Research Article

Alcohol dehydrogenase 3 transcription associates with proliferation of human oral keratinocytes


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Abstract. Gene expression underlying cellular growth and differentiation is only partly understood. This study analyzed transcript levels of the formaldehyde-metabolizing enzyme alcohol dehydrogenase 3 (ADH3) and various growth and differentiation-related genes in human oral keratinocytes. Culture of confluent cells both with and without fetal bovine serum inhibited colony-forming efficiency and induced a squamous morphology. Confluency alone decreased the transcript levels of ADH3, the proliferation markers cell division cycle 2 (CDC2) and proliferating cell nuclear antigen (PCNA), and the basal cell marker cytokeratin 5 (K5), but increased transcripts for the suprabasal differentiation markers involucrin (INV) and small proline-rich protein 1B (SPR1). These changes were variably influenced by serum, i.e., loss of CDC2 and PCNA was inhibited, loss of K5 promoted, increase of SPR1 transcripts inhibited, and increase of INV promoted. The extent and onset of the effects implied that ADH3 transcription serves as a proliferation marker and that confluency with or without serum exposure can serve to selectively analyze proliferative and differentiated cellular states.

Key words. Oral mucosa; keratinocyte; alcohol dehydrogenase; formaldehyde metabolism; squamous differentiation; proliferation; transcript profiling.

Squamous epithelia, including those of the oral cavity, eliminate cells primarily by desquamation to maintain a homeostatic balance with cells generated by proliferation [1]. During the basal to suprabasal transition, keratinocytes gradually lose their proliferative ability and undergo terminal squamous differentiation (TSD) before the cells or cell remains are shed from the outermost layer [1, 2]. Various genes/proteins signify proliferative and differentiated cellular states. Markers of proliferation include cell division cycle 2 (CDC2), also termed cyclin-dependent kinase 1, which constitutes a key component of the cell cycle control system, and proliferating cell nuclear antigen (PCNA), which is part of a multiprotein complex regulating DNA replication [3, 4]. Expression of the corresponding genes decreases when cells commit to TSD as cells then cease to divide [5–7]. The process of oral keratinocyte TSD is coupled with the expression of numerous structural proteins, including those involved in cornification, e.g., involucrin (INV) and small proline-rich proteins [8]. Among the approximately 20 cytokeratins identified so far, K5 is one of the principal basal keratins in stratified epithelia, yet other keratins, like K13, are associated with the induction of TSD [2, 8, 9]. Culture of keratinocytes and the application of different conditions variably mimic epithelial tissue homeostasis and physiological processes [10, 11]. Gene expression un-
nderlying loss of proliferation and onset of TSD is only partly understood. Transfer cultures of oral keratinocytes in serum-free medium are reminiscent of basal cells, and show high proliferative ability, relatively small cell size, intense expression of basal keratins but low expression of TSD markers [12, 13]. Addition of fetal bovine serum (FBS) and/or the transition from a proliferative, confluent to a post-confluent stage, induces irreversible growth arrest and TSD, associated with increased cell surface area and the expression of TSD markers [6, 12, 13]. An opportunity to investigate these processes further is provided by the variable expression of these under different culture protocols [reviewed in ref. 11]. Notably, results from other types of epithelia may not apply to oral epithelium due to tissue specific expression of structural proteins and differential responsiveness to toxic agents [6, 14, 15].

Formaldehyde is a toxic, mutagenic and carcinogenic chemical [16, 17]. Oral inhalation of vapor in tissue fixatives, tobacco smoke, and automotive emissions, usage of certain dental materials as well as the ingestion of fruits and other foods lead to oral exposure to formaldehyde [16]. Atmospheric formaldehyde even below permissible exposure limits causes micronuclei and chromosome breakage in oral buccal and nasal epithelium, implying a need to characterize the enzymatic defense against formaldehyde [18]. Alcohol dehydrogenase 3 (ADH3), also known as glutathione-dependent formaldehyde dehydrogenase, is the primary formaldehyde scavenger [16, 19]. Assessment of ADH3 expression in oral epithelium demonstrated transcripts in the basal and parabasal layers without detectable transcripts in upper layers [20]. In contrast, the ADH3 protein was present throughout the epithelium and, moreover, it remained expressed and metabolically functional in cultured oral keratinocytes under several conditions [20].

Previous analysis of contact-inhibited, differentiated cultured oral keratinocytes showed decreased levels of ADH3 transcripts compared to sparse, proliferative cells, but the coupling to alteration of proliferation and differentiation remained unclear [20]. The current study utilized oral keratinocytes under several growth protocols to analyze the association of ADH3 transcription with markers of proliferation and/or TSD, including those normally expressed at basal or more superficial positions in oral epithelium. The cultures were expanded from a sparse to a confluent state and then maintained at confluency for up to 15 days, with or without the presence of 2 and 10% FBS. Proliferative and differentiated states were initially assessed from changes in colony-forming efficiency (CFE) and induction of morphological changes. Numerical data for the transcript levels relative to the number of β-actin transcripts were then obtained for ADH3, CDC2, PCNA, K5, INV, and small proline-rich protein 1B (SPR1) using a recently established technique for quantitative RT-PCR (StaRT-PCR), shown to be reproducible among different laboratories [21]. Finally, Northern blot analysis was used to quantify the levels of β-actin transcripts and K13 under the different conditions; the latter gene served as a positive control since the level of the corresponding transcript was previously shown to increase upon serum exposure under confluency [6]. The overall results demonstrate several means of inducing TSD markers in oral keratinocytes and, moreover, indicate that ADH3 transcription terminates in association with a loss of proliferation without association with the onset or execution of TSD.

Materials and methods

Cell cultures

Procedures used to culture human oral keratinocytes, including the preparation of the serum-free epithelial medium with high amino acid supplementation (EMHA medium) have been described recently [11]. Buccal tissue was obtained from healthy donors (non-tobacco users) undergoing reconstructive surgery, with the approval of the ethical committee at Karolinska Institutet. Primary cell cultures were initiated from incubation of tissue for 18–24 h at 4°C with 0.17% trypsin in phosphate-buffered saline to form single cells and small aggregates. The mixture was resuspended in EMHA and plated onto fibronectin/collagen-coated dishes at an approximate density of 5 × 10² cells/cm². Cultures were transferred at 4–5 × 10³ cells/cm² in 60- or 100-mm Petri dishes. The various analyses were made with cells in second passage. Keratinocytes were seeded at 5 × 10³ cells/cm² to reach confluence (100%) at 6–8 days. The term confluence (100%) was regarded as the stage/moment when the cultures were (first) grown to fully occupy the dish surface area as determined from visual inspection and photographic documentation under a phase contrast microscope. Thereafter, the assessments were based on time, and cultures were analyzed at 5, 10 and 15 days at the post-confluent stage using EMHA with or without FBS (2 or 10%) as culture condition. Medium was exchanged at 2-day intervals.

Statistical analysis

The various results were based on a minimum of three separate experiments and statistical differences were determined by analysis of variance (ANOVA). Dunnett’s multiple-comparison post-test was subsequently used for comparison of multiple values to one reference value, and the Tukey-Kramer post-test was used for comparison of all results among multiple values.

Colony-forming efficiency

CFE was assayed at confluence and beyond (0, 5, 10 and 15 days). Briefly, the cells were removed using trypsin