnost u svim epitelnim slojevima, od bazalnog do superficijalnog, u 19/34 slučajeva, a kod DC slabu pozitivnost u srednjem i superficijalnom sloju u 7/25 slučajeva.*
Zaključak: *Odsustvo ekspresije S100A8 proteina je uočeno češće u KCOT nego kod RC i DC. Ova razlika može biti povezana sa neoplastičnom prirodom i potencijalno agresivnim ponašanjem kod odontogenih cističnih lezija.*

Ključne reči: proteini ćelijskog ciklusa, S100A8/9, odontogeni tumori

Introduction

Odontogenic keratocysts, considered develop-pmental abnormalities having origin from derivatives of the embryonic dental lamina, are unique among odontogenic cysts for their specific clinical and histologic features and their biologic behavior (1, 2): they are uni or multicystic, intraosseous tumors, with a characteristic lining of parakeratinized stratified squamous epithelium (3), with high proliferative activity and p53 expression (4). Allelic imbalance of tumor suppressor genes have been described in the majority of them (2, 3, 5). They can present extensive local invasion into adjacent structures and frequently they show recurrences (30-60%) (2, 5). Recently the WHO Working Group on odontogenic tumors recommended the term Keratocystic Odontogenic Tumor (KCOT) for these lesions instead of the traditional designation of OKC to address their neoplastic nature (3).

S100 are calcium-binding proteins of the EF-hand type that transmit calcium-dependent signals (6). These proteins are involved in a number of intracellular activities including membrane remodeling, calcium-related intracellular signaling, cytoskeleton dynamics, tissue homeostasis and formation of the cornified envelope of differentiated keratinocytes. They are also involved in the regulation of cell differentiation, energy metabolism, cytoskeletal membrane interactions and cell cycle progression . Twenty S100 proteins have been described. Their potential role in inflammatory processes and cancerogenesis is under discussion. These proteins are expressed in epithelial, endothelial and myeloid cells in response to inflammation (6). Phagocytes that express S100A8 have been found in many inflammatory diseases such as rheumatoid arthritis, inflammatory bowel and lung diseases (6-9). The levels of these proteins in gingival fluids of patients with some inflammatory diseases increase (10). In inflamed tissues, these proteins deposit onto the endothelium of venules associated with extravasating leukocytes (11).

These proteins are up-regulated in periodontitis and are detected at higher levels in gingival crevicular fluid and tissue specimens (12). The pattern of expression of these S100 proteins in the normal epithelium and in different conditions of deregulated differentiation strongly suggests that they are involved in epithelial differentiation pathways (6).

The aim of our study was to evaluate S100A8 protein expression in radicular cysts, dentigerous cysts, and KCOT to verify its potential role as a biological marker in defining the biological aggressiveness of odontogenic cysts.

Materials and methods

A total of 84 consecutive odontogenic cysts, 34 radicular cysts (RC), 25 dentigerous cysts (DC), and 25 keratocystic odontogenic tumors (KCOT) have been retrieved from the files of the Institute of Pathologic Anatomy and Histopathology of the Polytechnic University of the Marche, Ancona, Italy. No KCOT were associated with the Naevoid Basal Cell Carcinoma Syndrome (NBCCS).

Uvod

Odontogene keratociste, koje se smatraju razvojnim anomalijama poreklom od ostataka embrionalne dentalne lamine, jedinstvene su među odontogenim cistama zbog svojih specifičnih kliničkih i histoloških karakteristika i biološkog ponašanja.[1,2] To su uniili multicistični, intraosealni tumori sa karakterističnom pozicijom parakeratinizovanog, skvamoznog epitela[3], visoko proliferativnom aktivnošću i ekspresijom p53.[4] Alelni disbalans tumor-supresorskih gena opisan je u većini slučajeva.[2,3,5] Mogu pokazivati ekstenzivnu lokalnu invaziju u okolne strukture sa čestim recidivima (30-60%).[2,5] Nedavno je radna grupa o odontogenim tumorima SZO preporučila termin "keratocistični odontogeni tumor (KCOT)" za ove lezije umesto tradicionalnog OKC, u cilju isticanja njihove neoplastične prirode.[3]

S100 su kalcijum-vezujući proteini EF tipa koji prenose kalcijum-zavisne signale.[6] Ovi proteini su uključeni u veliki broj intracelularnih aktivnosti, kao što su membransko remodeliranje, intracelularni kalcijum-zavisni signali, dinamika citoskeleta, tkivna homeostaza i stvaranje omotača kod diferenciranih keratocista. Uključeni su i u regulaciju ćelijske diferencijacije, energetski metabolizam, interakcije citoskeletne membrane i progresiju ćelijskog ciklusa. Dvadeset S100 proteina je do sada opisano. Još uvek se diskutuje o njihovoj potencijalnoj ulozi u inflamatornim procesima i kancerogenezi. Ovi proteini pokazuju ekspresiju u epitelnim, endotelnim i mijeloidnim ćelijama u odgovoru na inflamaciju.[6] Fagociti koji eksprimiraju S100A8 su pronađeni u mnogim inflamatornim oboljenjima, kao npr. reumatodnom artritisu, zapaljenskim stanjima creva i pluća.[6-9] Nivoi ovih proteina rastu u gingivalnoj tečnosti pacijenata sa nekim inflamatornim oboljenima.[10] U inflamiranim tkivima, ovi proteini se nakupljaju u endotelu venula zajedno sa ekstravaziranim leukocitima.[11]

Ovi proteini rastu kod parodontitisa i otkrivaju se u većim količinama u gingivalnoj sulkusnoj tečnosti i uzorcima tkiva.[12] Obrazac ekspresije S100 proteina u normalnom epitelu i različitim stanjima poremećene diferencijacije snažno ukazuje na to da su uključeni u epitelijalnu diferencijaciju.[6]

Cilj ove studije je bio da ispita ekspresiju S100A8 proteina u radikularnim cistama, dentogenim cistama i KCOT i, eventualno, potvrdi njihova potencijalna uloga kao bioloških markera za određivanje biološke agresivnosti odontogenih cista.

Materijal i metod

Ukupno 84 odontogene ciste, 34 RC, 25 DC i 25 KCOT, su prikupljene na Institutu za patološku anatomiju i histopatologiju Politehničkog Univerziteta u Marheu, Ankona, Italija. Nijedan KCOT nije bio povezan sa Sindromom nevoidnog bazocelularnog karcinoma (NBCCS).

Epithelial dysplasia was not observed. Biopsy specimens, obtained during surgery, were fixed in 10% buffered formalin (24 to 48 h), dehydrated in graded alcohol, cleared in xylene and embedded in paraffin. The diagnosis was based on clinical, radiographic and histo-logical examinations. Using haematoxylin and eosin-stained sections, all histological slides were reviewed; the quality of the material was checked, and on a selection of the slides the immunohistochemical evaluation was performed.

Immunohistochemistry

Immunohistochemical staining was performed by using the standard avidin-biotin-peroxidase complex (ABC) method. Briefly, each 5-μm tissue section was deparaffinized, rehydrated, and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature. After rehydratation through a graded ethanol series, the sections were micro waved in zinc sulfate heptahydrate buffer at 90 °C for 10 minutes and then cooled to 30 °C. After incubation with normal goat serum for S100A8, the tissue sections were applied for 30 minutes and removed by blotting. The sections were then incubated with anti-S100A8 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a dilution of 1:100 in phosphate- buffered saline (PBS) containing 1% bovine serum albumin at 4 °C overnight, washed in PBS, and incubated with secondary antibody for 30 minutes at room temperature.

Immunohistochemistry was performed using the ABC system (Vectastain; Vector Laboratories, Burlingame, CA). The chromogen was 3,39-diaminobenzidine tetrahydrochloride applied as a 0.02% solution containing 0.0055% H2O2 in 50 mM ammonium acetate-citric acid buffer, pH 6.0. The sections were lightly counterstained with hematoxylin. A negative control was performed in all cases by omitting the primary antibody, and no detectable staining was evident. In normal tissue immunostaining of S100A8 was detected in the macrophages (positive control).

Evaluation of the staining for S100A8 protein

The S100A8-stained cells were evaluated in each case in all the epithelial layers. The DC, RC, and KCOT were subdivided into two groups: when more than 10% of the epithelial cells were positively stained, the specimen was considered to be S100A8 positive; when the positivity of the epithelial cells was less than 10%, the specimen was classified as S100A8 negative. Descriptive statistical analysis was performed for each group of cysts and the chi-square test was used to show statistically significant differences between the groups.

Nisu uočene epitelne displazije. Uzorci biopsija, dobijeni tokom hirurških intervencija, fiksirani su u 10% formalin (24 – 48 h), dehidrirani u alkoholu, očišćeni od ksilena i postavljeni u parafin. Dijagnoza je bazirana na kliničkom, radiografskom i histološkom nalazu. Uzorci su bojeni hematoksilin-eozinom i analizirani u pogledu kvaliteta materijala, a zatim je na odabranim uzorcima urađena imunohis-tohemijska analiza.

Imunohistohemija

Imunohistohemijsko bojenje je urađeno standardnom metodom pomoću kompleksa avidin-biotin-peroksidaze (ABC). Ukratko, svaki uzorak 5 μm debljine je deparafiniziran, rehidriran i inkubiran u svežem 0.3% hidrogen peroksidu u metanolu 30 min na sobnoj temperaturi. Posle rehidratacije kroz seriju različitih koncentracija etanola, preseci su tretirani na 90°C u cink-sulfat-heptahidratnom puferu 10 min, a zatim ohlađeni do 30°C.

Tkivni preseci su inkubirani sa antiS100A8 poliklonskim antitelima (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) u fosfatnom puferizovanim fiziološkom rastvoru (PBS) dilucije 1:100 koji je sadržavao i 1% albumin goveđeg seruma na 4°C preko noći, zatim su isprani u PBS-u i inkubirani sa sekundarnim antitelima 30 min na sobnoj temperaturi. Imunohistohemija je urađena primenom ABC sistema (Vectastain; Vector Laboratories, Burlingame, CA). Kao hromogen je primenjen 0.02% rasvor 3,39-diaminobenzidin tetrahidrohlorida koji je sadržao 0.0055% H_2O_2 u 50 mM puferu amonijum acetata i limunske kiseline, pH 6,0. Preseci su blago obojeni hematoksilinom. Negativna kontrola je urađena u svim slučajevima, izostavljanjem primarnih antitela, kada nije bilo uočljivo prebojavanje. U normalnom tkivu, imunobojenje S100A8 je bilo uočeno u makrofagima (pozitivna kontrola).

Ispitivanje prebojenosti za S100A8 protein

S100A8-prebojene ćelije su ispitivane u svim slučajevima u epitelnom sloju. DC, RC i KCOT su podeljeni u dve grupe: kada je više od 10% epitelnih ćelija bilo pozitivno prebojeno, uzorak je smatran pozitivnim na S100A8; kada je pozitivnost epitelnih ćelija bila prisutna u manje od 10% uzorka, on je klasifikovan kao negativan na S100A8. Deskriptivna statistička analiza urađena je u svakoj grupi cista, a χ^2 test je korišćen za utvrđivanje statistički značajnih razlika između grupa.

Results

Clinical features of the 82 odontogenic cysts are summarized in Table 1.

Variables	FC	RC	KCOT
N° of cases	25	34	25
Age (mean)	40 (range18-61)	52 (range21-66)	23 (range16-35)
Male	16	22	20
Female	9	12	5
Site **Maxillary** **Mandibular**	 11 14	 20 14	 5 20
Size (cm)	Range 1.5-2	Range 0,8-2	Range 1.5-3.8
Recurrence	0	0	4/25
Multiple	0	0	5/25

Table 1. *Clinical features of the 84 odontogenic cysts. FC = Dentigerous cysts, RC = radicular cysts, KCOT = keratocystic odontogenic tumors.*

Tabela 1. *Kliničke karakteristike 84 odontogene ciste. FC = Dentogene ciste, RC = radikularne ciste, KCOT = keratocistični odontogeni tumori.*

S100A8 expression in odontogenic cysts and the keratocystic odontogenic tumor is summarized in Table 2.

Rezultati

Kliničke karakteristike 84 odontogene ciste predstavljene su u Tabeli 1.

Ekspresija S100A8 u odontogenim cistama i KCOT sumirana je u Tabeli 2.

Table 2. *Correlation between S100A8 expression and histopatologic features of odontogenic cysts and keratocystic odontogenic tumor. RC = Radicular cysts, FC = Dentigerous cysts, KCOT = Keratocystic odontogenic tumors.*
Tabela 2. *Korelacija između ekspresije S100A8 i histopatoloških karakteristika odontogenih cista i keratocističnih odontogenih tumora. RC = radikularne ciste, FC = Dentogene ciste, KCOT = keratocistični odontogeni tumori.*

	N° of cases	*Positive*	*Negative*
RC	34	19	15
FC	25	7	18
PKCOT	25	0*	25

***: in 2 cases associated with inflammation a focal positivity in less than 10% of cells was observed.**
***: u 2 slučaja sa inflamacijom, primećena je fokalna pozitivnost u manje od 10% ćelija.**
Statistical analysis: KCOT versus Radicular and Dentigerous cysts ($p<0.0005$)

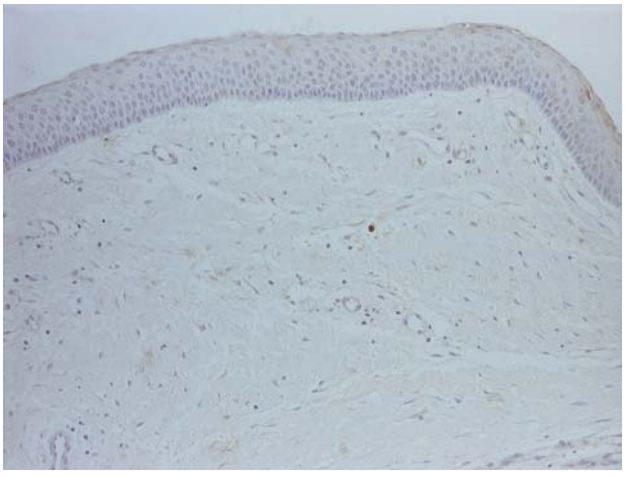

Figure 1. *KCOT: negativity of the all epithelial layers for S100A8 (magnification 200x).*

Slika 1. *KCOT: negativnost svih epitelnih slojeva prema S100A8 (uveličanje 200x).*

In KCOT epithelial cells of different layers were not immunoreactive for S100A8, except for a focal nuclear and cytoplasmic positivity in less than 10% of cells limited to the superficial layers in 2 cases associated with an inflammatory infiltrate.

U KCOT, epitelne ćelije različitih slojeva nisu bile imunoreaktivne na S100A8, osim kod izvesne fokalne nuklearne i citoplazmatske pozitivnosti u manje od 10% ćelija u superficijalnim slojevima u 2 slučaja koja su bila povezana sa inflamatornim infiltratom.

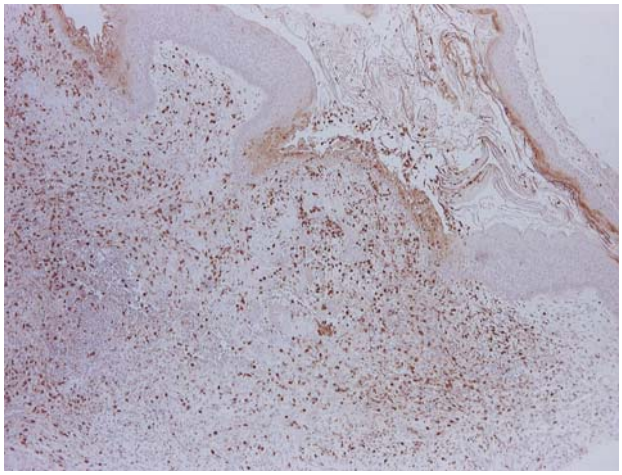

Figure 2. *In KCOT with the inflammatory infiltrate, focal cell positivity for S100A8 is present in the superficial layer (magnification 160x).*

Slika 2. *U KCOT sa inflamatornim infiltratom, mestimična ćelijska pozitivnost prema S100A8 prisutna je u površinskom sloju (uveličanje 160x).*

RC cases showed a variable positivity of all the epithelial layers, from the basal to the superficial, with more than 10% positive cells in 55% of cases. DC cases showed a weak nuclear and cytoplasmic positivity limited to the intermediate and superficial layers, and not in the basal cells. In DCs positivity was observed in more than 10% of cells in 28% of cases. A discrete number of positive cells in the stroma were present, mainly neutrophils and inflammatory cells, in the 3 groups without differences.

Slučajevi RC pokazali su varijabilnu pozitivnost u svim epitelnim slojevima, od bazalnog do superficijalnog, sa više od 10% pozitivnih ćelija u 55% slučajeva. Slučajevi DC pokazivali su slabu nuklearnu i citoplazmatsku pozitivnost ograničenu na srednje i superficijalne slojeve, ali ne i u bazalnim ćelijama. Kod DC, pozitivnost je uočena u više od 10% ćelija u 28% slučajeva. Ograničen broj pozitivnih ćelija je bio prisutan u stromi, pretežno neutrofili i ćelije zapaljenja, kod sve 3 grupe, bez razlike.

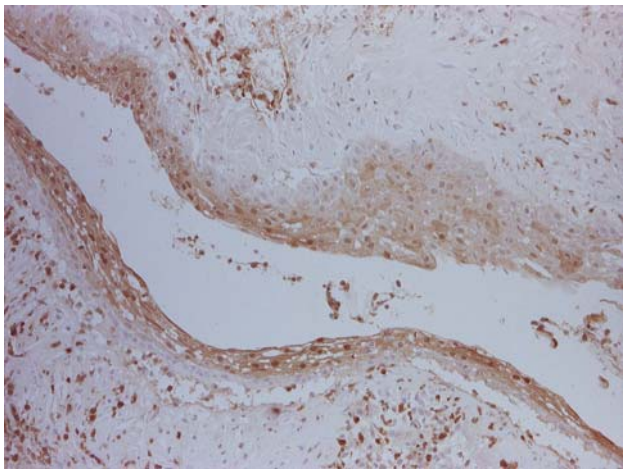

Figure 3. *A Dentigerous Cyst showing a variable positivity for S100A8 in the intermediate-superficial layers of the epithelium (magnification 160x).*

Slika 3. *Dentogena cista pokazuje varijabilnu pozitivnost prema S100A8 u srednjem i površinskom sloju epitela (uveličanje 160x).*

The fibroblasts were negative in all cases. Statistically significant differences of S100A8 expression in epithelial cells were observed between KCOT and the RC/DC group (chi-square test, $p<0.05$).

Fibroblasti su bili negativni u svim slučajevima. Statistički značajne razlike u ekspresiji S100A8 u epitelnim ćelijama uočene su između KCOT i RC/DC grupe (χ^2 test, $p<0.05$).

Discussion

In normal epidermis, S100A8 expression is correlated to the degree of keratinocyte differentiation, being lower in the basal layers compared to the superficial layers of differentiated cells (6,7). In gingival keratinocytes S100A8 is also related to the differentiation stage (13). In some inflammatory conditions, the expression of S100A8 is increased particularly in the stroma, such as in gingival overgrowth induced by cyclosporine A, where several positive cells were present inside the connective tissue, while, on the contrary, few cells were present in normal gingival (7). These positive cells corresponded either to activated macrophages or to neutrophils, reflecting the well-known gingival inflammation status associated with the cyclosporin-A treated specimens (7).

In the present study S100A8 was expressed in epithelial cells in 28% of radicular and 55% of dentigerous cysts, whereas the epithelial layers were negative in all KCOT cases. These findings brings us to hypothesize that the lack of epithelial S100A8 expression in KCOT is related to the neoplastic nature of these lesions. Down-regulation of S100A8 has been shown in carcinomas of the oral cavity associated with HPV-18 (14), in head and neck carcinomas, and it seems to be related in these condition to more aggressive behavior (15-17). Absence of S100A8 in epithelial cells should be listed as another biological marker that distinguishes KCOT from odontogenic cysts, like the higher expression of Bcl-2 protein (18), p53 (19), calretinin (20), and p63 (21-22) in the epithelial layers.

Conclusion

The small number of cases did not allow us to explore any difference of S100A8 expression in KCOT cases with recurrence. Larger series are needed to further investigations.

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Diskusija

U normalnom epidermu, ekspresija S100A8 povezana je sa stepenom diferencijacije keratinocita, pri čemu je manja u bazalnim nego u superficijalnim slojevima diferenciranih ćelija.[6,7] U gingivalnim keratinocitima, ekspresija S100A8 je takođe povezana i sa fazom diferencijacije.[13] U nekim inflamatornim stanjima, ekspresija S100A8 povećana je naročito u stromi, kao npr. kod hipertrofije gingive indukovane ciklosporinima A, gde su neke pozitivne ćelije prisutne u vezivnom tkivu dok, s druge strane, vrlo malo ćelija postoji u normalnoj gingivi.[7] Ove pozitivne ćelije su odgovarale bilo aktiviranim makrofagima bilo neutrofilima, potvrđujući dobro poznato stanje inflamacije gingive kod uzoraka tretiranih ciklosporinom A.[7]

U ovoj studiji, S100A8 se eksprimirao u epitelnim ćelijama u 28% radikularnih i 55% dentogenih cista, dok su epitelni slojevi bili negativni u svim KCOT slučajevima. Ova saznanja nas navode na hipotezu da je odsustvo ekspresije S100A8 u epitelu kod KCOT povezano sa neoplastičnom prirodom ovih lezija. Redukcija S100A8 je pokazana u karcinomima usne duplje koji su povezani sa HPV-18[14], u karcinomima glave i vrata, a izgleda da su povezani sa agresivnijim oblicima ovih stanja.[15-17] Odsustvo S100A8 u epitelnim ćelijama treba označiti kao još jedan biološki marker pomoću koga se KCOT razlikuju od odontogenih cista, pored veće ekspresije Bcl-2 proteina[18], p53[19], kalretinina[20] i p63[21,22] u epitelnim slojevima.

Zaključak

Mali broj uzoraka nije omogućio podrobnije ispitivanje različite ekspresije S100A8 u slučajevima KCOT sa recidivima. Veće serije su neophodne za dalje istraživanje.

References / Literatura

1. *Kolar Z, Geierova M, Bouchal J, Pazdera J, Zboril V, Turdy P.* Immunohistochemical analysis of the biological potential of odontogenic keratocysts. *J Oral Pathol Med* 2006; 35:75-80
2. *Agaram NP, Collins BM, Barnes L,* et al. Molecular analysis to demonstrate that odontogenic keratocysts are neoplastic. *Arch Pathol Lab Med* 2004; 128: 313-7
3. *Philipsen HP.* Keratocystic odontogenic tumour. In: *Barnes L, Eveson JW, Reichart P, Sidransky D* (EDS). Head and neck tumours. Pathology and Genetics. WHO Classification of tumours. *IARC Press, Lyon,* 2005: 306-7
4. *Henley J, Summerlin DJ, Tomich C, Zhang S, Cheng L.* Molecular evidence supporting the neoplastic nature of odontogenic keratocyst: a laser capturing microdissection study of 15 cases. *Histopathology* 2005; 47:582-6
5. *Roesch Ely M, Nees M, Karsai S,* et al. Transcript and proteome analysis reveals reduced expression of calgranulins in head and neck squamous cell carcinoma. *Eur J Cell Biol* 2005; 84:431-44
6. *Martinsson H, Yhr M, Enerback C.* Expression patterns of S100A7 (Psoriasin) and S100A9 (Calgranulin B) in keratinocyte differentiation. *Exp Dermatol* 2005; 14:161-8
7. *Echelard S, Hoyaux D, Hermans M,* et al. S100A8 and S100A9 calcium-binding proteins: localization within normal and cyclosporin A-induced overgrowth gingiva. *Connect Tissue Res* 2002; 43:419-24
8. *Stritz I, Jaresova M, Lacha J, Sedlacek J, Vitko S.* MRP 8/14 and procalcitonin serum levels in organ transplantation. *Ann Transplant* 2001; 6:6-9
9. *Lundy FT, Chalk R, Lamey PJ, Shaw C, Linden GJ* Quantitative anallysis of MRP-8 in gingival crevicular fluid in periodontal health and disease using microbore HPLC. *J Clin Periodontol* 2001; 28:1172-7
10. *Kido J, Nakamura T, Kido R,* et al. Alprotectin in gingival crevicular fluid correlates with clinical and biochemical markers of periodontal disease. *J Clin Periodontol* 1999; 26:653-7
11. *Robinson MJ, Tessier P, Poulsonr, Hogg N.* The S100 family heterodimer, MRP-8/14 binds with high affinity to heparin and heparan sulfate glycosaminoglycans on endothelial cells. *J Biol Chem* 2002; 277:3658-65
12. *Nisapakultorn K, Ross KF, Herzberg MC.* Calprotectin expression in vitro by oral epithelial cells confers resistance to infection by porphyromonas gingivalis. *Infect Immun* 2001; 69:4242-7
13. *Hayashi N, Kido J, Kido R,* et al. Regulation of calprotectin expression by interleukin-1alpha and tansforming growth factor-beta in human gingival keratinocytes. *J Periodontal Res* 2007; 42:1-7
14. *Lo WY, Lai CC, Hua CH,* et al. S100A8 is identified as a biomarker of HPV18-infected oral squamous cell carcinomas by suppression subtraction hybridization, clinical proteomics analysis, and immunohistochemistry staining. *J Proteome Res* 2007; 6:2143-51
15. *Hermani A, Hess J, De Servi B,* et al. Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. *Clin Cancer Res* 2005; 11:5146-52
16. *Cross SS, Hamdy FC, Deloulme JC, Rehman I.* Expression of S100 proteins in normal human tissues and common cancers using tissue microarrays: S100A6, S100A8, S100A9, and S100A11 are all overexpressed in common cancers. *Histopathology* 2005; 46:256-69
17. *Kong JP, Ding F, Zhou CN,* et al. Loss of myeloid-related proteins 8 and myeloid-related proteins 14 expression in human esophageal squamous cell carcinoma correlates with poor differentiation. *World J Gastroenterol* 2004; 10:1093-7
18. *Ohki K, Kumamoto H, Ichinohasama R, Sato T, Takahashi N, Ooya K.* PTC gene mutations and expression of SHH, PTC, SMO and GLI-1 in odontogenic keratocysts. *Int J Oral Maxillofac Surg* 2004; 33:584-92
19. *Piattelli A, Fioroni M, Rubini C.* Differentiation of odontogenic keratocysts from other odontogenic cysts by the expression of bcl-2 immunoreactivity. *Oral Oncology* 1998; 34:404-7
20. *Piattelli A, Fioroni M, Santinelli A, Rubini C.* p53 protein expression in odontogenic cysts. *J Endodon* 2001; 27:459-61
21. *Piattelli A, Fioroni M, Iezzi G, Rubini C.* Calretinin expression in odontogenic cysts. *J Endodon* 2003; 29:394-6
22. *Foschini MP, Cocchi R, Marucci G,* et al. High ΔN p63 isoform expression favours recurrences in odontogenic keratocyst – odontogenic keratocystic tumour. *Int J Oral Maxillofac Surg* 2006; 35:673-5

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