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SHORT COMMUNICATION

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The expression of α6 and β4 integrin genes are differentially regulated by *all-trans*-retinoic acid (RA) in cultured human keratinocytes

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Integrins are a family of heterodimeric cell adhesion receptors which mediate the interaction of cells with the extracellular matrix (ECM) and with other cells. The ligand specificity is provided by the α-subunits, although the β-subunits apparently play a role as well. A number of essential biological phenomena, such as growth, differentiation, migration and expression of specific genes, can be controlled by signal transduction via these receptors [5, 7]. Integrin α6β4 has been shown to be a component of hemidesmosomes of stratified squamous epithelium [14, 15] and the α6 and β4 integrin subunits are expressed by cultured normal human keratinocytes [9]. Even though the extracellular ligands for the α6β4 integrin are not entirely determined, the α6β4 integrin has been shown to be a laminin receptor in a colon adenocarcinoma cell line [8], and a receptor for both laminin and kalinin in transfected K562 erythroleukemic cells [11].

The obvious role of retinoic acid (RA) in the regulation of development and cell differentiation have focused efforts on elucidating the biological actions of RAs at the molecular level. Previous studies have demonstrated that RAs can modulate various cell surface receptors affecting, for example, the receptor-ligand affinity [16, 17]. This study was designed to elucidate the effects of RA on α6 and β4 integrin gene transcription, and protein distribution in normal human keratinocytes in vitro.

Primary cultures of normal human keratinocytes were established from adult skin as previously described [2]. The cultures were maintained in serum-free keratinocyte medium (Keratinocyte-SFM; Life Technologies, Paisley, UK) routinely supplemented with epidermal growth factor (EGF) (10 ng/ml) and bovine pituitary extract (BPE) (35–50 μg/ml), and containing a low calcium concentration (0.09 m*M*). Keratinocytes were grown to no more than 70–80% confluency. Keratinocytes of the second passage were plated on flasks 2 days prior to incubation with the test media (see below). Cell cultures were rinsed twice with keratinocyte-SFM without growth factor supplements before adding the test media (containing 10^{-6} *M* RA) (Sigma, St. Louis, Mo.) for 24 h. The identical culture conditions and combinations of testing media were used for cells grown for immunofluorescence studies on glass coverslips.

Isolation of keratinocyte RNA was carried out using guanidine isothiocyanate extraction as previously described [3]. For Northern transfer analyses, samples of total RNA were electrophoresed on 1.3% agarose gels under denaturing conditions and transferred to nylon filters (Biodyne Transfer membrane; Pall Biosupport, East Hill, N.Y.). Prior to transfer, the integrity of the RNA and even loading of the gels were verified by visualizing ribosomal RNA subunits with ethidium bromide staining. The following cDNAs were used as probes: a 3.8 kb probe covering the 3′ terminal part of the β4 integrin cDNA (kindly provided by M. Hemler, Dana-Farber Cancer Institute, Boston, Mass.), and a full-length α6 integrin cDNA [4]. A 28S rRNA oligonucleotide was used as a reference probe as previously described [1]. The probes were labeled radioactively with α-[^{32}P]dCTP by random-priming to a specific activity of at least 5×10^8 cpm/μg and used for hybridizations as previously described [10].

Cells cultured on glass coverslips were fixed and immunolabeled as previously described [12]. For the detection of β4 protein, mouse monoclonal antibody UM-A9 was used. This antibody identifies an epitope in the extra-

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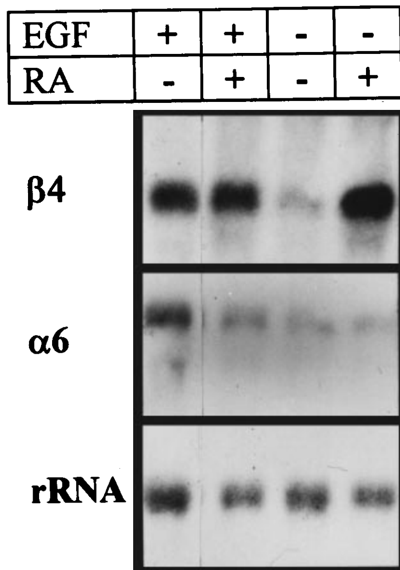

Fig. 1 Northern transfer analysis of RNAs isolated from cultured human keratinocytes maintained in media with and without EGF and RA. The same filter with intermittent washes was hybridized with cDNAs specific for human β4 integrin and α6 integrin subunits. A 26-base oligonucleotide specific for human 28S ribosomal RNA was used as a reference probe

cellular portion of β4 protein [6]. GoH3, a rat monoclonal antibody specific for the extracellular portion of the α6 subunit [13], was generously provided by Dr. Arnould Sonnenberg (University of Amsterdam, The Netherlands). Tetramethyl rhodamine isothiocyanate (TRITC)-conjugated rabbit antimouse IgG, used as the secondary antibody, was purchased from Dako (Glostrup, Denmark). The samples were observed and photographed with a Leitz Aristoplan microscope equipped with an epi-illuminator and filters suitable for TRITC fluorescence. In control immunoreactions, the primary antibody was omitted, replaced with 1% bovine serum albumin dissolved in phosphate buffered saline, or replaced with sera from non-immunized rabbits diluted 1:50 in phosphate buffered saline. A faint uniform background only was observed in all controls.

The steady-state levels of α6 and β4 mRNAs of cultured keratinocytes were first studied in cultures grown in media with or without EGF. Northern transfer analyses in-

Fig. 2 A–D The effect of RA on the expression of the β4 integrin subunit by cultured keratinocytes maintained in medium with (**C, D**), or without (**A, B**) EGF. Note the granular immunoreaction in the RA-treated samples (**B, D**), which may in part be representative of extracellular immunolabeling of the β4 integrin subunit (see text)

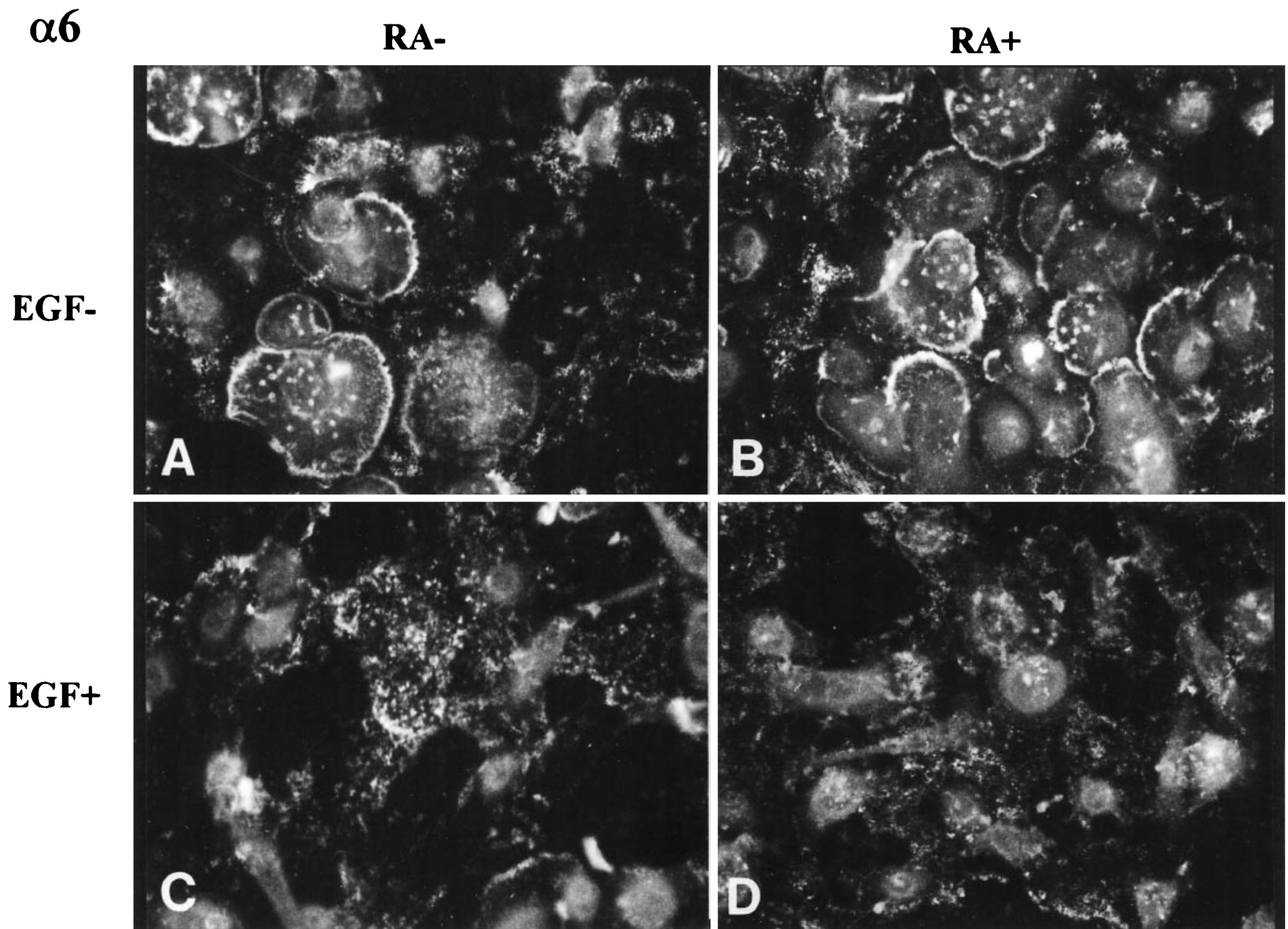

Fig. 3 A–D The effect of RA on the expression of the α6 integrin subunit by cultured keratinocytes maintained in medium with (**C, D**), or without (**A, B**) EGF. Note that RA does not exert any apparent effects on the α6 integrin distribution on keratinocytes cultured in either EGF-supplemented or EGF-depleted medium

dicated that the levels of both α6 and β4 mRNAs were markedly higher in keratinocytes cultured in medium supplemented with EGF compared to control cultures (Fig. 1).

In subsequent studies, RA was added to EGF-supplemented and EGF-depleted media, and the α6 and β4 mRNA levels were monitored by Northern hybridizations. The results revealed that RA exerted diverse effects on α6 and β4 integrin gene expression. Specifically, the levels of α6 integrin mRNA were at a barely detectable level in cultures maintained in medium supplemented with RA (medium containing RA and EGF, and medium containing RA but no EGF). In contrast, RA did not downregulate β4 mRNA levels in cultures maintained in medium containing EGF, and exerted a marked upregulatory effect on β4 integrin mRNA levels in cultures maintained in medium depleted of EGF.

RA induced redistribution of β4 integrin subunits. Specifically, RA-treated cultures were characterized by the presence of a punctate staining pattern (Fig. 2). Observation of the same samples with phase contrast microscopy revealed that the immunoreaction for β4 integrin was in part associated with extracellular material, apparently left behind by migrating keratinocytes. In marked contrast to findings on the β4 subunit, RA did not have any apparent effect on the localization of α6 integrin subunit either in EGF-depleted or EGF-supplemented cultures (Fig. 3). It is of interest to note that the expression of the β4 integrin subunit seemed to be differently regulated on the cell surface and at the mRNA level.

Our findings suggest that RA exerts separate effects on the transcription of the β4 integrin gene and on the subcellular distribution of the β4 integrin subunit.

In conclusion, our results suggest that treatment of cultured keratinocytes with RA induces marked changes in the β4 integrin expression both at the mRNA and protein levels. In contrast, the expression of α6 integrin was not similarly affected by RA.

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