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RESEARCH

Immunolocalization of Vimentin, Keratin 17, Ki-67, Involucrin, β-Catenin and E-Cadherin in Cutaneous Squamous Cell Carcinoma

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Abstract Skin squamous cell carcinoma (SCC) is a subtype of very aggressive skin cancers. To investigate if epithelial-mesenchymal transition (EMT), a process for epitheloid cells losing their polarity and cohesiveness and transform into spindle-shaped cells, occurs in skin SCC. By using immunofluorescence, we defined the immunolocalization of vimentin, Keratin 17, β-catenin, E-cadherin, Ki-67 and involucrin, in SCC samples. Our results show reduced activity of involucrin and E-cadherin, and increased expression of Ki-67, β-catenin, Keratin 17 and vimentin in SCC. These data propose that EMT really occurs in poorly differentiated SCC and keratin 17 and involucrin may be another two biomarkers for EMT.

Keywords Squamous cell carcinoma · Epithelial-mesenchymal transition · Immunolocalization

Introduction

Skin squamous cell carcinoma (SCC) is a subtype of very aggressive skin cancers that usually develops in sunexposed body regions, but can also affect a large number of organs such as the bladder, esophagus, lungs etc. However, little is known about the biology of these cells, which consequently makes difficult the generation of new specific therapies; actually, the standard treatments are based on surgery and subsequent radiotherapy. Epithelial-mesenchymal transition (EMT) [1, 2], which is defined as epitheloid cells lose their polarity and cohesiveness and transform into spindle-shaped cells that are more fibroblast- or myofibroblast-like [3–7].

The EMT is a key developmental program that is often activated during cancer invasion and metastasis [8]. A defining characteristic of EMT is the loss of epithelial phenotypes and the acquisition of a mesenchymal phenotype, including attenuation of E-cadherin, ZO-1, cytokeratins, laminin 1 and microRNA-200 family, and acquisition of N-cadherin, vimentin, β-catenin, fibronectin, snail, slug and α-SMA, etc. [7]. To investigate the possible role of EMT in development of SCC, SCC samples from 26 patients and 12 normal skin biopsies were studied.

Through immunofluorescent analysis, we have examined the expression of molecular biomarkers of EMT, including Vimentin, Keratin 17, β-catenin and E-cadherin, and proliferation marker Ki-67, differentiation marker Involucrin, in SCC samples.

Materials and Methods

Subjects

The study was approved by the Ethical Review Board of Second Affiliated Hospital, Zhejiang University School of Medicine. The experiments were undertaken with the understanding and written consent of each subject, and conforms with Declaration of Helsinki. In 26 patients (9 females, 17 males; mean age: 68±15 years) and 12 normal controls (5 females, 7 males, mean age 50±16 years). Biopsy specimens of cSCC and control specimens from an healthy skin site were obtained during surgical removal of the tumor and cosmetic surgery. Specimens were immediately stored in 10 % buffered formaldehyde.

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Immunofluorescence

Immunofluorescence was performed according to previous publication [9]. In brief, cSCC and normal skin biopsies were fixed in formalin, embedded in paraffin and cut into 4-μm serial sections using a microtome. Tissue sections were deparaffinized, rehydrated and antigen retrieval was performed by heating at 95 °C for 20 min in sodium citrate buffer (10 mM, pH 8.5). Blocking was performed with 10 % normal rabbit serum for 1 h at room temperature. Sections were then incubated with a mouse anti-human Vimentin, Keratin 17, Ki-67, Involucrin, β-catenin and E-cadherin antibody (Santa Cruz, CA, USA) overnight at 4 °C, followed by an Alexa Fluor 594-conjugated rabbit anti-mouse secondary antibody (AF-594, Invitrogen) for 2 h at room temperature. Immunofluorescent images were obtained using a fluorescence microscope (Olympus B202; Carson Group Inc., Markham, ON) with a digital camera.

Results

Immunolocalization of Ki-67, Keratin 17, Vimentin, E-Cadherin, β-Catenin and Involucrin in Normal Skin

In normal skin, Ki-67 were scattered in the nuclei of epidermal keratinocytes (Fig. 1a). Little or no signal of Keratin 17 (Fig. 1b) and Vimentin (Fig. 1c) is detected in normal epidermis. In the dermis, no signal of Keratin 17 is displayed. However, for Vimentin, strong signal could be seen in the dermis. Furthermore, intense homogenous membranous immunofluorescent signal of E-cadherin (Fig. 1d) and β-catenin (Fig. 1e) is stained in the whole layer of the epidermis, but not in the dermis. In addition, in normal skin, involucrin is exclusively detected in the granular keratinocytes and upper stratum spinous keratinocytes (Fig. 1f).

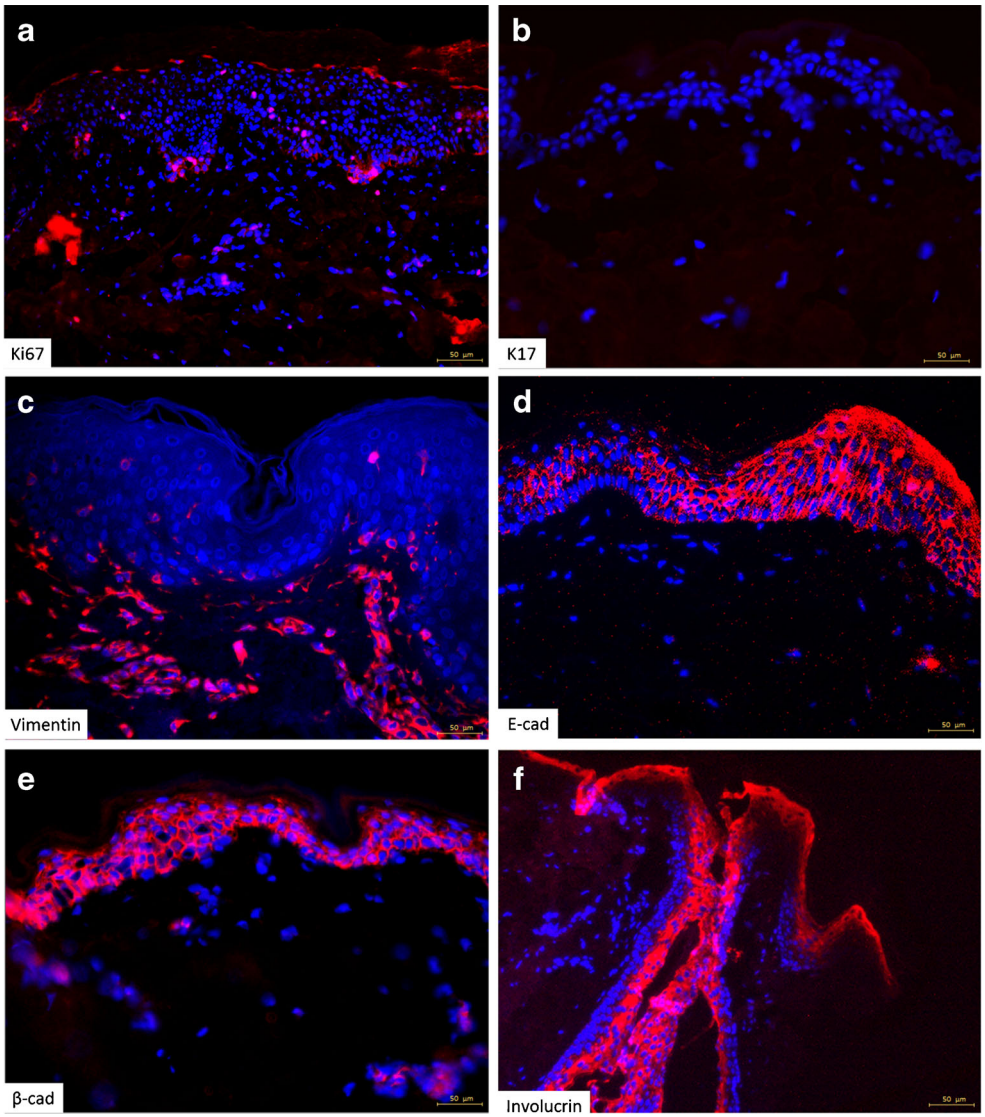

Fig. 1 Immunolocalization of **a** Ki-67, **b** Keratin 17, **c** Vimentin, **d** E-cadherin, **e** β-catenin, **f** Involucrin in normal skin

Immunolocalization of Ki-67, Keratin 17, Vimentin, E-Cadherin, β-Catenin and Involucrin in cSCC

In cSCC tissues, we also examined the immunolocalization of Ki-67, Keratin 17, Vimentin, E-cadherin, β-catenin and Involucrin. An obvious overexpression of Ki-67 in the nuclei of cSCC cells could be observed, especially in the poorly differentiated carcinoma cells. No signal of Ki-67 could be detected in these well differentiated cells, such as keratin pearl (Fig. 2a). In contrast to Ki-67, immunofluorescent signal of Keratin 17 is homogenous distributed in the whole cSCC tissue, not only in poorly differentiated cells, but also in well-differentiated cells (Fig. 2b). For Vimentin and β-catenin, little signal is visualized in well differentiated cells, but intense signal could be observed in poorly differentiated cells (Fig. 2c and e). However, E-cadherin and involucrin are reduced or absent in the poorly differentiated cells, but strong in well differentiated cells (Fig. 2d and f).

Discussion

SCC is generally a proliferative exophytic tumor growing moderately rapidly over months [10]. There are many factors that contribute to the aggressiveness, recurrence rate, and metastatic potential of squamous cell carcinomas. The nuclear Ki-67 protein is a cellular marker for proliferation [11, 12]. Previous studies had defined the expression of Ki-67 in cSCC [13]. Our study also demonstrated increased expression of Ki-67 in cSCC, as compared with normal epidermis. Furthermore, most Ki-67 localized in the poorly differentiated cells,

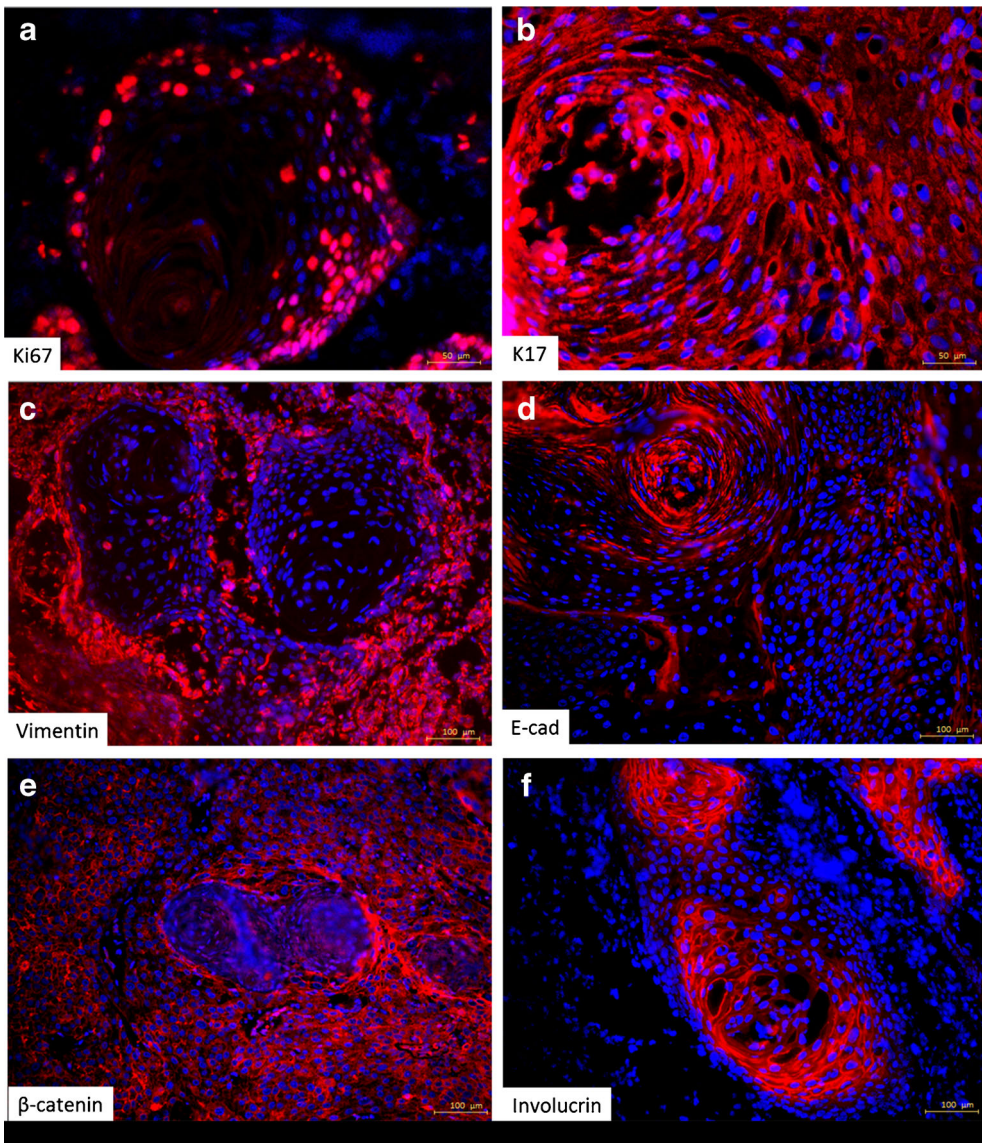

Fig. 2 Immunolocalization of **a** Ki-67, **b** Keratin 17, **c** Vimentin, **d** E-cadherin, **e** β-catenin, **f** Involucrin in squamous cell carcinoma

not in the well differentiated cells. This indicates that Ki-67 could be a marker for differentiation of SCC.

cSCC can express vimentin, especially in the poorly differentiated cells [14]. This finding underscores the need for caution in the use of immunohistochemical stains for tumor typing [14]. Vimentin is molecular marker for EMT. Acquisition of vimentin in SCC suggests that EMT really occurred in cSCC.

To further confirmed EMT in SCC, other two biomarkers of EMT, E-Cadherin and β-catenin, were investigated. Expression of E-cadherin is only limited in well differentiated cells, but reduced or absent in the poorly differentiated cells. On the contrary, β-catenin is strong in poorly differentiated cells, and absent in well differentiated cells. Increased β-catenin predisposes SCC patients to poor prognosis and survival [15]. These data further confirms that EMT may occur in SCC.

Keratin 17 could promote epithelial proliferation and tumor growth by polarizing the immune response in skin [16]. Keratin 17 expression may prove prognostically helpful when assessing dysplasia in epidermal tumors [17]. Our results show that in normal epidermis, no keratin 17 is detected. However, in SCC, a very strong expression of keratin 17 is demonstrated. This is in accordance with previous studies of oral SCC [18–20]. Therefore, Keratin 17 is a marker for proliferation and may be a biomarker for EMT.

Involucrin is identified as a protein precursor of the cross-linked envelope which turns out to be an early differentiation marker of the epidermal keratinocytes [21, 22]. Low or no quantity of involucrin is detected in poorly differentiated SCC. Therefore, involucrin is not only a marker for differentiation, but also may be a biomarker for EMT.

In conclusion, our results show reduced activity of involucrin and E-cadherin, and increased expression of Ki-67, β-catenin, Keratin 17 and vimentin. These cellular processes, including loss of differentiation, increase in proliferation and increase in invasive capacities, is similar to many endpoints of EMT. These data propose that EMT really occurs in poorly differentiated SCC and keratin 17 and involucrin may be another two biomarkers for EMT.

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References

1. Thiery JP, Sleeman JP (2006) Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7(2): 131–142. doi:10.1038/nrm1835
2. Said NA, Williams ED (2011) Growth factors in induction of epithelial-mesenchymal transition and metastasis. Cells Tissues Organs 193(1–2):85–97. doi:10.1159/000320360
3. Kalluri R, Neilson EG (2003) Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 112(12):1776–1784. doi:10.1172/JCI20530
4. Hugo H, Ackland ML, Blick T, Lawrence MG, Clements JA, Williams ED, Thompson EW (2007) Epithelial–mesenchymal and mesenchymal–epithelial transitions in carcinoma progression. J Cel Physiol 213(2):374–383. doi:10.1002/jcp.21223
5. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119(6):1420–1428. doi:10.1172/JCI39104
6. Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA (2009) Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest 119(6):1438–1449. doi:10.1172/JCI38019
7. Zeisberg M, Neilson EG (2009) Biomarkers for epithelial-mesenchymal transitions. J Clin Invest 119(6):1429–1437. doi:10.1172/JCI36183
8. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133(4): 704–715. doi:10.1016/j.cell.2008.03.027
9. Man XY, Finnson KW, Baron M, Philip A (2012) CD109, a TGF-beta co-receptor, attenuates extracellular matrix production in scleroderma skin fibroblasts. Arthritis Res Ther 14(3):R144. doi:10.1186/ar3877
10. Marks R (1996) Squamous cell carcinoma. Lancet 347(9003):735–738
11. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H (1984) Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133(4):1710–1715
12. Scholzen T, Gerdes J (2000) The Ki-67 protein: from the known and the unknown. J Cel Physiol 182(3):311–322. doi:10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9
13. Pich A, Chiusa L, Navone R (2004) Prognostic relevance of cell proliferation in head and neck tumors. Ann Oncol: official journal of the European Society for Medical Oncology/ESMO 15(9):1319–1329. doi:10.1093/annonc/mdh299
14. Iyer PV, Leong AS (1992) Poorly differentiated squamous cell carcinomas of the skin can express vimentin. J Cutan Pathol 19(1):34–39
15. Chang HW, Lee YS, Nam HY, Han MW, Kim HJ, Moon SY, Jeon H, Park JJ, Carey TE, Chang SE, Kim SW, Kim SY (2012) Knockdown of beta-catenin controls both apoptotic and autophagic cell death through LKB1/AMPK signaling in head and neck squamous cell carcinoma cell lines. Cell Signal. doi:10.1016/j.cellsig.2012.12.020
16. Depianto D, Kerns ML, Dlugosz AA, Coulombe PA (2010) Keratin 17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin. Nat Genet 42(10):910–914. doi:10.1038/ng.665
17. Proby CM, Churchill L, Purkis PE, Glover MT, Sexton CJ, Leigh IM (1993) Keratin 17 expression as a marker for epithelial transformation in viral warts. Am J Pathol 143(6):1667–1678
18. Wei KJ, Zhang L, Yang X, Zhong LP, Zhou XJ, Pan HY, Li J, Chen WT, Zhang ZY (2009) Overexpression of cytokeratin 17 protein in oral squamous cell carcinoma in vitro and in vivo. Oral Dis 15(1): 111–117. doi:10.1111/j.1601-0825.2008.01501.x
19. Toyoshima T, Koch F, Kaemmerer P, Vairaktaris E, Al-Nawas B, Wagner W (2009) Expression of cytokeratin 17 mRNA in oral squamous cell carcinoma cells obtained by brush biopsy: preliminary results. J Oral Pathol Med: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology 38(6):530–534. doi:10.1111/j.1600-0714.2009.00748.x
20. Kitamura R, Toyoshima T, Tanaka H, Kawano S, Kiyosue T, Matsubara R, Goto Y, Hirano M, Oobu K, Nakamura S (2012) Association of cytokeratin 17 expression with differentiation in oral squamous cell carcinoma. J Cancer Res Clin Oncol 138(8):1299–1310. doi:10.1007/s00432-012-1202-6
21. Rice RH, Green H (1977) The cornified envelope of terminally differentiated human epidermal keratinocytes consists of cross-linked protein. Cell 11(2):417–422
22. Bhargava G, Rifas L, Makman MH (1979) Presence of epidermal growth factor receptors and influence of epidermal growth factor on proliferation and aging in cultured smooth muscle cells. J Cell Physiol 100(2):365–374. doi:10.1002/jcp.1041000217