ORIGINAL ARTICLE

Asian Pacific Journal of
Allergy and Immunology

APJAI

BCAP 31 expression and promoter demethylation in psoriasis

Kriangsak Ruchusatsawat,[1] Laddawan Thiemsing,[1] Apiwat Mutirangura,[2] Jongkonnee Wongpiyabovorn[3]

Abstract

Background: Psoriasis is the disease of abnormal keratinocyte differentiation and apoptosis. Alterations in DNA methylation leading to keratinocyte hyperproliferation is one of the proposed pathogenic mechanisms of psoriasis. B-cell receptor associated protein (BCAP31) has been reported to be involved in the proliferation and apoptosis of keratinocytes. Up-regulation and changing in BCAP31 promoter methylation has been reported to be associated with some cancers. To date, there has been no study of psoriasis.

Objective: We investigated BCAP31 protein expression and the status of BCAP31 promoter methylation in psoriasis.

Method: Ten patients with psoriasis and 10 healthy subjects were enrolled. The immunohistochemistry was performed on paraffin-embedded tissue section to detect BCAP31 protein expression and compared between psoriasis and normal skin. The laser capture micro-dissected keratinocyte were analyzed using bisulfite PCR method and cloning and sequencing.

Results: Increased BCAP31 protein expression was observed in psoriatic epidermis compared with normal epidermis. Interestingly the methylation level of the BCAP31 promoter was significantly lower in patients with psoriasis compared with healthy subjects ($p < 0.001$, % psoriasis vs. normal skin methylation = 14.94 vs. 60.61).

Conclusions: The present study demonstrated increase expression of BCAP31 protein related to BCAP31 DNA demethylation in psoriasis. Future study is needed to indicate the mechanism of BCAP31 promoter demethylation and its potential use as a novel treatment for psoriasis in the future.

Keywords: Promoter methylation, Protein expression, Immunohistochemistry, BCAP31 and psoriasis

From:
[1] National Institute of Health, Department of Medical Sciences, Nontaburi 11000, Thailand
[2] Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
[3] Center of Excellence in Immunology and Immune Mediated Diseases, Division of Immunology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Corresponding author:
Jongkonnee Wongpiyabovorn
Division of Immunology, Department of Microbiology
Faculty of Medicine Chulalongkorn University
Rama 4 Road Bangkok 10330 Thailand
E-mail: jongkonnee.w@chula.ac.th

Introduction

Psoriasis is a chronic, inflammatory skin disease found in approximately 1-3% of the world's population.[1,2] The characteristics of psoriasis are inflammation in dermis with abnormal epidermis.[3,4] The keratinocytes of psoriatic skin lesions reveal hyperproliferation, incomplete differentiation and abnormal apoptosis. Both genetic and environmental factors play roles in the induction and maintenance of disease.[5,6] Recent cumulative data shown that DNA methylation is one of the main pathogenic mechanisms of psoriasis. Changes in the methylation of retrotransposons and promoters of several genes such as SHP-1, ID4, p15, p21 and p16^{ink4a} have been reported in psoriasis.[7-10]

B-cell receptor associated protein 31 (BCAP31) is a polytopic endoplasmic reticulum (ER) membrane protein. The cytoplasmic tail of BCAP31 contains a death effector domain. BCAP31 has roles in the over-expression of MHC class I, and the transport of selected proteins from the ER.[11,12] Both caspase-1 and caspase-8 can cleave BCAP31. The full-length protein of BCAP31 provides anti-apoptotic activity, while the cleaved fragment is presumed to promote apoptosis in the Keratin–forming tumor cell line HeLa (KB cell line) and Human lung carcinoma cell line (H1229 cell line).[13-15] BCAP31 has been reported to be involved in the proliferation and apoptosis of keratinocytes. For example, BCAP31 binds to

E5 protein of HPV 16 and 31 to regulate the proliferative ability of HPV-infected keratinocytes.[16] The current study demonstrated that BCAP31 was the target of caspase-1 in UVB-induced apoptosis of keratinocytes.[13] Up-regulation of BCAP31 has been reported in early and advanced hepatocellular carcinoma.[17] Recently, BCAP31 amplification was proved to be correlated with cellular immune response and longer survival in ovarian cancer.[18] Furthermore, BCAP31 promoter methylation has been defined as being associated with hormone receptor status and survival in breast cancer.[19,20]

Abnormal proliferation and apoptosis of keratinocytes is the main pathogenesis of psoriasis, and UVB phototherapy is one of the most effective therapies in psoriasis. Therefore, we investigated BCAP31 protein expression and the methylation status of BCAP31 promoter in keratinocytes from patients with psoriasis compared with normal subjects. These data might clarify a probable mechanism for the abnormal proliferation and apoptosis of keratinocytes in psoriasis and the efficiency of UVB phototherapy in psoriasis.

Methods

Patients and healthy controls

Skin biopsies of ten patients diagnosed with chronic plaque type psoriasis by experienced dermatologist at King Chulalongkorn Memorial Hospital (5 males, 5 females) and 10 normal subjects (2 male, 8 females) were analyzed in the study. The severity of psoriasis was classified according to the Psoriasis Area and Severity Index (PASI). All patients were free from systemic skin therapies for at least 4 weeks or topical skin therapies for at least 2 weeks prior to sample collection. Patients with a familial or personal history of autoimmune disease and cancer were excluded from the study. Normal skin was recruited from elective plastic surgery cases. The study was approved by the ethical committee of the King Chulalongkorn University. All participants provided informed consent. The demographic data are shown in **Table 1**.

Table 1. Patient and control demographic

Sample group	Sex (Male/Female)	Age (years) (Mean ± SD)	PASI
Normal controls (n= 10)	2/8	53.25 ± 4.50	-
Patients with psoriasis (n= 10)	5/5	49.40 ± 10.49	23.21 ± 12.34

Immunohistochemistry

Formalin-fixed skin sections from 8 patients with psoriasis and 5 normal subjects were analyzed by immunohistochemistry technique as previous described.[8] Briefly, tissue section on positively charged glass slides were baked overnight at 60°C and carried by Ventana cell Conditioning 1 solution (Ventana Medical Systems, Tucson, AZ). Then, the tissue sections were microwaved at 900-W for 15 minutes and blocked for endogenous peroxidase and biotin, followed by incubation with antibody against BCAP31 protein (Abcam, Cambridge, MA; at dilution 1:2000). The staining was performed using a Ventana Benchmark LT automated immunostainer. Normal human lung tissue was used as a positive control, and omission of the primary antibody as a negative control. The staining was assigned as pathologist visual scoring according to the percentage of cells with BCAP31 staining as follows: 0 (no staining), 1 (<10% of cell staining), 2 (10-50% of cell staining), or 3 (>50% of cell staining).[21]

Cell isolation

Keratinocytes from paraffin-embedded tissues from 10 patients with psoriasis and 10 normal subjects were separated using The PALM MicroLaser Microdissection System (P.A.L.M. MicroLaser Technologies AG, Burnried, Germany). The micro-dissected keratinocytes were removed from the slide by cutter pulse and collected in a micro-tube.

DNA preparation and Bisulfite-modification

All samples were extracted DNA using QIAamp DNA mini kit™ (QIAGEN). Then, 500 ng of DNA was bisulfite-treated using the EZ DNA methylation Kit™ (Zymo Research, Orange, CA, USA) according to the manufacturer's specifications. The bisulfite-treated DNA samples were stored at -20˚C until use.

Bisulfite Cloning and Sequencing

The methylation status of BCAP31 Promoter was analyzed using bisulfite cloning and sequencing technique as previously described.[22] The amplification was carried out in nested PCR with 2 consecutive primer sets. For nested PCR, 1µL of modified DNA was amplified using first round primers (forward 5'-GTA GGG TTT TTT GGT TAG TAG-3' reverse 5'-ACT ACT ATA AAA AAA TTC ATT AC-3') and second round primers (forward 5'-ATT TTT AGA GGG TAG GAT T-3' reverse 5'-ACT ACT ATA AAA AAA TTC TAT TAC-3'; product size 492 bp). The DNA was amplified by PCR at 95˚C for 4 min followed by 8 cycles of 95˚C for 1 min, 50˚C for 2 min, and 72˚C for 3 min, 30 cycles of 95˚C for 30 sec, 50˚C for 2 min, 72˚C for 1.5 min and final extension at 72˚C for 10 min. The PCR products were separated on a 2% agarose gel and purified by the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). Then, the purified-PCR products were ligated into a pGEM-T-easy vector (Promega). Sequencing of cloned PCR products was done with the ABI Prism BigDye or Dye Terminator cycle sequencing kits (Perkin Elmer, Foster City CA).

Statistical analysis

The overall percentage of methylation of all 46 CpG positions and the percentage of methylation of each CpG position were compared between groups by independent t-test and paired t-test, (sig 2-tailed) respectively, using the SPSS software for windows version 20.0 (SPSS Inc., Chicago, IL, USA). A p-value of < 0.05 was considered to be significant.

Results

Increase BCAP31 protein expression in psoriatic epidermis

All normal skin sections revealed mild to moderate staining of BCAP31 in the epidermis, especially in the lower part, whereas all of the skin sections from patients with psoriasis

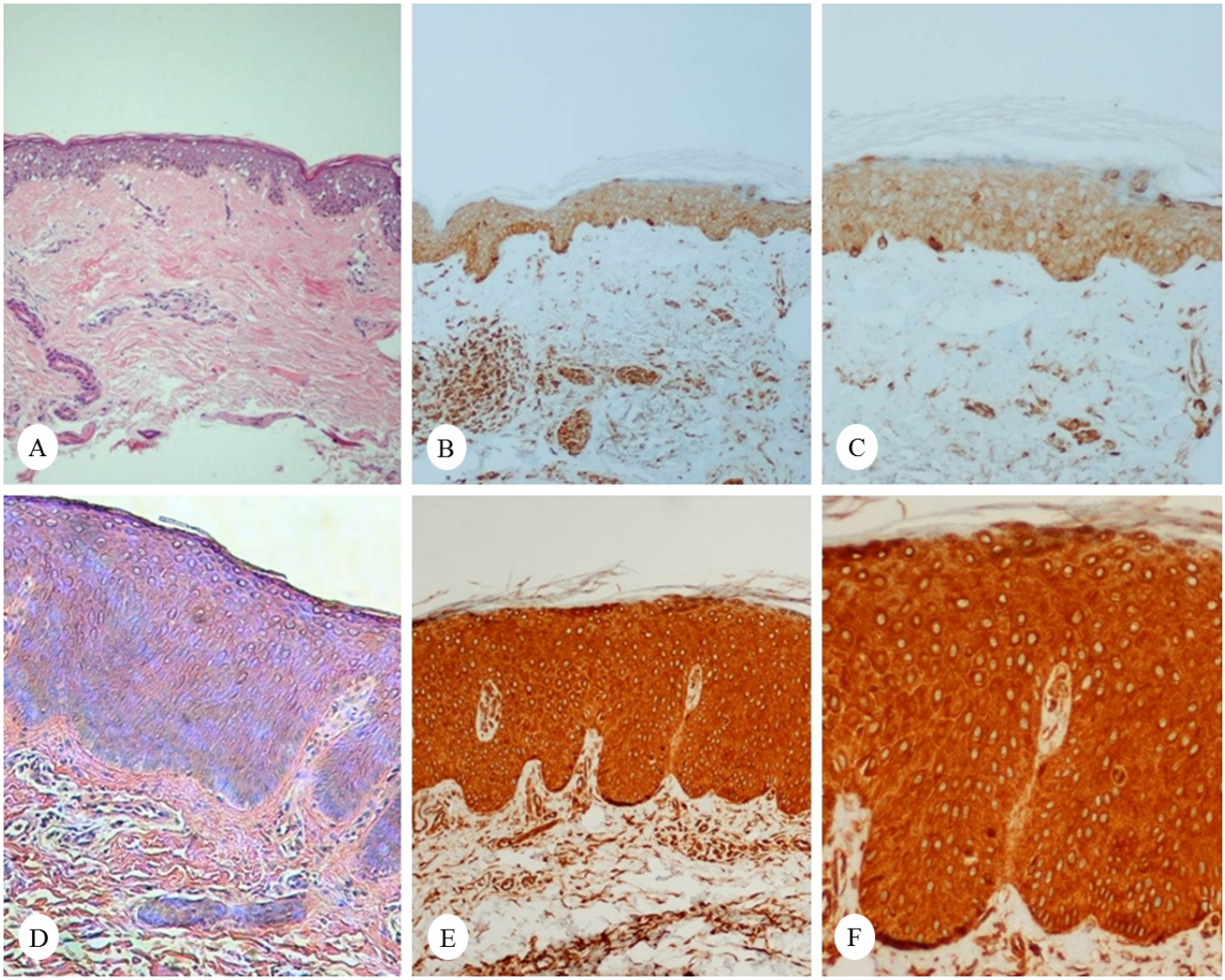

Figure 1. Immunohistochemical staining of BCAP31 protein in skin section from patients with psoriasis and normal subjects (A-C), normal subjects (D-F), psoriasis

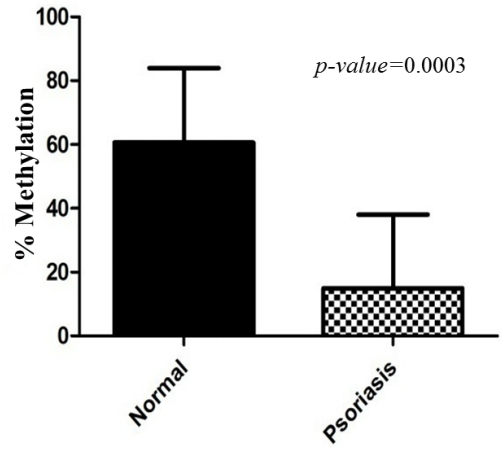

Figure 2. BCAP31 promoter methylation in epidermis from 10 patients with psoriasis and 10 normal subjects. The percentage of BCAP31 methylation was compared between psoriasis and normal epidermis. T indicates standard deviation (SD)

revealed diffusely dense staining of BCAP31 in all epidermal layer (**Figure 1**). The mean pathologist visual score of patients with psoriasis was higher than normal subjects (mean score ± SD of psoriasis vs. normal = 3.0 ± 0 vs. 1.8 ± 1.095). There is no significant correlation between the epidermal thickness and BCAP31 protein expression (data not shown).

Demethylation of BCAP31 promoter in psoriatic keratinocyte

The overall methylation of 46 CpG positions of the BCAP31 promoter of keratinocytes from patients with psoriasis was significantly lower than in normal subjects (% methylation of psoriasis vs. normal = 14.94 vs. 60.61, $p = 0.0003$) (**Figure 2**). The methylation levels of each CpG site (46 CpGs in total) were also compared between psoriasis and normal subjects (**Table 2**). Keratinocytes from psoriatic patients revealed a significantly lower level of methylation of most CpG sites than normal subjects (35 of 46 CpG sites) ($p < 0.05$).

Table 2. The percentage of methylation of each CpG sites (total 46 CpG) were compared between psoriasis and normal subject

Position	Normal (%methylation ±SD)	Psoriasis (%methylation±SD)	*p-value*	Position	Normal (%methylation ± SD)	Psoriasis (%methylation ± SD)	*p-value*
1	56.0±43.0	2.0±6.32	0.001	24	66.0±45.26	14.0±31.34	0.0079
2	74.0±38.93	12.0±31.55	0.001	25	30.0±44.47	4.0±8.43	0.0860
3	76.0±29.51	20.0±33.99	0.001	26	50.0±46.43	4.0±12.65	0.0073
4	80.0±28.28	18.0±31.90	0.0002	27	82.0±28.98	24.0±37.48	0.0011
5	46.0±40.06	22.0±33.27	0.162	28	54.0±37.06	12.0±27.00	0.0214
6	62.0±41.58	18.0±31.90	0.016	29	60.0±42.16	16.0±30.98	0.0160
7	48.0±40.22	18.0±31.90	0.081	30	24.0±37.48	2.0±6.32	0.0838
8	72.0±34.25	16.0±32.39	0.001	31	74.0±38.93	22.0±37.06	0.0068
9	32.0±43.41	4.0±12.65	0.065	32	82.0±28.98	14.0±34.34	<0.0001
10	38.0±43.67	10.0±21.60	0.085	33	78.0±37.06	16.0±30.98	0.0007
11	66.0±41.15	22.0±37.06	0.022	34	78.0±30.48	22.0±37.06	0.0017
12	38.0±44.67	12.0±27.00	0.133	35	84.0±29.51	24.0±37.48	0.0009
13	70.0±40.28	24.0±36.27	0.015	36	66.0±37.77	16.0±30.98	0.0046
14	84.0±29.51	20.0±37.71	0.0005	37	30.0±40.28	8.0±13.98	0.1201
15	48.0±46.38	10.0±25.39	0.035	38	80.0±33.99	24.0±37.48	0.0026
16	70.0±40.28	24.0±37.48	0.016	39	48.0±43.37	24.0±37.48	0.1964
17	82.0±34.58	16.0±30.98	0.0003	40	36.0±45.02	12.0±27.00	0.1654
18	46.0±41.15	18.0±31.90	0.106	41	78.0±28.98	22.0±37.06	0.0014
19	82.0±34.58	18.0±31.90	0.0004	42	78.0±28.98	24.0±37.48	0.0020
20	48.0±46.38	8.0±25.30	0.0278	43	80.0±29.81	18.0±31.90	0.0003
21	82.0±28.98	12.0±31.55	<0.0001	44	40.0±43.20	6.0±13.50	0.0289
22	66.0±41.15	16.0±32.39	0.0074	45	76.0±29.51	22.0±37.06	0.0020
23	50.0±46.43	10.0±25.39	0.0280	46	16.0±32.39	8.0±13.98	0.4825

Discussion

BCAP31 is an integral membrane protein that is ubiquitously expressed in the ER. It is a member of the Bcl-2 family of proteins that plays an important role in apoptosis.[14,23] A previous study demonstrated that BCAP31 is a target of high-risk HPV E5 and A4 proteins in HPV-infected keratinocytes and is crucial for sustaining proliferative ability in differentiating cells.[15,16] Our study demonstrated the increased BCAP31 protein expression in psoriatic keratinocyte compared with normal keratinocyte. A previous study showed that BCAP31 is involved in UVB-induced apoptosis of keratinocytes. Moreover, BCAP31 has been proven to be a target molecule of the E5 protein and plays an important role in the maintenance of proliferative competence of HPV-infected keratinocytes.[16] Abnormal keratinocyte apoptosis is one of the important pathogenic processes of psoriasis. Several reported indicated that alterations in the pro-apoptotic and anti-apoptotic molecules of keratinocytes, such as the up-regulation of Bcl-xL, c-FLIP, and survivin and down-regulation of caspase-9, are the conceivable main mechanisms leading to abnormal keratinocyte apoptosis in psoriasis. Moreover, significant down-regulation of some anti-apoptotic molecules and up-regulation of pro-apoptotic molecules had been observed after psoriatic treatments.[24-26] Thus, it is possible that increase BCAP31 protein is one of the mechanism controlling abnormal hyperproliferative and abnormal apoptosis of keratinocyte in psoriasis.

DNA Methylation is one of the main epigenetics mechanisms regulating gene expression in many diseases such as cancer, autoimmune and some inflammatory diseases. Current publications illustrated the alterations in global and specific promoter DNA methylation of several genes related to the abnormal proliferation and differentiation of keratinocytes in psoriasis. For instance, hypomethylation of long interspersed element-1 (LINE-1) has been reported to be associated with the down-regulation of several apoptotic genes in keratinocytes of patients with psoriasis. In addition, promoter hypermethylation of *inhibitor of differentiation 4* (ID4) was reported to be associated with parakeratosis in psoriatic epidermis.[8,27] Changes

in BCAP31 promoter methylation have been reported to be biomarkers in a number of malignancies. Nevertheless, there is no information about BCAP31 promoter methylation in the other group of diseases. In this study, we firstly demonstrated demethylation of BCAP31 promoter in keratinocyte from patients with psoriasis associated with increased BCAP31 protein expression. Thus, it is likely that increase BCAP31 protein expression in psoriatic keratinocyte, is the consequence of BCAP31 promoter demethylation causing up-regulation of BCAP31 mRNA expression. However, the mechanism of BCAP31 promoter demethylation remains unknown.

In conclusion, the present study demonstrated BCAP31 promoter demethylation, which may be a fundamental mechanism in increased BCAP31 protein expression in keratinocytes from patient with psoriasis. BCAP31 could be involved in a crucial molecular pathogenesis of the hyperproliferation and abnormal apoptosis of psoriatic keratinocytes. Nevertheless, further studies are needed to identify the significant biological relevance of BCAP31 in psoriasis and the mechanism of BCAP31 promoter demethylation, which may provide clues for innovative therapies for psoriasis in the future.

Acknowledgements

This work was supported by a research grant from the Government Research Budget (2016) and Thailand Research Fund (TRG 5480010)

References

1. Greaves MW, Weinstein GD. Treatment of psoriasis. N Engl J Med. 1995;332:581-8.
2. Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. J Clin Invest. 2004;113: 1664-75.
3. Bowcock AM, Krueger JG. Getting under the skin: the immunogenetics of psoriasis. Nat Rev Immunol. 2005;5:699-711.
4. Luba KM, Stulberg DL. Chronic plaque psoriasis. Am Fam Physician. 2006;73:636-44.
5. Burden AD, Javed" S, Bailey M, Hodgins M, Connor M, Tillman D. Genetics of psoriasis: paternal inheritance and a locus on chromosome 6p. The Journal of investigative dermatology. 1998;110:958-60.
6. Bos JD, De Rie MA. The pathogenesis of psoriasis: immunological facts and speculations. Immunol Today. 1999;20:40-6.
7. Ruchusatsawat K, Wongpiyabovorn J, Shuangshoti S, Hirankarn N, Mutirangura A. SHP-1 promoter 2 methylation in normal epithelial tissues and demethylation in psoriasis. J Mol Med (Berl). 2006;84:175-82.
8. Ruchusatsawat K, Wongpiyabovorn J, Protjaroen P, Chaipipat M, Shuangshoti S, Thorner PS, et al. Parakeratosis in skin is associated with loss of inhibitor of differentiation 4 via promoter methylation. Hum Pathol. 2011;42:1878-87.
9. Zhang K, Zhang R, Li X, Yin G, Niu X. Promoter methylation status of p15 and p21 genes in HPP-CFCs of bone marrow of patients with psoriasis. European journal of dermatology : EJD. 2009;19:141-6.
10. Chen M, Chen ZQ, Cui PG, Yao X, Li YM, Li AS, et al. The methylation pattern of p16INK4a gene promoter in psoriatic epidermis and its clinical significance. The British journal of dermatology. 2008;158:987-93.
11. Paquet ME, Cohen-Doyle M, Shore GC, Williams DB. Bap29/31 influences the intracellular traffic of MHC class I molecules. J Immunol. 2004;172:7548-55.
12. Cacciagli P, Sutera-Sardo J, Borges-Correia A, Roux JC, Dorboz I, Desvignes JP, et al. Mutations in BCAP31 cause a severe X-linked phenotype with deafness, dystonia, and central hypomyelination and disorganize the Golgi apparatus. Am J Hum Genet. 2013;93:579-86.
13. Sollberger G, Strittmatter GE, Grossi S, Garstkiewicz M, Auf dem Keller U, French LE, et al. Caspase-1 activity is required for UVB-induced apoptosis of human keratinocytes. The Journal of investigative dermatology. 2015;135:1395-404.
14. Wang B, Nguyen M, Breckenridge DG, Stojanovic M, Clemons PA, Kuppig S, et al. Uncleaved BAP31 in association with A4 protein at the endoplasmic reticulum is an inhibitor of Fas-initiated release of cytochrome c from mitochondria. J Biol Chem. 2003;278:14461-8.
15. Kotnik Halavaty K, Regan J, Mehta K, Laimins L. Human papillomavirus E5 oncoproteins bind the A4 endoplasmic reticulum protein to regulate proliferative ability upon differentiation. Virology. 2014;452-453:223-30.
16. Regan JA, Laimins LA. Bap31 is a novel target of the human papillomavirus E5 protein. Journal of virology. 2008;82:10042-51.
17. Elsemman IE, Mardinoglu A, Shoaie S, Soliman TH, Nielsen J. Systems biology analysis of hepatitis C virus infection reveals the role of copy number increases in regions of chromosome 1q in hepatocellular carcinoma metabolism. Mol Biosyst. 2016;12:1496-506.
18. Sankaranarayanan P, Schomay TE, Aiello KA, Alter O. Tensor GSVD of patient- and platform-matched tumor and normal DNA copy-number profiles uncovers chromosome arm-wide patterns of tumor-exclusive platform-consistent alterations encoding for cell transformation and predicting ovarian cancer survival. PLoS One. 2015;10:e0121396.
19. Benevolenskaya EV, Islam AB, Ahsan H, Kibriya MG, Jasmine F, Wolff B, et al. DNA methylation and hormone receptor status in breast cancer. Clin Epigenetics. 2016;8:17.
20. Fleischer T, Edvardsen H, Solvang HK, Daviaud C, Naume B, Borresen-Dale AL, et al. Integrated analysis of high-resolution DNA methylation profiles, gene expression, germline genotypes and clinical end points in breast cancer patients. Int J Cancer. 2014;134:2615-25.
21. Rizzardi AE, Johnson AT, Vogel RI, Pambuccian SE, Henriksen J, Skubitz AP, et al. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. Diagn Pathol. 2012;7:42.
22. Grunau C, Hindermann W, Rosenthal A. Large-scale methylation analysis of human genomic DNA reveals tissue-specific differences between the methylation profiles of genes and pseudogenes. Hum Mol Genet. 2000;9:2651-63.
23. Ng FW, Nguyen M, Kwan T, Branton PE, Nicholson DW, Cromlish JA, et al. p28 Bap31, a Bcl-2/Bcl-XL- and procaspase-8-associated protein in the endoplasmic reticulum. J Cell Biol. 1997;139:327-38.
24. Elango T, Thirupathi A, Subramanian S, Ethiraj P, Dayalan H, Gnanaraj P. Methotrexate treatment provokes apoptosis of proliferating keratinocyte in psoriasis patients. Clinical and experimental medicine. 2016.
25. Kokolakis G, Giannikaki E, Stathopoulos E, Avramidis G, Tosca AD, Kruger-Krasagakis S. Infliximab restores the balance between pro- and anti-apoptotic proteins in regressing psoriatic lesions. The British journal of dermatology. 2012;166:491-7.
26. Wang WH, Zhang L, Li LF, Sun TT. Ichthyosis hystrix Lambert type and Curth-Macklin type are a single entity with affected (KRT1 mutation) or unaffected (KRT10 mutation) palms and soles? European journal of dermatology : EJD. 2016.
27. Yooyongsatit S, Ruchusatsawat K, Noppakun N, Hirankarn N, Mutirangura A, Wongpiyabovorn J. Patterns and functional roles of LINE-1 and Alu methylation in the keratinocyte from patients with psoriasis vulgaris. J Hum Genet. 2015;60:349-55.