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Keratinization is associated with the expression of a new protein related to the desmosomal cadherins DGII/III

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Amino acid sequencing of a 48/46 kDa glycoprotein from human plantar callus, recognised by antisera raised against the desmosomal cadherins DGII/III, has revealed N-terminal homology to the DNA-derived sequence of human and bovine DGII/III. However, a tryptic fragment has homology only with a bovine clone. We propose that there are two classes of DGII/III-like molecule, that represented by the bovine cDNA clone and the 48/46 kDa protein, a monoclonal antibody against which stains mainly the suprabasal layers of human epidermis, and that represented by the human cDNA clone, identified by a monoclonal antibody which stains uniformly the living layers of the epidermis.

Desmosome; Cell adhesion; Cadherin; Epidermis; Keratinization

1. INTRODUCTION

Epidermal differentiation (keratinization) involved dramatic changes in ultrastructure and biochemical composition as cells migrate from the basal layer through the spinous and granular layers, to terminally differentiate as squames of the stratum corneum. The cell surface changes that have been most clearly defined in biochemical terms thus far, have been the characterisation of the major new proteins, involucrin and loricrin, which are synthesised and assembled into a cornified submembranous envelope [1]. We now show that subtle yet equally dramatic changes also occur in candidate adhesion glycoproteins of the desmosome junctions.

Changes in the morphology of cell adhesions have of course already been well characterized during this process [2–4]. The desmosome junctions are prominent in the basal layer; they increase in number and change in dimensions and electron density before final breakdown in the later stages of keratinization. By the time the stratum corneum is reached, there is an impermeable and highly protective layer in which desmosomes appear to play little part in cell-cell adhesion. Some changes have been described in the composition of the proteins of the desmosomal plaque, which is thought to provide a link with the intermediate filament network, [5–7], although reports that desmoplakin DPII was restricted to stratified epithelia [8] have now been refuted [9].

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As part of a programme to study the molecular basis of cell-cell adhesion, we have cloned and sequenced the major desmosomal glycoproteins DGI (desmoglein) [10] and DGII/III (desmocollins) [11] from cDNA libraries prepared with cultured human keratinocyte mRNA. This has demonstrated that these proteins have extensive homology with the cadherin family of cell-cell adhesion molecules, and makes both DGI and DGII/III candidate adhesion molecules. DGII and DGIII are produced by alternative splicing of a single gene, DSC, which we have assigned to chromosome 9 [12]. Similar DGI [13] and DGII/III [14] clones have also been obtained from bovine muzzle epidermal cDNA libraries. We now report evidence that a protein related to the desmosomal glycoproteins DGII/III, but distinct from them, is expressed in the later stages of epidermal differentiation, whereas DGII/III themselves are expressed throughout the epidermis. Since DGII/III are candidate adhesion molecules, this finding may presage a change in the adhesive capacity of desmosomes as keratinization proceeds.

2. MATERIALS AND METHODS

2.1. *N-terminal sequencing*

ConA binding glycopeptides from plantar callus [15] were resolved by SDS-PAGE and transferred to Immobilon membrane (Millipore) using 10 mM 3-[cyclohexylamino]-1-propane sulphonic acid, pH 11.0, containing 10% methanol. After staining with Amido black and destaining the 48/46 kDa band was excised and sequenced on an Applied Biosystems sequencer (model 473A) using a tangential flow cartridge.

2.2. *Tryptic fragment sequencing*

For the derivation of tryptic peptides, the 48/46 kDa band was electroeluted from preparative 10% SDS gels and dialysed against 20 mM

ammonium bicarbonate, pH 8.5, 0.05% SDS. The protein was digested with 2% (w/w) TPCK-trypsin (Worthington) overnight at 37°C. The digest was lyophilised twice, redissolved in SDS-PAGE sample buffer and resolved using the SDS-PAGE system of Schagger and von Jagow [16]. The fragments were transferred to Immobilon membrane and sequenced.

2.3. *Sequence comparisons*

Amino acid sequence comparisons were carried out using the University of Wisconsin Computer Group programmes [17] running on NIMR's Sun-4 computers. The sequence data used are available from EMBL/Genbank/DDBJ under the accession numbers X56807 (human DGII/III) [11] and X56966/7/8 (bovine DGII/III) [14].

2.4. *Indirect immunofluorescence*

Unfixed frozen sections of human breast skin were stained with mouse monoclonal antibodies (1 in 5 dilution) as described previously [18] using fluorescein-conjugated rabbit anti-mouse IgG as second antibody. The monoclonal antibody LH50 (an IgG) was generated against the 48/46 kDa glycopeptides from plantar callus using standard procedures [19]. CE1, and IgG monoclonal generated against bovine DGII/III has been described previously [11].

3. RESULTS AND DISCUSSION

DGII/III-related glycoproteins have previously been identified in the conA-binding material isolated from various human epidermal sources [15], by using antisera raised against a major 78 kDa glycoprotein from pig epidermis [18]. This latter glycoprotein was thought to result from degradation of DGII/III during terminal epidermal differentiation [20]. It is recognised by anti-DGII/III polyclonal sera prepared against DGII/III purified from bovine muzzle desmosomes [21] in both immunoblotting and immunoprecipitation analyses, and antisera directed against the 78 kDa glycopeptide specifically stained desmosomes in immunogold-labelling. Likewise, the immunologically related glycopeptides of 48/46 kDa from human epidermal sources, e.g. plantar callus, have been used to raise sera which label desmosomes in immunogold localization. Indeed anti-DGII/III polyclonal sera recognise these glycopeptides, and anti-48/46 kDa sera produce a similar immunoblotting pattern to that produced by authentic anti-bovine DGII/III, recognising a 115kD/100 kDa glycoprotein. However, although a monoclonal antibody raised against the 48/46 kDa protein, LH50, and a monoclonal antibody raised against bovine DGII/III (CE1) both recognised bovine protein bands in the 115–100 kDa range, those recognised by CE1 usually appeared more diffuse than the bands recognised by LH50, and had slightly different mobilities (King, unpublished work).

Therefore to throw more light on the nature of this human 48/46 kDa glycopeptide, especially to determine whether it was indeed a degradation product of DGII/III, we have performed amino acid sequencing. Amino terminal sequence was similar, although not identical to the DNA-derived sequence of human DGII and DGIII (Fig. 1) [11], but was more similar to that of the bovine clones [14] than of the human clones, even though the peptides were from human material. Moreover, the sequence of a tryptic fragment from the 48/46 kDa peptide was not found in the DGII or DGIII sequences derived from the human cDNA clones. (The closest match is shown in Fig. 1, but since this is at residue 579, a peptide encompassing this and the N-terminus would be larger than the size of the 48/46 kDa peptide.) We have now, however, found a good match of the sequence of this tryptic peptide with the DNA-derived sequence of a bovine DGIII clone reported by Collins et al. [14] (Fig. 1), taking into account the species difference between the two sequences. Though the comparison was less striking, other peptides derived from the human 48/46 kDa peptide also matched the bovine better than the human DGIII and were found in the first 150 N-terminal amino acids.

We believe, therefore, that the 48/46 kDa glycopeptide is a degradation product of a human protein analogous to the protein coded by the bovine cDNA DGII/III isolated by Collins et al. [14] but actually distinct from the protein coded by our human DGII and DGIII cDNA clones. The fact that homology between the human and bovine amino acid sequences derived from the cDNA clones is scattered throughout the molecule, rather than arranged in groups, would argue for these proteins being the result of a gene duplication and subsequent evolutionary divergence rather than alternative splicing of a single gene. These two DGII/III proteins have: (i) very similar molecular weights; (ii) the same domain structures, including transmembrane, calcium-binding and cytoplasmic domains; (iii) the same alternative splicing pattern and the same unusual mechanism to insert a stop codon; *yet* identity is a mere 52% at the amino acid level, far lower than would be expected from just inter-species variation, especially when the human and bovine DGI are identical to the extent of 78%. The level of homology

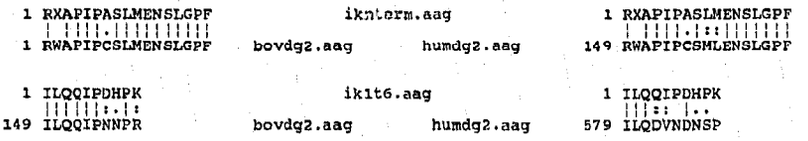

Fig. 1. Computer-generated comparisons between the amino acid sequences of the N-terminus (iknterm.aag) and of a tryptic fragment (ik1t6.aag) of the 48/46 kDa human glycoprotein, with the DNA-derived sequences of a human DGII cDNA clone [11] (humdg2.aag) and a bovine cDNA clone [14] (bovdg2.aag).

between the two genes is probably not enough to result in cross-hybridization in genomic Southern blots under stringent washing conditions, and certainly we saw only one band in such hybridizations using a short 118 bp probe [11]. Also we did not see any discrepancies in the chromosomal assignments performed using PCR on rodent-human somatic cell hybrids [12]. In any case, if there are two genes, they may lie on the same chromosomal arm. A precedent for this is the fact that DSP, coding for desmoplakin, a major desmosomal plaque protein, is assigned to 6p21-ter [12], and BPAG1, coding for the hemidesmosomal bullous pemphigoid antigen which has homology to desmoplakin, also maps to chromosome 6, at 6p11-6p12 [22]. It may therefore be that gene duplication and subsequent evolutionary divergence have played a major role in fulfilling the needs for a variety of proteins with differing functions in these types of cell junction. What these functions are for the two types of DGII/III molecules we have begun to examine by determining their location with immunofluorescence microscopy using Mabs specific to these molecules. The results of these experiments (Fig. 2) show that a monoclonal antibody generated against the 48/46 kDa glycopeptide produced strong staining at the surface of suprabasal cells in human epidermis, whereas staining with an anti-bovine

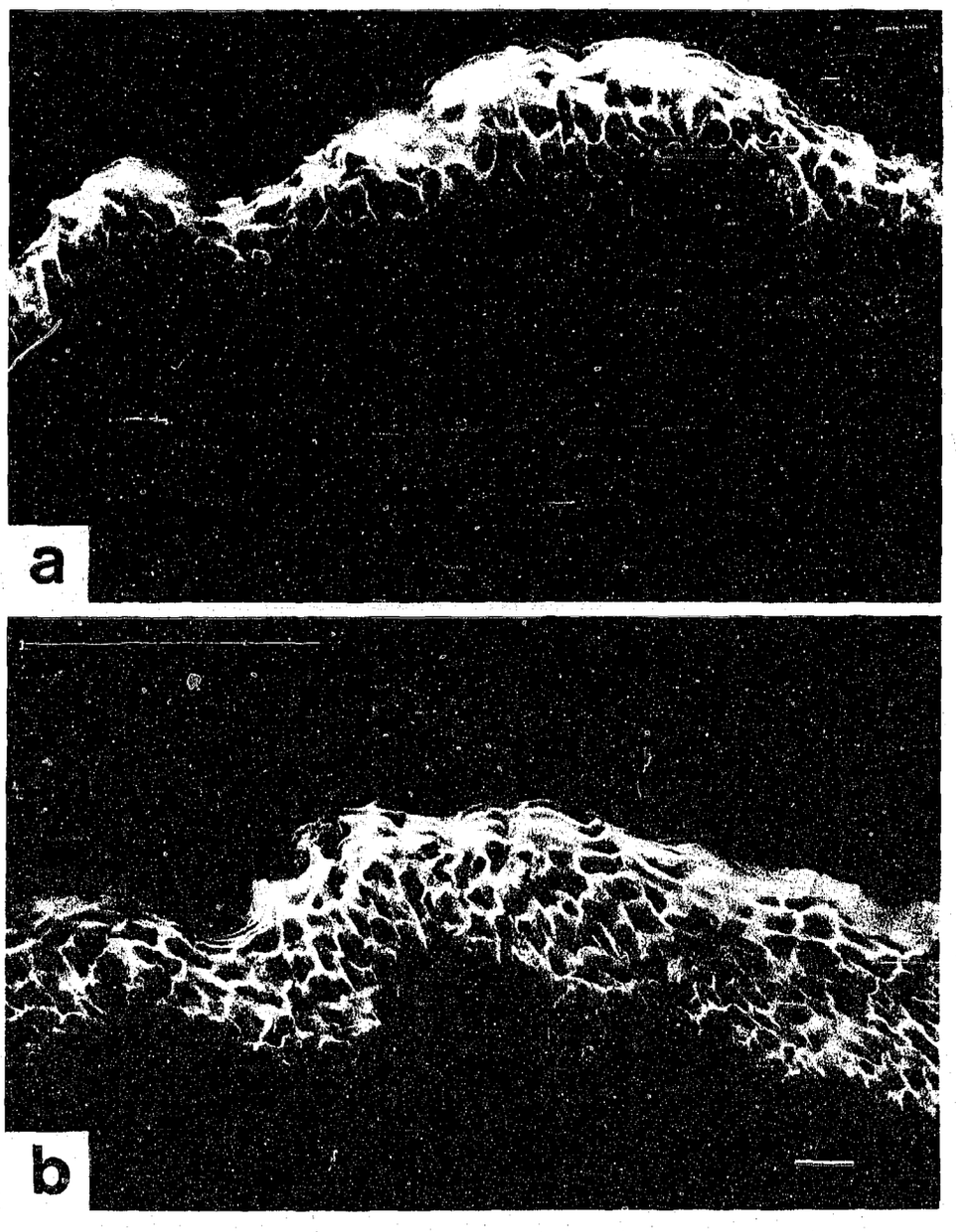

Fig. 2. Immunofluorescent staining of human skin with (a) LH50, a monoclonal antibody generated against 48/46 kDa glycopeptides from callus, which gives stronger staining in the upper layers of the epidermis and (b) CE1, generated against bovine DGII/III, which gives more uniform staining throughout the epidermal layers. The bar is 20 μm.

DGII/III monoclonal antibody was more uniform throughout the living layers of the tissue. The latter antibody may, of course, be recognising more than one type of related molecule, so that the distribution of the protein coded by our DGII/III clones may in fact be more restricted than is apparent. It therefore appears that differential expression of these distinct types of desmosomal cadherins varies throughout the epidermis, which implies changes in desmosome-mediated adhesion. Still other DGII/III related genes may be unveiled by further analysis.

We propose naming the proteins coded by the DGII/III cDNA clones reported by us [11] and by Collins et al. [14] as differentiation-specific variants of Desmosomal Cadherins II and III, abbreviated to D-cadherins II and III, with DGI being D-cadherin I. When the specificity of tissue expression has been more rigorously defined, unambiguous nomenclature can be developed accordingly.

REFERENCES

[1] Mehrel, T., Hohl, D., Rothnagel, J.A., Longley, M.A., Bundman, D., Cheng, C., Lichti, U., Bisher, M.E., Steven, A.C., Steinert, P.M., Yuspa, S.H. and Roop, D. (1990) Cell 61, 1103-1112.
[2] White, F.H. and Gohari, K. (1984) J. Submicrosc. Cytol. 16, 407-422.
[3] Chapman, S.J. and Walsh, A. (1990) Arch. Dermatol. Res. 282, 304-310.
[4] Skerrow, C.J., Clelland, D.G. and Skerrow, D. (1989) J. Cell. Sci. 92, 667-677.
[5] Tsukita, S. and Tsukita, S. (1985) J. Cell. Biol. 101, 2070-2080.
[6] Kapprell, H.P., Owaribe, K. and Franke, W.W. (1988) J. Cell. Biol. 106, 1679-1691.
[7] Hieda, Y., Tsukita, S. and Tsukita, S. (1989) J. Cell. Biol. 109, 1511-1518.
[8] Cowin, P., Kapprell, H.P. and Franke, W.W. (1985) J. Cell. Biol. 101, 1442-1454.
[9] Angst, B.D., Nilles, L.A. and Green, K.J. (1990) J. Cell. Sci. 97, 247-257.
[10] Wheeler, G.N., Parker, A.E., Thomas, C.L., Ataliotis, P., Poynter, D., Arnemann, J., Rutman, A.J., Pidsley, S.C., Watt, F.M., Rees, D.A., Buxton, R.S. and Magee, A.I. (1991) Proc. Natl. Acad. Sci. USA (in press).
[11] Parker, A.E., Wheeler, G.N., Arnemann, J., Pidsley, S.C., Ataliotis, P., Thomas, C.L., Rees, D.A., Magee, A.I. and Buxton, R.S. (1991) J. Biol. Chem. (in press).
[12] Arnemann, J., Spurr, N.K., Wheeler, G.N., Parker, A.E. and Buxton, R.S. (1991) Genomics (in press).
[13] Koch, P.J., Walsh, M.J., Schmelz, M., Goldschmidt, M.D., Zimbelmann, R. and Franke, W.W. (1990) Eur. J. Cell. Biol. 53, 1-12.
[14] Collins, J.E., Legan, P.K., MacGarvie, J., Holton, J.L. and Garrod, D.R. (1991) J. Cell. Biol. (in press).
[15] King, I.A., Wood, M.J. and Fryer, P.R. (1989) J. Invest. Dermatol. 92, 22-26.
[16] Schagger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368-379.
[17] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387-395.
[18] King, I.A., Tabiowo, A. and Pope, F.M. (1986) Biochem. J. 237, 405-414.
[19] Kohler, G. and Milstein, C. (1975) Nature 256, 495-497.
[20] King, I.A., Tabiowo, A. and Fryer, P.R. (1987) J. Cell. Biol. 105, 3053-3063.
[21] Gorbsky, G. and Steinberg, M.S. (1981) J. Cell. Biol. 90, 243-248.
[22] Sawamura, D., Nomura, K., Sugita, Y., Mattei, M.-G., Chu, M.-L., Knowlton, R. and Uitto, J. (1990) Genomics 8, 722-726.