Abstracts of the Symposium on Epidermolysis Bullosa: Molecular Biology and Pathology of the Cutaneous Basement Membrane Zone

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OF MICE AND MEN: THE GENETICS OF EPIDERMOLYSIS BULLOSA SIMPLEX, complex. E. Funah, P.A. Côtier et al. Howard Hughes Medical Institute, Department of Molecular Genetics & Cell Biology, and Biochemistry & Molecular Biology, The University of Chicago, Chicago, IL 60637. University of Texas Health Science Center, Houston, TX 77030. Northwestern University Medical School, Chicago, IL 60614. University of Kentucky, Lexington, KY 40536.

The keratin K5 and K14 are the major structural proteins of the basal cells of epidermis. They form obligate coiled-coil heterodimers, and ~20,000 of these subunits then associate to form 10 nm cytoskeletal filaments. Over the years, we have isolated and characterized the human cDNAs and genes encoding these two keratins, and we have used molecular genetics to elucidate the sequences that are critical for keratin filament assembly in vitro and for cytoskeletal network formation in vivo. Using transgenic mice as a model system, we have targeted the expression of a range of mutant human keratin genes to mouse epidermis. These animals exhibit phenotypes bearing striking similarities to the group of EBs that gives rise to such mutations. The degree to which these mutations perturb keratin filament assembly correlates directly with the severity of the mouse EBS phenotype. These animals have been instrumental in our elucidating (1) how mutant keratins cause keratin network perturbation and basal cell cytolysis, (2) how EBS basal cells regenerate epidermis and without apparent scarring despite their potential for cytolysis, and (3) how a number of prior misconceptions for the biochemistry of EBS were made. Finally, we have discovered keratin mutations in two different EBS patients and we provide genetic and functional evidence to demonstrate that these mutations are responsible for the EBS phenotype.


Epidermolysis Bullosa of the Dowling-Meara type (DB-Eb) is characterized by herpetiform blistering of intraepidermal blisters and a marked degree of palmo-plantar hyperkeratosis. Only sporadic clinical reports have been published so far in the literature. We review here, the clinical features of 22 of our patients affected with DB-Eb (10 males and 12 females) ranging from birth to age 51.

There is a unique clinical aspect of blisters in DB-Eb: serous or haemorrhagic blisters in a herpetiform distribution, healing in a centrifugal sequence, and pronounced inflammatory reaction like an anacrine around the blister, and post-bullous residual pigmentation. These blisters can be generalized on the trunk, face and extremities.

One case was found at birth to have palmo-plantar hyperkeratosis, nail dystrophy, widespread skin blistering, and mucosal involvement. The other cases were less affected at birth and had variable skin involvement which generally improved with age. The most characteristic features, permitting diagnosis of DB-Eb at first glance in children and young adults, were recurrent blisters of the chin, arase-like eruptions near the inguinal and axillary folds, and post-bullous residual pigmentation.

Although palmar and plantar keratoderma are not usually present before the age of 7, if they do occur, the sole involvement is observed first followed by patchy palmar hyperkeratosis.

Electron microscopy of EB keratin filaments in vitro shows the presence of a disorganized network of keratin filaments.


On 16 patients presenting with DM-Eb underwent biopsies of non blistering skin. The site of biopsy had been previously traumatized by rubbing. 5 biopsies were found to have an intraepidermal split at the level of the basal layer. 11 biopsies did not show any separation. In both instances the biopsies shared common features: the basal membrane zone was extremely tortuous and festooned. Thick bundles of tonofilaments were attached to hemidesmosomes. The tonofilaments in the cytoplasm were extremely abundant and arranged in perinuclear shells or thick bundles. In the 5 biopsies where a split was observed it occurred between the nucleus and the basal cytoplasmic membrane of the keratinoocyte of the basal layer just above the thick bundles of tonofilaments attached to the hemidesmosomes. Some tonofilament clumps were free in the cleaved area. In these split cells, the mitochondria were altered, vacuolated with loss of their crests. In all the eleven biopsies where no separation was observed, the horsetails bundle of tonofilaments were present as well as the festooned basal membrane zone and in a few biopsies the mitochondrial distortion was seen. The tonofilament anomaly and the festooned basal membrane zone can be an ultrastructural diagnostic marker of the disease even in the absence of a split. In addition, the observation in split skin and in some non-split skin of mitochondrial anomalies suggest that abnormal mitochondrial function through cellular damage might be associated with the primary defect of the cytoskeleton and is a sign of predamage.

A FUNCTION FOR KERATINS AND A COMMON THREAD AMONG EPIDERMOLYSIS BULLOSA SIMPLEX DISEASES, P.A. Côtier, M.E. Hutton, R. Vassar, E. Fuchs, Departments of Molecular Genetics and Cell Biology and Biochemistry and Molecular Biology, The University of Chicago, Chicago, 1160637.

Keratins are the most complex family of proteins that form intermediate-sized filaments in the cell cytoplasm. Keratin filaments are obligatory heteropolymers comprised of type I and type II chains, and each keratin pair is expressed in a developmentally-, tissue-, and differentiation-specific fashion in all epithelia of the body. The epithelium manifests its protective function by building an extensive network of keratin filaments composed of the keratin pairs K5 (58 Kd) and K14 (50 Kd) (mitotically-active basal layer) and K1 (67 Kd) and K10 (56.5 Kd) (suprabasal, differentiating cell layers). An understanding of the role(s) of K5 and K14 in epidermal basal cells is lacking despite major research efforts over the last decade. We have recently demonstrated that transgenic mice expressing a truncated gene encoding a filament assembly-disrupting mutant of K14 exhibited a phenotype bearing a striking resemblance to a subclass (Dowling-Meara) of human skin disorders known as epidermolysis bullosa simplex (EBS) (Cell 64: 365-380, 1991). The extent to which subtypes of EBS are genetically related is unknown, although they all exhibit skin blistering as a consequence of basal cell cytolysis. We have now examined transgenic mice expressing a range of keratin mutants which perturb keratin filament assembly to varying degrees. These studies provided new insights regarding the etiology of different forms of EBS, the augmentation of the blistered epidermis without scarring, and the function of the keratin filament network in epidermal basal cells.
epidermolysis bullosa simplex (Koeberer) family of Irish origin (1). Notably, positive loci were obtained for APOA2 (2 < 1.94, 0.15) and AT3 (2<1.11, 0.0). The markers D15S13 and D15S66 also produced positive loci scores of 1.03 (0.5) and 1.12 (0.0) respectively. Compilation of these data into a multilocus analysis produced a lod score just maximizing at 3 at the locus for AT3, further substantiating previous tentative indications of linkage between EB and Fr (2). While these data provide strong evidence for a 1q locus, further evidence will be required to identify the gene at a single locus. We will present an update on linkage in this family, particularly with regard to analysis of markers derived from chromosomes 12 and 17, now shown in other pedigrees to be linked to dominant simplex forms of the disease (3,4).


EVIDENCE THAT THE DOWLING-MEARA AND WEBER-COCKAYNE SUBTYPES OF EPIDERMOLYSIS BULLOSA SIMPLEX ARE NOT ALLELIC
K. Stephens, V.P. Saberi, E.M. Wiseman, L. Ramsdell, R.J. Livingston, P.J. Ehrlich, University of Washington and Children’s Hospital and Medical Center, Seattle, WA.

Using genetic linkage analysis, we tested whether the gene responsible for the Dowling-Meara subtype of epidermolysis bullosa simplex (EBM-M) is allelic to the gene causing the Weber-Cockayne subtype (EBM-WC) which was recently reported by Epstein and colleagues (2) to be tightly linked to the D12S14 locus. We determined the genome of 1D5S14 on 25 individuals from three families in which the patients of EBM-M was biopsy-confirmed. Two-point linkage analysis of the D12S14 and the EBM-WC loci gave a maximum LOD score of 2.0 at a recombination fraction of 0.05. Significant evidence in favor of excluding the EBM-M gene from being located within 4% recombinant on either side of the D12S14 locus was obtained. On the three combined families, a LOD score of 2.0 was obtained at a recombination fraction of 0.05 providing significant evidence for excluding the EBM-M gene from being located within 4% recombinant on either side of the D12S14 locus. When LOD scores were calculated for each individual family, the three EBM-M-D12S14 lod scores were 2.0 or less. These data provide strong evidence for excluding the gene(s) responsible for the EBM-M phenotype in these two families from being tightly linked to the D12S14 locus taken under the liberal hypothesis that mutation at any one of several loci may result in an EBM-M phenotype.

ULTRASTRUCTURAL HETEROGENEITY OF KERATIN FILAMENT CLUMPING IN EPIDERMOLYSIS BULLOSA SIMPLEX DOWLING-MEARA
Jyme T. Smith1, Virginia P. Syber2, D. Martin Carter2, Andrew N. Linn2, Karen A. Holbrook1, University of Washington, Seattle, WA and 1Rockefeller University Hospital, New York NY.

Clumping of the skin in epidermolysis bullosa simplex (EBS) occurs within the basilar layer. In the Dowling-Meara (DM) subtype of EBS keratin filament clumping in basal keratinocytes is a characteristic ultrastructural finding. Intracellular cytolysis has been suggested as causative for blister formation in various types of EBS, however, in EBM-M the basilar keratinocytes may be abnormal, structurally weakened cytoskeleton that results in loss of integrity. Some cases from 17 individuals (range from 2 to 76 years) in 11 families with EBM-M were studied by electron microscopy to compare the organization and distribution of keratin filaments. Circumscribed dense aggregates of keratin filaments with rounded borders were present in basal keratinocytes and to a lesser extent in lower spinous cell layers. Keratin filament aggregates were also seen above clumps of the cytoskeleton. The overall distribution of keratin filaments throughout the cytoplasm was restricted, leaving large portions of basal keratinocytes devoid of normal cytoskeleton. There was variation among individuals, in the shape and size of clumps, and the presence of dense keratin filament whorls. A few individuals with EBM-M also showed distinct and abundant suprabasal keratin filament nuclear capping. In most cases keratin filament distribution appeared normal in the remainder of the epidermis. The variable structural findings suggest that EBS-M is phenotypically heterogeneous.
aggregation was also found in cultured DM-EBs keratinocytes. The overall distribution of TF expression in DM-EBs was very similar to the known distribution of the basal keratin, K5 and K14. The TF abnormalities seen included thickened TF bundles as well as round, basket-weave or irregular-shaped clumps ranging in size from less than 1 μm to more than 5 μm in diameter. Immunoelectron microscopy showed dense labelling within the TF clumps of both basal and suprabasal cells using antibodies to keratin 5 and 14. Suprabasal keratin (K10) labelling was noted in uncapped suprabasal TFs. These observations suggest that DM-EBs may be due to a disorder in the formation of the keratin filament network in keratinocytes expressing K5 or K14 or both. This then leads to a decreased resistance to mechanical shearing and subsequent blister formation.

**CONCLUSION**

A therapeutic trial with 5-hydroxy-tryptamine-2 antagonists in a mother and her child affected by EB-DS is reported. The mother had several bullae on the hands and face and the child (9 months old) showed severe eruption of bullae on palms, soles, face and diaper area in a characteristic herpetiform distribution. In November 1990 both began the treatment with piperperone (an anti 5-HT-2 drug), according to a previous report, at the dose of 0.016 mg/day and 1 mg/day respectively for the mother and for the child. After an initial worsening, the lesions dramatically improved in a period of three weeks until a complete clearing of the bullae in both patients. Two months later we were forced to withdraw the treatment because of relevant side effects, except for sedation in the mother and slow new neformation development for the baby. Lesions reappeared in a three week period. In order to avoid these side effects, another SHT-2 antagonist, cyproheptadine was started in April 1991. Clinical efficacy was almost the same as with piperperone although a longer period of induction has been observed and the improvement was less dramatic. Both patients used a standard dose, 5 mg/day for the baby and 8 mg/day for the mother with an almost complete clearing of the lesions without any sign of side effects, namely the child had a normal growth and motor development. We can speculate that the direct receptoral effect of SHT-2 antigogists could play a pivotal role in restoring the normal function of basal keratinocytes and we propose to use cyproheptadine in all the patients suffering from a severe form of EB-DS.

**MOLECULAR GENETICS OF THE CUTANEOUS BASEMENT MEMBRANE ZONE**


(1) Thomas Jefferson University, Philadelphia, PA. (2) University of Wisconsin, Milwaukee, WI, France.

The cutaneous basement membrane (BMZ) consists of several collagenous and non-collagenous macromolecules which are necessary for the stable association of the epidermis and dermis. Some of them also serve as candidate genes in epidermolysis bullosa, a group of heritable blistering disorders. In this study, we have isolated cDNAs corresponding to human COL7A1, BPAG1 and BPAG2 sequences from keratinocyte cDNA expression libraries by immunoscreening and probe hybridization. The identities of the genes were confirmed by comparison with protein sequences and by selection of antibodies which bind to epitopes expressed in the fusion proteins. The coding sequences were mapped by chromosomal in situ hybridizations and Southern analyses of DNA from human x rodent hybrid cells. COL7A1 was mapped to 10q11-12, while BPAG1 and BPAG2 loci were on 10q11-12 and 10q24-25, respectively. This information, together with previous mapping information reporting the type IV collagen, laminin and nidogen loci on different chromosomes, indicates that the BMZ genes are widely dispersed within the human genome.

**MOLECULAR CHARACTERIZATION OF HEMIDESMOSONAL PROTEINS**

G.J. Giudic, D.J. Emerly and L.A. Diaz, Department of Dermatology, Medical College of Wisconsin, Milwaukee, WI, USA.

Bullous pemphigoid (BP) and herpes gestationis (HG) are two clinically related autoimmune skin diseases that are characterized by the presence of subepidermal blisters resulting from a disruption of the adhesive interactions between the epidermis and the dermis. Autoantibodies from patients suffering from these disorders recognize two hemidesmosomal proteins, BP180 and BP230, both of which have been characterized using a molecular genetic approach. BP230 exhibits significant structural similarities with the desmosomal plaque protein, desmoplakin I, a finding which has been reported by several laboratories. Our laboratory has also recently cloned and sequenced BP180 which has led to the identification of several relevant structural features. A single putative membrane-spanning domain was identified near the midpoint of the BP180 autoantigen, while the C-terminal half was shown to consist of a series of 15 domains with primary structures characteristic of the triple helicalforming region of collagens (16am repeats of glycine-X-Y triplets). Situated between the transmembrane domain and the long collagenous region is an ankyrin domain that is a topological feature of proteins that are capable of binding to multiple partners in a manner similar to the mRNA. The close association of BP180 and BP230 with the chicken-specific type VII collagen suggests that the latter may play a role in the development of BP and HG.

Dystrophic EB is often associated with abnormalities in the anchoring fibrils, morphologically recognizable attachment structures, which provide stability to the association of the cutaneous basement membrane and the underlying dermis. Since type VII collagen is the major component of the anchoring fibrils, we tested for genetic linkage of dominant dystrophic EB (DDEB) and the type VII collagen gene (COL7A1). Inheritance of a COL7A1 RFLP, detected by Pvull restriction enzyme digestion, was followed in three Finnish DDEB families. Close genetic linkage of COL7A1 and DDEB was demonstrated, with a maximum LOD score of 8.77 at a recombination fraction of zero. COL7A1 was mapped to chromosome 3p by somatic cell hybrid analysis and by chromosomal in situ hybridization. The localization of DDEB loci was confirmed by the linkage of the disease to D3S30 and D3S32, two RFLPs on chromosome 3p21. Since there were no meiotic recombinants between COL7A1 and DDEB in this study, the hypothesis that COL7A1 is the mutant gene in these three families.

TYPE VII COLLAGEN: DNA LINKAGE STUDY ON A FAMILY WITH DOMINANT DYSTROPHIC EPIDERMOLYSIS BULLOSA. Kazuo Nomura, Takamitsu Sugawara, Takashi Saio, Hajime Nakano, Kazuo Umeki, Katsuko Tamasawa, and Jouni Uitto. Department of Dermatology, Hiroshi University School of Medicine, Hiroshi, Japan; and Jefferson Medical College, Philadelphia, PA.

Ultrastructural observations of dominant dystrophic epidermolysis bullosa (DDEB) have demonstrated rudimentary structure and diminished number of anchoring fibrils in the dermo-epidermal junction. Type VII collagen is the major component of the anchoring fibrils, and it may, therefore, be a candidate gene for mutations in some families with DDEB. Quite recently, DNA linkage analysis revealed genetic linkage between a Finnish family with DDEB and Pvull restriction endonuclease polymorphism in the type VII collagen gene (COL7A1) on chromosome 3p (Ryhanen et al., Am. J. Hum. Genet. 47:797-802, 1990).

In this study, we attempted to determine whether the mutation link to the COL7A1 locus or not in a Japanese family with DDEB. DNA was isolated from affected and nonaffected individuals in the family. Southern hybridizations of DNA digested with PvuII and Pvull endonuclease with a 1.9 kb type VII collagen cDNA revealed co-segregation of PvuII polymorphisms in this pedigree, but the 2 was only -0.4 at 4. Thus, the data are consistent with the COL7A1 locus for DDEB, and further analyses with flanking markers at 3p are underway.


Generalized recessive dystrophic epidermolysis bullosa (RDEB) is a severe inherited autosomal disease characterized by fragile skin and blisters. Alterations in collagenase have been reported in skin from affected patients. We used a genetic linkage approach to test the hypothesis that this disease is due to a defect in the collagenase gene in nine affected families. Analysis of amplified genomic DNA fragments of the collagenase gene by means of denaturing gradient gel electrophoresis (DGGE) allowed us to detect intragenic polymorphisms, which were characterized by direct genomic sequencing. Segregation analysis of these polymorphic sites showed exclusion of linkage between the collagenase gene and generalized RDEB phenotype in a family with consanguineous parents and three affected children. However, the possibility of linkage with the collagenase gene in the other eight families tested could not be excluded, indicating LOD scores ranging from 0.35 to 0.73 at a recombination fraction (r) = 0, and the maximum lod score for all the families being 0.79 at a recombination fraction (r) = 0.10. Overall, these results support the hypothesis that the genetic defect in this family, does not lie in the collagenase gene. The type VII collagen gene, which encodes the major component of anchoring fibrils, is the next candidate gene being investigated.

TRANSFORMING GROWTH FACTOR-β STIMULATES COLLAGEN VII EXPRESSION IN NORMAL HUMAN KERATINOCYTES, BUT NOT IN KERATINOCYTES DERIVED FROM DYSTROPHIC EPIDERMOLYSIS BULLOSA MUTILANS SKIN. Adrian Kong, Dorna Lushajati, and Leena Brinkerhoff-Tuderman. Division of Dermatology, University Hospital, Zurich, Switzerland, and University Hospital, Helsinki, Finland.

Normal keratinocytes and fibroblasts express collagen VII, the major component of anchoring fibrils, at a low level in vitro. However, in co-cultures, a mutual stimulation of collagen VII synthesis by keratinocytes and fibroblasts is observed. One possible mediator of such a stimulation is transforming growth factor-β (TGF-β). In this study, we investigated the effect of this cytokine on collagen VII expression in keratinocytes derived either from normal human skin or from a patient with epidermolysis bullosa miliaris (EBM) keratocytes. Keratinocytes were grown to semi-conflueney in serum-free medium supplemented with ascorbate. Recombinant human TGF-β1 was added at concentrations of 0.1-20 ng/ml for 3 days. In normal keratinocytes, a dose-dependent stimulation of collagen VII expression, as assessed per μg DNA, was found. The maximal stimulation was obtained with 20 ng/ml TGF-β, and resulted in an approximately 7-fold increase. In time-course experiments, a stimulation of collagen VII expression was observed already after 12 h, with a steady increase up to 3 days. In contrast, keratinocytes derived from EB skin did not show collagen VII expression. Stimulation of the EB keratinocytes with TGF-β could not be studied with either normal or EB fibroblasts did not result in an unambiguous dose-response relationship. One possible mediator of epithelial-mesenchymal interactions, may be the synthesis of basement membrane components by cutaneous cells.


Transforming growth factor-β (TGF-β) has been shown to enhance the expression of the extracellular matrix gene, type VII collagen, in several collagen. In this study, the effects of TGF-β1 and TGF-β2 on the expression of the gene for type VII collagen, the major component of anchoring fibrils, in human epidermal cell cultures were examined. Incubation of human epidermal keratinocytes or oral epidermoid carcinoma KB cells with TGF-β1 or TGF-β2

INTRACELLULAR EXPRESSION OF TYPE VII COLLAGEN DURING WOUND HEALING IN SEVERE RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA AND NORMAL HUMAN SKIN. J.A. McGrath1, W. L. Leight, R. A. J. Early, Dept. of Cell Pathology, Institute of Dermatology, St. Thomas's Hospital, London, and 1 Dept. of Experimental Dermatology, The Royal London Hospital, London.

The severe form of recessive dystrophic epidermolysis bullosa (RDEB) is characterized morphologically by an absence of recognizable anchoring fibrils at the dermo-epidermal junction and blister formation below the lamina densa. Anchoring fibrils are mainly composed of type VII collagen (COL7) and an absence of immunofluorescence staining with anti-COL7 antibodies has been widely used in the diagnosis of RDEB. It is not clear, however, whether the abnormality of TGF-β in RDEB represents complete absence, altered synthesis, aberrant assembly or excessive breakdown of the TGF-β molecule. Immunofluorescent studies using the LH-7 fluorescent monoclonal antibody (recognizing the globular region of TGF-β) were performed on biopsies of intact skin and healing skin 7-14 days after dermabrasion injury in five patients with DDEB and in five normal human skin controls. Baseline immunofluorescence showed completely absent staining of the epidermis, dermis and dermo-epidermal junction in RDEB samples and bright linear dermo-epidermal junction fluorescence in normal human skin. In all the 7-14 day post-wounding biopsies of normal human skin, TGF-β expression was noted in basal cells as well as in a continuous or interrupted linear distribution at the basement membrane zone. In all the corresponding RDEB samples, LH-7 fluorescence was also seen with varying intensity within basal and lowermost suprabasal cells and occasionally at the dermo-epidermal junction. The levels of intracellular type IV collagen were seen in both groups but only in those samples taken 7-8 days after injury; later biopsies showed merely continuous dermo-epidermal junction staining. No skin specimens had intracellular anti-laminin fluorescence. The finding of anti-TGF-β immunoreactive staining within the epidermis of RDEB during wound healing suggests that these keratinocytes have an inherent capacity to synthesize TGF-β, or at least the non-collagenous globular region of the molecule. This result, if confirmed, may imply that the expression of TGF-β might be on a component of anchoring fibrils. Further work with molecular markers should help to determine whether the TGF-β abnormality is at the translational or post-translational level.
MARKEDLY (up to 6.3-fold) elevated the \( \lambda (1) \) collagen mRNA levels. This elevation was accompanied by enhanced synthesis of type VII collagen, as demonstrated by indirect immunofluorescence with a monoclonal antibody. The results indicate that TGF-\( \alpha \)-1 and TGF-\( \beta \) have similar biological activities with respect to enhanced type VII collagen gene expression. This synthetic synergy with TGF-\( \alpha \) or other cytokines may enhance the assembly of anchoring fibrils in patients with dystrophic forms of EB.

**MUTANT PS3 IN SQUAMOUS CARCINOMA ARISING IN PATIENTS WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA.** P. H. MCKEE, S. SLATER, C. HOBBS, J. A. GRACIAT, R. W. REID, DEPARTMENT OF HISTOPATHOLOGY, ST THOMAS` HOSPITAL MEDICAL SCHOOL, LONDON, UK. *INSTITUTE OF DERMATOLOGY AND ST JOHN`S HOSPITAL DERMATOLOGY CENTRE, LONDON, UK.

The presence of mutant ps3 protein is associated with a wide variety of human malignant tumours including colon, bronchial and mammary carcinoma. It has a greater than 90% homology to the PS3 protein compared to the native, wild-type, and can thus be readily recognised in routinely processed histological tissues using immuno-cytochemical methodology. In this study, we have investigated 23 squamous carcinomas arising in six patients with recessive dystrophic epidermolysis bullosa for the presence of ps3 mutant protein using a rabbit polyclonal anti-ps3 antibody and a streptavidin peroxidase system. Sixteen tumors were well differentiated. Of these, only one was positive for mutant ps3 protein and this was focal along the advancing border. One tumor was well to moderately differentiated. The presence of ps3 protein was associated with a wide variety of human malignant tumors including colon, bronchial and mammary carcinoma. In all cases, the presence of ps3 protein was associated with a high degree of malignancy.

**THE EXPRESSION OF BASEMENT MEMBRANE COMPONENTS IN CYLRIMODUS AND DERIVED CELLLINES.** J. T. LEE, C. SAXTON, P. P. PURKIS, P. WHITEHEAD, ICRF Skin Tumour Laboratory, London, UK.

Cylindromas are uncommon benign tumours occurring predominantly on the scalp and upper trunk. They are thought to derive from appendageal (sweet gland) epithelium. Multiple tumours from 3 patients have been examined for their reactivity with a panel of monoclonal antibodies to epithelial, basement membrane, matrix metalloproteinases, fibronectin and collagen types IV and VII. All 3 cases were positive for both basement membrane components (types IV and VII collagen, laminin, tenascin) and novel BMZ antigens; LG3, GDA JF3, and F25. Patient derived antiserum to bullous pemphigoid antigen did not react with any of the BMZ components. Cylindromas appear to express both simple epithelial keratins 8 and 18 and keratocytic keratins 5 and 14, a phenotype of a subset of sweat gland luminal epithelial cells. The BMZ of cylindromas is present in all known keratinocyte BMZ antigens. A subset of cylindroma cells within the tumours expressed high levels of intracellular BMZ proteins.

**DISSECTED CYLINDROMAS HAVE BEEN CULTURED USING STANDARD RHINELAND/GRAY TECHNIQUES THROUGH MULTIPLE PASSAGES.** Cylindroma keratinocytes grew well and produced myoid-like cells (p02) which, by lipopectomy, provided an immortalised cell line (CIL-1). Cylindroma keratinocytes produced a high level of type VII collagen in the culture supernatant on immunoblotting with LIT-7. Type VII collagen was found in the matrix. Cell morphology and cultured keratinocytes exhibits intracellular expression of type VII collagen, LAD antigen, and other BMZ antigens, excepting type IV collagen. Cylindroma keratinocytes (CIL-1) were screened for clones corresponding to BMZ antigens. The complex of overexpressing cDNAs delineated 8,930 bp of nucleotide sequences which contained an open reading frame encoding 2,649 amino acids. Analysis of the deduced amino acid sequences predicted a putative signal peptide of 43 amino acids and the presence of a membrane associated sequence of 17 amino acids. Several potential sites for N-glycosylation as well as a potential phosphorylation site were also identified. The protein kinase mediated phosphorylation were identified. Three peptide segments were predicted to be highly antigenic, potentially serving as epitopes for immunisation of a vaccine. Cylindroma keratinocytes were identified with a high degree of homology with sequences in desmoplakin I, a component of the desmosomal cytoskeleton, were detected in the cytoskeleton terminal end of the molecule. In addition, the expression of the three autoantigens characterized by heptad repeats encoded an alpha-helical coiled coil dimeric structure in the central portion of the protein. These data suggest that BPA1 may be a membrane associated protein which plays a role in the assembly of basal keratinocytes to the underlying basement membrane.
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