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Expression of CK8 and CK17, specific epithelial markers, by oral squamous cell carcinoma cell lines

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Objectives Oral squamous cell carcinoma (OSCC) is among the most common cancers worldwide. This study aimed to assess the expression of CK8 and CK17 by OSCC cell lines in Iranian patients.
Methods This observational study was conducted on three OSCC cell lines with a minimum of 45 passages. Cells isolated from three patients with OSCC were cultured and passaged. Expression of CK8 and CK17 epithelial markers was assessed using reverse-transcription polymerase chain reaction. Data were presented qualitatively.
Results The results confirmed the expression of CK8 and CK17 mRNA in three groups. Expression of these markers was different in the three specimens based on their degree of malignancy.
Conclusion Since CK8 and CK17 were expressed in different superficial and basal layers in the three specimens, epithelial origin of these cells was confirmed. This study can pave the way for further cancer research and designing new treatments.
Keywords carcinoma, cell line, mouth, squamous cell

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most widely occurring cancers worldwide[1] and is the eighth most common cancer in males throughout the world. However, it is not ranked among the top 10 cancers in females.[1,2] Different populations show variable incidence and mortality rates for OSCC and it is generally more common in developing countries.[1,3]

Standard treatments for these patients include surgery, chemotherapy, and radiotherapy. Despite the attempts for more efficient diagnosis and treatment of squamous cell carcinoma (SCC), 5-year survival of SCC patients in the past three decades remains around 50–60%.[4] Advances in treatment of these patients requires adequate knowledge about the main mechanisms of tumorigenesis and spread of tumoral cells.Culture of a cell line is one method for evaluation of tumor cell behavior in molecular level. HeLa cells isolated from tumors are highly valuable for biochemical and genetic research and evaluation of immunologic properties of cells. Homogeneity of the sample, numerous passages of cells, low cost, no ethical issues and more importantly, enabling the study of living cells are among the advantages of using these cell lines.[5]

Several markers have been identified for assessment of the properties and confirmation of SCC cell line such as Sox2, Oct4, CD44, CD133, MDM2, P53, and vascular endothelial growth factor.[6–11]

In general, tumor markers are divided into epithelial, connective tissue, and salivary gland types. Cytokeratins (CKs) are among the epithelial markers. As tumor markers, they have two main applications namely differentiating epithelial tumors from non-epithelial tumors and determining the type of epithelial tumor.[12] CK is a filament-forming protein that provides structural support for cells. In human epithelial cells, 20 subgroups of CKs are expressed and their type depends on the type of cell and location of CK in the cytoplasm.[13] Several studies have assessed the expression of different types of CKs in OSCC cell lines.[14,15]

CK8 has the widest range of expression in different types of epithelial and cancer cells among 20 types of CKs recognized so far.[13] CK8 is expressed on the surface of carcinoma cells in mammals in contrast to normal epithelial cells.[16,17] Also, increased expression of CK8 has been reported in colon, pancreatic, breast, and lung cancer.[18–20]

CK17 is a marker used for differentiation of basal cell layer in the epithelial complex. Its expression increases in laryngeal, pharyngeal, and lung cancer compared to normal tissue.[21]

Studies on this topic have been mainly conducted in the United States and have mostly focused on isolation of pharyngeal SCC cell line. Thus, this study aimed to assess the expression level of CK8 and CK17 markers in three SCC cell lines to describe the molecular profile of OSCC cell lines.

Materials and Methods

This *in vitro* observational study was conducted on OSCC cell lines retrieved from the cell bank of Shahid Beheshti University of Medical Sciences. The inclusion criterion was OSCC cell lines with a minimum of 45 passages. The exclusion criteria were poor quality of the specimen, no growth and proliferation in the culture medium and observing cell death under inverted microscope. The study protocol was approved in the ethics committee of our university.

Three specimens of OSCC were evaluated in this study. The first specimen had been obtained from the mandibular right alveolar mucosa of a 59-year-old female diagnosed with moderately differentiated OSCC. The second specimen belonged to a 74-year-old female with a previous history of

maxillary right alveolar mucosa OSCC with the diagnosis of recurrent well-differentiated SCC. The third specimen had been taken from the mandibular left gingiva of a 79-year-old male with the diagnosis of primary well-differentiated SCC.

Cell Culture

Specimens were cut into 2.5 × 2.5 cm^2 pieces and placed under a biological hood for culture. The specimens were first rinsed with antibiotic solution containing penicillin, streptomycin, and amphotericin four times to decrease the risk of contamination and increase the success of culture. The specimens were placed in capped dishes and minced by a scalpel. Enzyme solution containing 0.08% type I collagenase was added and after 3–5 min, the mixture was vibrated for 5 min. The mixture was then incubated for 30 min. The enzyme was then deactivated by complete culture medium containing 15% fetal bovine serum and centrifuged at 1,200 rpm for 5 min (C-28A; Boeco). The supernatant was discarded and 5 mL of culture medium (Dulbecco's modified Eagle's medium + Fungizone + antibiotic) was added to each of the four flasks. Using pipet filler, released cells in small flasks and unlysed cells in larger flasks were cultured and incubated (Thermo Scientific Heva Cell 150i). The culture media were evaluated daily and refreshed weekly. After 24 days, cells were passaged for the first time. After 45 passages, cells were frozen and passaged again for 14 times.

Designing CK8 and CK17 Primers and Reverse-Transcription Polymerase Chain Reaction

RNA Extraction

The cells were dissolved in 1 mL of Trizol, transferred into an Eppendorf tube and stored at room temperature for 5 min. For each 2 mL of the solution, 300 mL of chloroform was added to the mixture and stirred for 15 s followed by centrifugation at 12,000 *g* at 4°C. After centrifugation, the superficial aqueous phase was transferred to a new microtube for RNA extraction. Isopropanol was added in the same volume as the solution to the microtube. The contents of the tube were mixed and stored on ice for 45 min followed by centrifugation at 12,000 *g* for 15 min at 4°C. The supernatant was discarded and 1 mL of 75% ethanol was added to the sediment. The mixture was centrifuged at 7,500 *g* for 8 min at 4°C. The supernatant was discarded and the microtube containing the sediment was stored at room temperature for 10 min in order for the cell mass to dry. DNase containing DNase I (2 mL), DNase I (2 mL), RNase inhibitor (1 mL), and DEPC water 15 mL was added to the dried cell mass.

The solution was incubated at 37°C for 30 min. To inactivate DNase, 2 μL of EDTA was added to the solution and incubated at 65°C for 10 min. The concentration of RNA was determined by a spectrophotometer and the RNA was stored at −70°C.

Synthesis of cDNA from the Extracted RNA

For each 1 μL of extracted RNA, 1 μL of oligo DT primer and 10 μL of deionized water were added. The ingredients were mixed and vortexed for a few seconds. The mixture in the microtube was stored at 70°C for 5 min and was then immediately placed on ice. While placed on ice, the followings were added to the microtube: 4 μL of 5× reaction buffer, 1 μL of ribonuclease inhibitor and 2 μL of 10 mM dNTP mix. The microtube was stored at 37°C for 5 min. Next, 1 μL of reverse transcriptase (200 U/μL) was added and the microtube was placed in a thermocycler and heated as follows: 60 min at 42°C and 10 min at 70°C. After completion of cDNA synthesis in thermocycler, the tube containing cDNA was stored at 4°C.

Conduction of PCR

First, CK8 and CK17 gene primers were designed as target genes using NCBI website and confirmed using BLAST. The sequence of these primers is presented in Table 1.

The components of 25 μL PCR reaction solution included 100 ng genomic DNA, 1.5 pM/L MgCl2, 5 nM/L dNTPs, 2.5 pM/L of each primer, 2.5 μL of PCR buffer and one unit of Taq DNA polymerase.The PCR protocol included primary denaturation at 94°C for 5 min, 32 cycles including 30 s at 94°C, 1 min at 58°C for CK8 (the temperature was 59°C for CK19), 1 min at 72°C and final elongation phase at 72°C for 5 min. The PCR products were separated on agarose gel 2 containing SYBRsafe florescent dye and visualized with ethidium bromide staining. Data obtained from reverse-transcription polymerase chain reaction (RT-PCR) were expressed descriptively.

Also, to further confirm the cell line, morphology of the cells was evaluated under an inverted microscope (TS100; Nikon, Tokyo, Japan).

Results

Culture of OSCC cells from all the three specimens was done successfully. Morphology of the cells was evaluated under an inverted microscope and it was found that the cultured cells had epithelial origin.

After the conduction of RT-PCR, one specimen was excluded from the study due to poor quality of cell line. Figure 1 shows electro-photograph of the results of RT-PCR of CK8 and CK17 genes. CK8 was expressed by all three cell lines. In the first specimen (moderately differentiated SCC), CK8 had a higher expression compared to that in the remaining two specimens. The expression of CK17 was observed in all three specimens as well. CK17 showed higher expression in the first and second specimens, which were more invasive than the third specimen.

Table 1. **Sequence of CK8 and CK17 primers**

Primer	Primer sequence
CK8 Forward	5′-ATCGACATCGCCACCTACAG-3′
CK8 Reverse	5′-AGCTCAAACCACCCGCATAG-3′
CK17 Forward	5′-GAGAGGATGCCCACCTGACT-3′
CK17 Reverse	5′-TCCTCAGCGGGTGGTCTG-3′

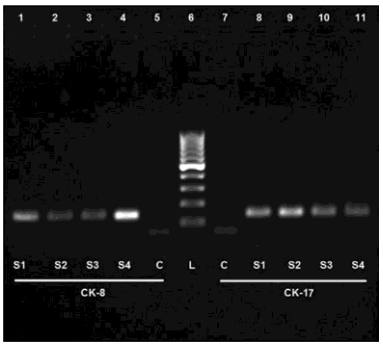

Fig 1. **Electron micrograph of the four primary specimens; S1: Moderately-differentiated SCC; S2: Recurrent well-differentiated SCC, S3: Primary well-differentiated SCC; S4: Excluded due to no regrowth.**

Figures 2–4 show photomicrographs of the three cell lines at different time points after culture.

Discussion

By isolation of stem cells and recognition of the biology of normal and cancer cells, attempts were made to determine the behavior of tumors and find new treatment modalities based on cellular and molecular evidence. At first, studies were focused mainly on paraffin blocks of patients. However, due to differences with *in vivo* conditions, the results of these studies could not be generalized to the clinical setting to accurately reveal the actual behavior of tumors *in vivo*. Over time, cell proteins in paraffin blocks are degraded and this negatively affects the results of immunohistochemistry and PCR studies on these blocks. Thus, researchers tried to culture the cells. Since cancer cells are different from normal cells in growth and differentiation, they can be purified and used as immortal cell lines. These cells can be cultured for unlimited times and used for assessment of tumor behavior with no risk for patients.[22]

The first studies on cell lines were started in 1960 in the United States.[23] Since SCCs of the cervix and esophagus are more common than OSCC, the primary studies focused on culture of SCC cells from the cervix. After 5 years, tissue specimens were obtained from patients with well-differentiated SCC of the cervix and successfully cultured. This cell line is currently known as the HeLa cells. This cell line has been passaged close to 1 million times and has been confirmed.[24–26]In the next phase, emphasis was placed on cell line isolation from SCC of the pharynx, which resulted in extraction of three cell lines. Two cell lines were isolated in the United States from two well differentiated specimens known as HSCC1 and HSCC2 and are non-purchasable for cellular studies.[27,28] The third cell line was extracted in Ireland from a well-differentiated SCC specimen commercially known as FADU, which can be purchased and this cell line has been extensively used for research purposes.[29] Well-differentiated SCC cell line has been used in all previous studies for cell culture since it is easy to culture and has a tendency for organization of cells next to each other resulting in faster stabilization of this cell line in culture medium.

In the recent years, it has been confirmed that the clinical behavior of tumors with different grades is variable. Also, tumors are different in terms of potential for growth, destruction and metastasis as well as tumor response to radiotherapy and chemotherapy. Thus, different cell lines are required for research purposes.[30,31]

Inour study, in contrast toprevious studies, three completely different specimens were used for culture. These cells had

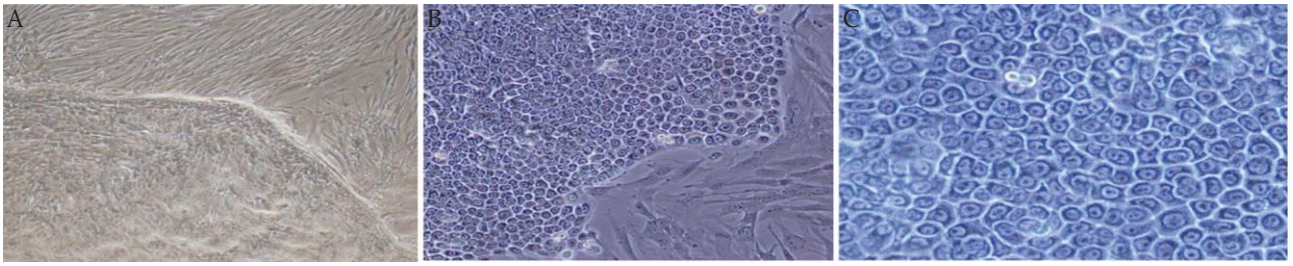

Fig 2. **Photomicrograph of OSCC1 (A) Four days after culture at 200× magnification; (B) four days after first trypsinization at 200× magnification; (C) final purified cell line at 400× magnification.**

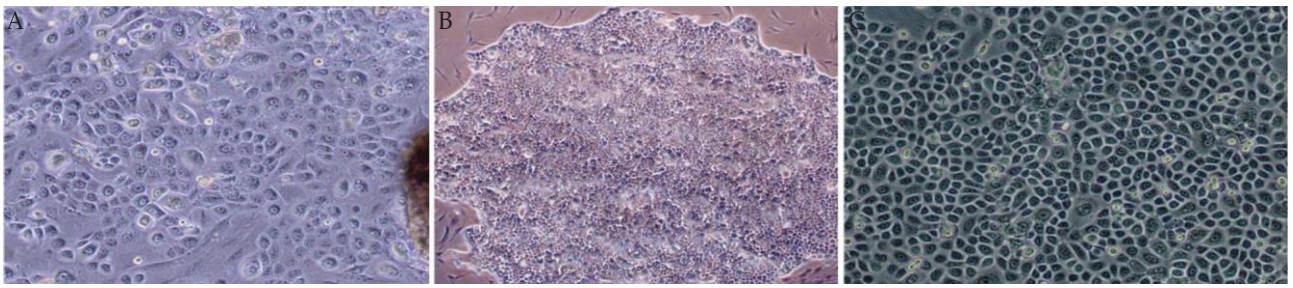

Fig 3. **Photomicrograph of OSCC2 (A) Four days after culture at 200× magnification; (B) four days after first trypsinization at 200× magnification; (C) final purified cell line at 400×.**

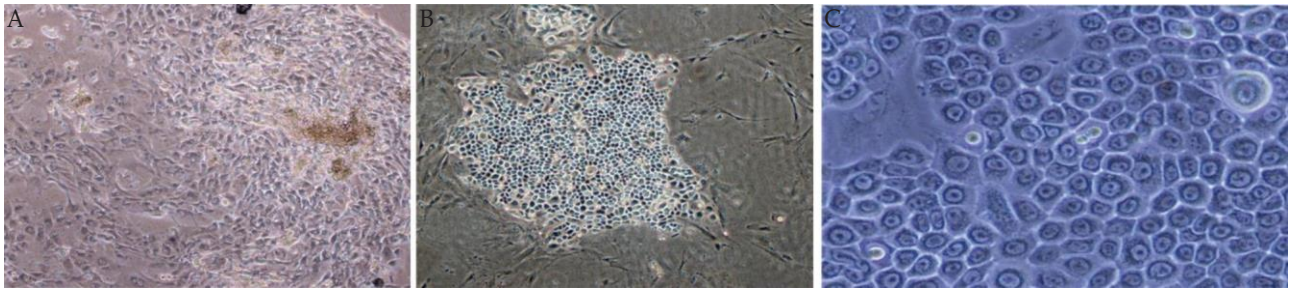

Fig 4. **Photomicrograph of OSCC3 (A) Four days after culture at 200× magnification; (B) four days after first trypsinization at 200× magnification; (C) final purified cell line at 400×.**

different grades and three distinct properties: First, the underlying and confounding factors were standardized in the three specimens and equal culture conditions were provided for all the three. Second, none of the specimens had metastasis at the time of surgery and third, it was possible to compare growth pattern among the specimens. The proliferation rate of cells in culture medium was different from one another and followed the clinical pattern of tumor growth. Comparison of the three cultured specimens revealed that OSCC1 had moderate growth and disseminated growth pattern while OSCC2 (tumor recurrence) had higher proliferation rate and more scattered growth pattern. The OSCC3 had lower proliferation rate than the other two and a more regular growth pattern. Comparison of the three specimens revealed that tumor grade had a direct association with growth pattern of cells in culture medium. On the other hand, it has been confirmed that tumor grade directly relates to prognosis and metastasis potential of tumor. Thus, it may be concluded that growth pattern of cells in the culture medium has a direct association with prognosis and metastasis.

The PRMI culture medium is better than phosphate buffered saline for growth and proliferation of cells and enhances the culture conditions for cell proliferation especially in-between passage of cells.[4]

In our study, to register the cell line, we tried our best to use specific markers for epithelial cells. Thus, CK8 and CK17 cytokeratin markers were used. One of these markers is for the superficial layer and the other one is for the basal layers of epithelium. Use of these markers enabled assessment of different epithelial layers. Nobusawa et al.[32] showed strong expression of CK17 in moderate dysplasia and *in situ* carcinoma, which suggests that CK17 expression is a marker with high specificity for neoplastic changes. According to Escobar-Hoyos et al.[33] CK17 has 94% sensitivity and 86% specificity for differentiation of OSSC from normal mucosa. Also, CK17 is correlated with poorer prognosis in SCC patients. There is a hypothesis that expression of CK17 in SCC affects stem cell properties of tumor and can be a predictor of treatment failure. In our study, increased expression of CK17 was noted in the majority of SCCs, HSIL and immature metaplasia while Kitamura et al.[34] and Toyoshima et al.[35] showed higher expression of CK17 in well-differentiated types of SCC. But, in our study expression of CK17 was equally higher in recurrent well-differentiated and moderately-differentiated types.

Expression of both CK8 and CK17 has been noted in carcinoma of the cervix and their expression increases from normal tissue to invasive carcinoma. Both markers have high specificity for malignant transformation while their sensitivity is low. Also, CK8 can serve as a specific marker for malignant transformation in pre-invasive stage.[12] However, Carrilho et al.[36] reported increased expression of CK8 and CK17 in invasive carcinomas. Martens et al.[37] showed that CK8 alone or in combination with CK17 can confirm higher invasion. Brotherick et al.[38] demonstrated high expression of CK8 in metastatic SCC. Since in our study expression of CK8 was higher in moderately differentiated SCC specimen compared to the other two well-differentiated types, our results confirmed the findings of previous studies.

Cells obtained in our study can serve as a source for studies on treatment of different head and neck cancers particularly OSCC. Studies on the efficacy of drugs and treatment modalities require large volume of cells, which may not be obtained from tissue specimens. By isolation and purification of these cells, an unlimited cell source can be achieved. On the other hand, by purifying these cells, their pure response to treatment protocols can be identified because several parameters related to tissues, such as cells and their secretions can interfere with the treatment protocol as a confounder and affect the results. Studies on cell lines are useful for treatment of cancer and assessment of tumor behavior.

Two out of three isolated cells were cultured for the first time in Iran. RT-PCR was also performed to confirm the cell lines had epithelial origin. Future studies are required for immunohistochemical assessment of isolated cells. Also, immortalization of the cells should be performed to introduce them as cell lines. This study was a preliminary study to pave the way for evidence-based introduction of new treatment protocols to improve prognosis of OSCC patients.

Conclusion

Considering the expression of CK8 and CK17 in superficial and basal layer of epithelium in our three specimens and the differences in their expression based on the malignancy grade, these markers can be used as epithelial specific markers with more specificity in malignancies. Also, cells obtained from this study can be a reliable source for researches in the field of head and neck cancers, especially OSCCs to introduce cell lines. This study can be a basis for further cancer research and designing new treatments.

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Conflicts of Interest

No conflict of interest to declare. n

References

1. Petersen PE. Oral cancer prevention and control—The approach of the World Health Organization. Oral Oncol. 2009;45:454–460.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011r;61:69–90.
3. de Camargo Cancela M, de Souza DL, Curado MP. International incidence of oropharyngeal cancer: a population-based study. Oral Oncol. 2012;48:484–490.
4. Hamid S, Lim KP, Zain RB, Ismail SM, Lau SH, Mustafa WM, et al. Establishment and characterization of Asian oral cancer cell lines as in vitro models to study a disease prevalent in Asia. Int J Mol Med. 2007;19:453–460.
5. Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer. 2005;114:806–816.
6. Kendall K, Repp MR, Jilka T, Kingsley K. Biomarker screening of oral cancer cell lines revealed sub-populations of CD133-, CD44-, CD24- and ALDH1-positive cancer stem cells. J Cancer Res Ther. 2013;1:111–118.
7. Lin CJ, Grandis JR, Carey TE, Gollin SM, Whiteside TL, Koch WM, et al. Head and neck squamous cell carcinoma cell lines: established models and rationale for selection. Head Neck. 2007;29:163–188.
8. Zhang Z, Filho MS, Nor JE. The biology of head and neck cancer stem cells. Oral Oncol. 2012;48:1–9.

9. Felthaus O, Ettl T, Gosau M, Driemel O, Brockhoff G, Reck A, et al. Cancer stem cell-like cells from a single cell of oral squamous carcinoma cell lines. Biochem Biophys Res Commun. 2011r;407:28–33.
10. Ue T, Yokozaki H, Kagai K, Higashikawa K, Yasui W, Sugiyama M, et al. Reduced expression of the CD44 variant exons in oral squamous cell carcinoma and its relationship to metastasis. J Oral Pathol Med. 1998;27:197–201.
11. Sano T, Hikino T, Xue Q, Saito T, Kashiwabara K, Oyama T, et al. Immunohistochemical inactivation of p14ARF concomitant with MDM2 overexpression inversely correlates with p53 overexpression in oral squamous cell carcinoma. Pathol Int. 2000;50:709–716.
12. Kudo Y, Takata T, Ogawa I, Sato S, Nikai H. Expression of p53 and p21CIP1/WAF1 proteins in oral epithelial dysplasias and squamous cell carcinomas. Oncol Rep. 1999y;6:539–545.
13. Mohamed KM, Le A, Duong H, Wu Y, Zhang Q, Messadi DV. Correlation between VEGF and HIF-1alpha expression in human oral squamous cell carcinoma. Exp Mol Pathol. 2004;76:143–152.
14. Choontharu MM, Binda A, Bhat S, Sharma SM. Role of tumor markers in oral squamous cell carcinoma: review of literature and future consideration. SRM J Res Dent Sci. 2012t;3:251–256.
15. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell. 1982v;31:11–24.
16. Crowe DL, Milo GE, Shuler CF. Keratin 19 downregulation by oral squamous cell carcinoma lines increases invasive potential. J Dent Res. 1999;78:1256–1263.
17. Fillies T, Jogschies M, Kleinheinz J, Brandt B, Joos U, Buerger H. Cytokeratin alteration in oral leukoplakia and oral squamous cell carcinoma. Oncology reports. 2007;18:639–644.
18. Godfroid E, Geuskens M, Dupressoir T, Parent I, Szpirer C. Cytokeratins are exposed on the outer surface of established human mammary carcinoma cells. J Cell Sci. 1991 Jul;99:595–607.
19. Hembrough TA, Vasudevan J, Allietta MM, Glass WF 2nd, Gonias SL. A cytokeratin 8-like protein with plasminogen-binding activity is present on the external surfaces of hepatocytes, HepG2 cells and breast carcinoma cell lines. J Cell Sci. 1995;108:1071–1082.
20. Sundstrom B, Stigbrand T. A two-site enzyme-linked immunosorbent assay for cytokeratin 8. Int J Cancer. 1990;46:604–607.
21. Abd El-Rehim DM, Pinder SE, Paish CE, Bell J, Blamey RW, Robertson JF, et al. Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol. 2004;203:661–671.
22. Karlsson C, Karlsson MG, Effects of long-term storage on the detection of proteins, DNA, and mRNA in tissue microarray slides. J Histochem Cytochem. 2011;59:1113–1121.
23. Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG. The unlabeled antibody method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem. 1970;18:315–333.
24. Lechner MG, Lade S, Liebertz DJ, Prince HM, Brody GS, Webster HR. Breast implant-associated ALK-negative, T-cell, anaplastic, large-cell lymphoma: establishment and characterization of a model cell line (TLBR-1) for this newly emerging clinical entity. Cancer. 2011;117:1478–1489.
25. Hermann RM, Fuzesi L, Pradier O, Christiansen H, Schmidberger H. Presence of human papillomavirus-18 and Epstein-Barr virus in a squamous cell carcinoma of the tongue in a 20-year-old patient. Case report and review of the current literature. Cancer Radiother. 2004g;8:262–265.
26. Higa M, Kinjo T, Kamiyama K, Chinen K, Iwamasa T, Arasaki A, et al. Epstein–Barr virus (EBV)-related oral squamous cell carcinoma in Okinawa, a subtropical island, in southern Japan—Simultaneously infected with human papillomavirus (HPV). Oral Oncol. 2003;39:405–414.
27. Erdem NF, Carlson ER, Gerard DA. Characterization of gene expression profiles of 3 different human oral squamous cell carcinoma cell lines with different invasion and metastatic capacities. J Oral Maxillofac Surg. 2008;66:918–927.
28. Issa A, Le TX, Shoushtari AN, Shields JD, Swartz MA. Vascular endothelial growth factor-C and C–C chemokine receptor 7 in tumor cell-lymphatic cross-talk promote invasive phenotype. Cancer Res. 2009;69:349–357.
29. Sok JC, Kuriakose MA, Mahajan VB, Pearlman AN, DeLacure MD, Chen FA. Tissue-specific gene expression of head and neck squamous cell carcinoma in vivo by complementary DNA microarray analysis. Arch Otolaryngol Head Neck Surg. 2003;129:760–770.
30. Warshamana-Greene GS, Litz J, Buchdunger E, Garc-a-Echeverr-a C, Hofmann F, Krystal GW. The insulin-like growth factor-I receptor kinase inhibitor, NVP-ADW742, sensitizes small cell lung cancer, cell lines to the effects of chemotherapy. Clin Cancer Res J. 2005;11:1563–1571.
31. Adamsen BL, Kravik L, de Angelis PM. Cellular response to chemoradiotherapy, radiotherapy and chemotherapy in two colorectal cancer cell lines. Radiat Res. 2009;171:562–571.
32. Nobusawa A, Sano T, Negishi A, Yokoo S, Oyama T. Immunohistochemical staining patterns of cytokeratins 13, 14, and 17 in oral epithelial dysplasia including orthokeratotic dysplasia. Pathol Int. 2014n;64:20–27.
33. Escobar-Hoyos LF, Yang J, Zhu J, Cavallo JA, Zhai H, Burke S, et al. Keratin 17 in premalignant and malignant squamous lesions of the cervix: proteomic discovery and immunohistochemical validation as a diagnostic and prognostic biomarker. Mod Pathol. 2014;27:621–630.
34. Kitamura R, Toyoshima T, Tanaka H, Kawano S, Kiyosue T, Matsubara R, et al. Association of cytokeratin 17 expression with differentiation in oral squamous cell carcinoma. J Cancer Res Clin Oncol. 2012;138:1299–1310.
35. Toyoshima T, Vairaktaris E, Nkenke E, Schlegel KA, Neukam FW, Ries J. Cytokeratin 17 mRNA expression has potential for diagnostic marker of oral squamous cell carcinoma. J Cancer Res Clin Oncol. 2008;134:515–521.
36. Carrilho C, Alberto M, Buane L, David L. Keratins 8, 10, 13, and 17 are useful markers in the diagnosis of human cervix carcinomas. Hum Pathol. 2004;35:546–551.
37. Martens J, Baars J, Smedts F, Holterheus M, Kok MJ, Vooijs P, et al. Can keratin 8 and 17 immunohistochemistry be of diagnostic value in cervical cytology? A feasibility study. Cancer. 1999r;87:87–92.
38. Brotherick I, Robson CN, Browell DA, Shenfine J, White MD, Cunliffe WJ, et al. Cytokeratin expression in breast cancer: phenotypic changes associated with disease progression. Cytometry. 1998;32:301–308.

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