HB1

Mutations in the *MRP6* Gene Cause Pseudoxanthoma Elasticum.

Franziska Ringpfeil, Mark G. Lebwohl, and Jouni Uitto.
Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, PA; Department of Dermatology, Mt. Sinai School of Medicine, New York, NY.

Pseudoxanthoma elasticum (PXE), the prototypic heritable connective tissue disorder affecting the elastic structures in the body, manifests with cutaneous, ophthalmologic and cardiovascular findings, with considerable morbidity and mortality. The molecular basis of PXE has remained unknown, but the disease locus has recently been mapped to a ~500 kb interval on chromosome 16p13.1, without evidence for locus heterogeneity. In this study, we report pathogenetic mutations in *MRP6*, a member of the ABC transporter gene family, in 12 kindreds with PXE. The mutation detection strategy consisted of heteroduplex scanning of coding sequences in the *MRP6* gene, which were amplified by PCR using genomic DNA as template, followed by direct nucleotide sequencing. A total of 18 mutant *MRP6* alleles were disclosed in the 12 probands with PXE. These genetic lesions consisted of either single bp substitutions resulting in missense, nonsense or splice site mutations, or large deletions resulting in allelic loss of the *MRP6* locus. A particular stop codon mutation, R1141X, occurred independently in six unrelated kindreds. None of the mutations were present in 50 unrelated, unaffected control individuals. Examination of clinically unaffected family members in five multiplex families identified heterozygous carriers, consistent with an autosomal recessive inheritance pattern. Collectively, identification of mutations in the *MRP6* gene provides the basis to examine the pathomechanisms of PXE, and allows development of DNA-based carrier detection in families with a history of this disease, as well as diagnosis prior to the onset of the clinical manifestations.

HB2

Collagen 17A1 Gene Correction Using Spliceosome Mediated RNA Trans-splicing (SMaRT™) Technology.

G. Dallinger*, M. Puttaraju†, L.G. Mitchell†, K.B. Yancey‡, H. Hintner* and J.W. Bauer*.
**Department of Dermatology, General Hospital, Salzburg, Austria; †INTRONN LLC, Durham, NC, USA; ‡Dermatology Branch, National Institutes of Health, Bethesda, MD, USA.*

As previously demonstrated, spliceosome mediated RNA trans-splicing (SMaRT™) can be used to correct mutant pre-messenger RNA (pre-mRNA) expressed from a mini-gene of the cystic fibrosis transmembrane conductance regulator gene (CFTR). That work demonstrated the feasibility of SMaRT™ as a tool to target and reprogram any chosen intron containing gene at the pre-mRNA level. We chose epidermolysis bullosa (EB) as a model system to test the possibilities of this technique in skin gene therapy. In GABEB a recessive mutation 4003delTC located on exon 52 of the COL17A1 gene was selected as the target for gene repair. Pre-therapeutic molecules (PTM's) with high trans-splicing efficiency were selected using a β-galactosidase repair model. To test the corrective possibilities, we constructed a COL17A1 mini-gene target containing Exon 51, Intron 51 and Exon 52 followed by FLAG epitope-sequence at the 3′end of Exon 52. For trans-splicing experiments we co-transfected 293T cells and a GABEB cell line which both do not produce detectable amounts of endogenous COL17A1 mRNA with plasmids expressing target (COL 17A1 mini-gene) and PTM6 consisting of *trans*-splicing domain followed by the cDNA sequence spanning Exons 52 to 56 of COL17A1. To analyse the accuracy and efficiency of cis- and trans-splicing, RT-PCR reactions were performed using a primer within Exon 51 as the forward primer and a FLAG-reverse primer for the detection of cis-splicing, or an Exon 53-reverse primer for detection of trans-splicing. Sequence analysis of the RT-PCR (Exon 51-Exon 53) product demonstrated the accurate trans-splicing between COL 17A1 target and PTM6. These results strongly suggest the feasability of SMaRT™ to correct the genetic defects which cause EB.

HB3

Pathogenesis in Pemphigus Vulgaris: A Central Role for the Armadillo Protein Plakoglobin.

E Müller, R Caldelari, A de Bruin, D Baumann, C Bierkamp, V Balmer and M.M. Suter.
Institute of Animal Pathology, University of Berne, Berne, Switzerland; Centre de Biologie du Developpement, Toulouse, France.

Autoantibody binding to desmoglein 3 induces loss of intercellular adhesion and blister formation in pemphigus vulgaris (PV). Two hypothesis are currently favored to explain the underlying molecular mechanism: i) disruption of adhesion through steric hindrance, or ii) interference of desmosomal cadherin-bound antibody with intracellular events which we speculated to involve plakoglobin. To investigate the second hypothesis we established keratinocyte cultures from plakoglobin knock-out ($PG^{-/-}$) embryos and compared their responsiveness to PV IgG with that of normal $PG^{+/+}$ keratinocytes. Both cell subtypes displayed desmosome-mediated adhesion and bound PV IgG at their cell surface. Keratin retraction and loss of adhesion, the hallmarks of PV lesions were however only observed in $PG^{+/+}$ keratinocytes. When full-length plakoglobin was reintroduced into $PG^{-/-}$ cells, responsiveness to PV IgG was restored. Investigating the subcellular distribution of plakoglobin in $PG^{+/+}$ cells, we found that PV IgG binding disturbed the localization of plakoglobin at the membrane, affected its steady-state level in the cytoplasm and perturbed its differentiation-induced nuclear import. The impaired nuclear trafficking of this armadillo protein in the presence of the PV antibody was further paralleled by decreased transcriptional transactivation of a reporter gene under control of a promoter containing multiple TCF binding sites. Our results demonstrate that steric hindrance is not sufficient to induce loss of adhesion in response to PV antibodies and provide evidence for a molecular mechanism involving plakoglobin in the pathogenesis of PV. These findings represent the first example of disease development which is dependent on plakoglobin and involves disturbed localization of this armadillo protein at the membrane and in the nucleus.

HB4

Microphthalmia and Loss of Coat Pigmentation from Transgenic Expression of a Neurogenic Factor in Pigment Cell Precursors.

TJ Hornyak, J Gavin, Z Jiao, and *EB Ziff.
Department of Dermatology, Henry Ford Health Science Center, Detroit, MI and *Department of Biochemistry and HHMI , NYU Medical Center, New York, NY.

A neuronal determination factor was expressed in the developing melanocyte lineage to explore determinants of melanocyte development from the neural crest. Transgenic mice expressing the neurogenic bHLH factor MASH-1 from the *dopachrome tautomerase (Dct)* promoter were generated using a 3.2 kb fragment of *Dct* upstream regulatory sequence linked to a FLAG epitope-tagged version of the murine *Mash1* gene. A single microphthalmic founder with a mosaic coat color phenotype produced non-pigmented, microphthalmic F_1 progeny. The F_1 progeny transmitted the transgene with approximately 50% frequency to F_2 progeny that shared the phenotype. Analysis of the eye of transgenic embryos at E11.5 revealed the presence of a developing lens and neuroretinal layer. However, the structure corresponding anatomically to the location of the retinal pigmented epithelium (RPE) was supplanted by a non-pigmented layer, termed RPE-like layer, that is more than one cell thick. At E14.5, the neuroretina is elongated, and the RPE-like layer extends anterior to the lens, forming a single continuous structure when examined in transverse sections. The ocular and coat color phenotypes of the *Dct::Mash1* transgenic line demonstrate that expression of *Mash1* in pigment cell precursors alters the development or differentiation of both non-neural crest-derived cells of the RPE and neural crest-derived melanocytes. These alterations result in the failure of proper eye development and in a dominantly inherited mutant coat color phenotype, respectively.

HB5

Cloning and Initial Characterization of Human Epsin 3, a Novel Matrix-Induced Keratinocyte Specific Transcript.

KD Spradling, TJ Burke, J Lohi, and BK Pilcher.
Depts. of Cell Biology and Dermatology, UT Southwestern Medical Center, Dallas, TX.

In injuries that disrupt the basement membrane, wound edge keratinocytes contact dermal extracellular matrix (ECM) and are activated to undergo global changes in gene expression, switching from a proliferation/differentiation program to one that supports repair of the tissue defect. Type I collagen, an abundant dermal ECM protein, plays an important role in keratinocyte activation as changes in cell:matrix interactions induce transcripts required for re-epithelialization. Indeed, keratinocyte contact with collagen induces the expression of MMP-1, a matrix metalloproteinase expressed invariantly by activated keratinocytes at the wound edge that is essential for migration across the denuded dermis. ddRT-PCR was used to identify other activation-specific genes that are modulated in keratinocytes following contact with collagen. cDNA clone GAP4G1, a novel transcript markedly induced (20-fold) following collagen contact, was sequenced and found to have 100% identity to nucleotides 10237-10379 of human chromosome 17 clone hCIT.22_K_21. Subsequent amplification and translation of the full-length cDNA revealed epsin 3, a novel 632 amino acid protein closely related to, but distinct from members of the epsin protein family. Epsins 1 and 2 are constitutively and ubiquitously expressed proteins that function as molecular bridges required for clathrin-mediated endocytosis. Interestingly, and in contrast to other known epsins, we found that epsin 3 is expressed specifically and invariantly by collagen-activated keratinocytes, whereas expression was not found in any other cell or tissue examined. Moreover, we determined that the triple helical conformation of collagen was required for induction as plating cells on gelatin failed to promote epsin 3 expression. Lastly, similar intracellular signaling pathways were required for collagen induction of both MMP-1 and epsin 3, suggesting a common mechanism leading to expression of both transcripts following matrix contact. Thus, we have identified a novel epsin that is induced following keratinocyte activation due to alterations in cell:matrix interactions, suggesting that this transcript serves a unique and required role during epidermal repair.

HB6

Comparative Analysis of the Global Transcriptional Profile of Human Skin-Homing Memory T Lymphocytes vs. Non-Skin Homing Memory and Naive T Lymphocytes.

Alex S. Carcamo, Robert C. Fuhlbrigge, Robert Hong, Steven R. Gullans and Thomas S. Kupper.
Harvard Skin Disease Research Center, Brigham and Women's Hospital, Boston, MA

T lymphocytes play a central role in the pathogenesis of a number of inflammatory, autoimmune and neoplastic skin diseases. Transcriptional profiling can provide substantial insights into the mechanisms regulating the development and function of the T cell populations involved in these disorders. We have applied oligonucleotide microarray technology (GeneChip Probe Array System, Affymetrix) to perform the first global comparison of gene expression between skin-related and non-skin related T cell populations. We report a comparative simultaneous analysis of 7100 human gene expression products between resting naïve (CD45RA+), skin-homing memory (cutaneous lymphocyte antigen (CLA)+, CD45RO+) and non-skin homing memory (CLA-, CD45RO+) T cells. Overall, we identified 21 genes with statistically significant expression level differences (? 2-fold) in memory T cells compared with naïve cells and 11 genes with differential expression in skin-homing (CLA+) vs. non-skin homing (CLA-) memory T cells. 1,076 genes displayed identical levels of expression ($p < 0.001$) across all three T lymphocyte populations including several genes that have not previously been reported in the context of T lymphocyte function and a sizable number that have not yet been functionally characterized. To test the utility of this technique in the analysis of cutaneous disease, the gene expression profile of T cells obtained from a patient with leukemic cutaneous T cell lymphoma (CTCL) (CD4+, CD45RO+, CD7-, CD25+, CLA+) was compared with that of normal non-transformed CLA+ memory T cells. 290 genes with statistically significant increased expression in CTCL cells were identified. Using a novel integrated information management system (linked to GeneBank, Locus Link, SWISS-PROT, OMIM, TIGR, Medline, etc) developed in collaboration with a team of software engineers we have annotated and functionally clustered this set of over-expressed genes. Our analysis indicates that several of these genes may play a pathogenic role in CTCL. Genome-wide parallel analysis of the expression patterns of T cell populations involved in skin diseases has the potential to provide unique insights into the multiple gene-gene interactions underlying the complex nature of these disorders.