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Original contribution

Parakeratosis in skin is associated with loss of inhibitor of differentiation 4 via promoter methylation☆,☆☆

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Summary Parakeratosis refers to incomplete maturation of epidermal keratinocytes, resulting in abnormal retention of nuclei in the stratum corneum. It occurs in many diseases of the skin, particularly in psoriasis. Down-regulation of inhibitor of differentiation 4 messenger RNA has been demonstrated in psoriatic skin, but the specificity and mechanism for this finding are unknown. In this study, we addressed specificity by immunohistochemical staining for inhibitor of differentiation 4 protein in skin disorders showing parakeratosis, including: psoriasis (n = 9), chronic eczema (n = 6), and squamous cell carcinoma (n = 7). In these conditions, parakeratotic keratinocytes in the upper layers of the skin lacked inhibitor of differentiation 4 protein expression, whereas keratinocytes in the lower layers were densely stained, in contrast to diffuse expression in normal skin. Because promoter hypermethylation of *inhibitor of differentiation 4* has been described in several cancers, we determined the methylation pattern of the *inhibitor of differentiation 4* promoter in psoriasis and compared this with squamous cell carcinoma. We found a novel methylation pattern of the *inhibitor of differentiation 4* promoter in both conditions.

☆ Conflict of interest: The authors declare no conflict of interest.

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Inhibitor of differentiation 4 promoter methylation was significantly increased in psoriasis (34.8%) and squamous cell carcinoma (21.8%), compared with normal skin (0%). Moreover, cells in the upper and lower parts of psoriatic epidermis were, respectively, hypermethylated and nonmethylated, at the *inhibitor of differentiation 4* promoter. Comparable studies in several cell lines confirmed that hypermethylation of the promoter was associated with loss of *inhibitor of differentiation 4* messenger RNA and protein expression. Our study demonstrates a previously unreported link between gene-specific promoter hypermethylation and abnormal cellular differentiation in several skin diseases. This mechanism might provide clues for novel therapies for skin disorders characterized by parakeratosis.
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1. Introduction

The skin is a highly regulated organ maintaining tight control over proliferation, differentiation, and apoptosis. During terminal differentiation in which granular cells turn to cornified cells, keratinocytes lose nuclei and keratohyaline granules, and keratin filaments become compacted. Parakeratosis is an abnormal occurrence in which keratinization of epithelial cells is incomplete, with abnormal retention of keratinocyte nuclei in the stratum corneum. Parakeratosis is found in many skin diseases and is visible clinically as scale. One of the most striking examples is psoriasis, characterized by confluent parakeratosis infiltrated by neutrophils [1]. The precise mechanism of the induction and maintenance of parakeratosis remains unclear. Some insight has come from recent complement DNA microarray studies that have identified down-regulation of inhibitor of differentiation 4 (*ID4*) messenger RNA (mRNA) in psoriatic skin [2,3].

ID4 is a member of the basic helix-loop-helix family and is expressed in the thyroid, brain, fetal tissue, and some nervous system tumor cell lines [4]. The protein behaves as a dominant negative inhibitor of gene transcription [5]. ID4 is an important factor for cell proliferation and differentiation [6,7], apoptosis [8], and regulation of oligodendrocyte development and differentiation [9]. Down-regulation of ID4 protein can induce tumor cell dedifferentiation in colorectal cancer. *ID4* may act as a tumor suppressor gene in lymphoma [10] and carcinomas of the stomach [11], breast [12], and colorectum [13]. Conversely, *ID4* can behave as an oncogene in prostate [14] and bladder cancers [15]. Transcriptional silencing of *ID4* by promoter hypermethylation has been reported in many cancers, including cholangiocarcinoma, breast cancer, lymphoma, and gastric adenocarcinoma [11,12,16,17].

The particular mechanism by which *ID4* is down-regulated in psoriasis is unknown, as well as the specificity of such a finding. We therefore compared expression of ID4 at the protein level in psoriasis to other skin disorders that show parakeratosis. We also determined the methylation status of the *ID4* promoter in psoriasis and compared this with cutaneous squamous cell carcinoma (SCC) that was characterized by parakeratosis. Lastly, we studied a series of cell lines to correlate promoter methylation, mRNA levels, and protein expression for ID4.

2. Materials and methods

2.1. Patients and controls

Biopsies of 28 subjects seen at King Chulalongkorn Memorial Hospital were used for this study. The biopsies included 9 cases of chronic plaque psoriasis, 7 of SCC, 6 of chronic eczema, and 6 of normal skin. All patients were free from systemic skin therapies for at least 4 weeks or topical skin therapies for at least 2 weeks before sample collection. Normal skin was obtained from elective plastic surgery cases. No patient had any personal or family history of autoimmune disease. The study was approved by the ethics committee of King Chulalongkorn University, and all participants provided informed consent.

2.2. Cell lines

Six epithelial cell lines (Hela, HEp2, SiHA, RKO, SW480, and HaCaT) and 5 hematopoietic cell lines (Daudi, Jurkat, Molt4, HL-60, and K562) were used in this study. All cell lines were cultured in Dulbecco modified Eagle medium or RPMI 1640 (Gibco BRL, Life Technologies, Pairly, UK) supplemented with 10% heat-inactivated fetal bovine serum (Sigma, St Louis, MO) and antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin). All cells were grown at 37°C in 5% CO_2 under humidified conditions

2.3. Immunohistochemistry

Immunohistochemistry was performed on 4-μm sections of formalin-fixed skin specimen from 9 patients with chronic plaque psoriasis, 7 patients with SCC, 6 patients with chronic eczema, and 6 healthy volunteers. The tissue sections were mounted on positively charged glass slides and baked overnight at 60°C. Heat-induced epitope retrieval was carried out using Ventana Cell Conditioning 1 solution

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