HB16

EXPRESSION OF A DOMINANT NEGATIVE TYPE II TGFβ RECEPTOR IN THE EPIDERMIS OF TRANSGENIC MICE DOCUMENTS ITS ROLE IN MEDIATING GROWTH INHIBITION. X.-J. Wang, D.A. Greenhalgh, J.A. Bickenbach, D.S. Bundman, R. Derynck* and D.R. Roop, Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, TX and *Department of Growth and Development, University of California at San Francisco, CA.

Transforming growth factor β (TGFβ) is a potent growth inhibitor of normal keratinocytes. Homodimers of type II TGFβ receptors and heteromers of the type I and II receptors are believed to mediate TGFβ effects, however, it is not clear whether the Ser/Thr -kinase domain of the type II receptor is exclusively required to mediate the growth inhibitory effect of TGFβ in the epidermis. To test this, and to establish an in vivo model for studying the role of the TGFβ pathway in skin carcinogenesis, we have generated transgenic mice which overexpress a dominant negative type II TGFβ receptor (ΔβRII) in the epidermis. The ΔβRII transgene was generated by deleting the Ser/Thr -kinase domain of the receptor, but retaining the ligand binding domain and the transmembrane domain for dimerization. The truncated mouse loricrin promoter (ML), which expresses transgenes in both basal and suprabasal layers of the epidermis, was utilized to target the ΔβRII to the epidermis (ML.ΔβRII). Newborn ML.ΔβRII mice exhibited a gross phenotype of a thickened and wrinkled skin, and histologically the epidermis was markedly hyperplastic. The skin of these mice began to peel within three days, indicating progression to a hyperkeratotic phenotype. In vivo labelling with BrdU showed a 3-fold increase in the mitotic rate over controls, with the labeled nuclei occurring in both basal and suprabasal cells. These mice also exhibited aberrant expression of keratin 6 in the epidermis. These data document the role of the type II TGFβ receptor in mediating TGFβ-induced growth inhibition in the epidermis. To date, the ML.ΔβRII mice are viable and capable of producing progeny with the neonatal phenotype. Since mutations generating dominant negative forms of the type II TGFβ receptor have recently been detected in squamous cell carcinomas, these transgenic mice should be useful in studying loss of functional type II TGFβ receptors at various stages of skin carcinogenesis.

HB11

IDENTIFICATION OF LABEL-RETAINING CELLS IN HUMAN SCALP EPIDERMIS AND HAIR FOLLICLE. Melpo Christofidou-Solomidou*, Motha Kalyanit, Steven M. Albelda*, George Cotsarelis+, Depts of *Medicine and +Dermatology, University of Pennsylvania Medical Center, Philadelphia, PA.

The epidermis and hair follicle are thought to contain slow-cycling stem cells that are responsible for repopulating these self-renewing tissues. The transition of the hair follicle from telogen to anagen results in the formation of a new lower follicle that subsequently produces a new hair. In humans, the location of stem cells giving rise to the regenerated hair follicle and hair is not clear. In mice, hair follicle stem cells have been identified experimentally as label- retaining cells (LRC'S) in the bulge area of the hair follicle. The purpose of this study was to identify stem cells in human scalp by localizing LRC's in skin grafts transplanted onto severe combined immunodeficient (SCID) mice. Three weeks after grafting, BrdU was delivered continuously for 2 weeks using intraperitoneal minipumps. During this time many follicles were in transition from telogen to anagen. We reasoned that hair follicle stem cells would be labeled at this point as they transiently proliferated to give rise to the new lower follicle. After chasing for 4 months, any rapidly proliferating cells would dilute their label and only slow-cycling (stem) cells should remain labeled. After the chase period, no LRC's were found in the hair follicle bulb or lower outer root sheath (ORS) below the insertion of the arector pili muscle. LRC's were only detected in the ORS near or above the insertion site of the arector pili muscle. LRC's were also identified in the interfollicular epidermis within the rete ridges and in the peri-infundibular area. These results suggest that, similar to the mouse hair follicle, stem cells are located in the bulge area of the human hair follicle, and not in the transient portion of the follicle. These results also support the concept that epidermal stem cells are located in rete ridges and in the peri-infundibular region of interfollicular epidermis.

HB12

MOLECULAR BASIS OF VARIEGATE PORPHYRIA: FRAMESHIFT MUTATIONS IN THE PROTOPORPHYRINOGEN OXIDASE GENE. HaMut Lam, Laryssa Dragan, Hui C. Tsou, Hans Merk*, Monica Peacocke, Günter Goerz**, Shigeru Sassa^, Maureen Poh-Fitzpatrick, David R. Bickers and Angela M. Christiano. Dept. of Dermatology, Columbia University, New York, NY; *Dept. of Dermatology, University Hospital, Aachen, Germany; ** Dept. of Dermatology, Heinrich Heine University, Düsseldorf, Germany; ^The Rockefeller University Hospital, New York, NY.

The porphyrias are disorders which result from the inherited or acquired dysregulation of one of the eight enzymes in the heme biosynthetic pathway. Variegate porphyria (VP), is characterized by deficiencies in protoporphyrinogen oxidase (PPO), and has recently been genetically linked (Z=6.62) to the PPO gene on chromosome 1q21. VP is usually inherited as an autosomal dominant trait with half-normal levels of PPO, although rare recessive cases with <10% PPO activity have been reported. Cutaneous manifestations are present in affected individuals, and consist of blistering, fragility, scarring of sun-exposed skin and post-imflammatory hyperpigmentation. The photosensitivity may exist alone or together with neurovisceral symptoms which characterize the acute hepatic porphyrias. We have identified two different frameshift mutations in the PPO gene in two unrelated patients with VP. The first is an apparently *de novo* 2 bp insertion in exon 3, and the second is a 1 bp deletion in exon 10. Both PPO mutations result in a frameshift and downstream premature termination codon, and both patients are heterozygous for these mutations, explaining why they have approximately 50% levels of residual PPO activity. The enzyme deficiencies elicited by these mutations are likely to result from haploinsufficiency due to nonsense-mediated mRNA decay of the mutant allele, and/or by dominant-negative interference of small amounts of mutant polypeptide with the wild-type. These two independent cases establish that premature termination codons in the PPO gene result in dominantly inherited VP, and provide the basis for the design of enzyme replacement strategies for this disorder in the future.

HB10

MUTATIONS IN THE PLECTIN GENE CAUSE EPIDERMOLYSIS BULLOSA WITH MUSCULAR DYSTROPHY. W.H. Irwin McLean, Leena Pulkkinen, Frances J.D. Smith, Elizabeth L. Rugg, Florencia Bullrich, Robert E. Burgeson, Satoshi Amano, David L. Hudson, Katsushi Owaribe, John A. McGrath, Robin A.J. Eady, Irene M. Leigh, E. Birgitte Lane, Angela M. Christiano and Jouni Uitto. Univ. of Dundee; CBRC Harvard Univ., Boston, MA; Nagoya Univ.; St. John's Inst. of Dermatology, London, U.K.; Royal London Hospital, and Jefferson Medical College, Philadelphia, PA.

Plectin, a high molecular weight cytomatrix protein, is an integral part of hemidesmosomes, the basal cell-basement membrane attachment complex, as well as a component of the sarcolemma in muscle. Epidermolysis bullosa (EB) is a heterogenous group of mechano-bullous diseases, and a specific variant (EB-MD) demonstrates tissue separation at the level of the hemidesmosomes, and is associated with muscular dystrophy. Recently, we have shown negative immunofluorescence staining with a monoclonal antibody HD-1, which recognizes a plectin epitope, in the skin and muscle of patients with EB-MD, suggesting that plectin is the gene/protein system for underlying mutations in these patients. We have cloned the human plectin cDNA and gene, which was mapped by FISH to the most telomeric portion of chromosome 8, corresponding to band 8q24. The predicted protein possesses an actin binding domain at the N-terminus, a central rod domain and a C-terminal intermediate filament binding domain. Sequencing of plectin cDNA amplified from keratinocyte or fibroblast mRNA by RT-PCR revealed homozygous frameshift mutations in two patients. In one case, a homozygous 8 bp deletion was noted while the second case was homozygous for a 8 bp insertion, both resulting in truncation of the protein within the central rod domain. In both cases, the clinically unaffected consanguineous parents were heterozygous carriers of the same mutations. These results establish, for the first time, the molecular basis of EB-MD, and clearly demonstrate the important structural role of plectin in intermediate filament-hemidesmosome adherence. The role of plectin in a similar membrane-cytoskeletal association in the sarcolemma would explain the combined phenotype of skin fragility and progressive muscle degeneration in EB-MD.

HB17

INTERLEUKIN 7 DEFICIENT MICE. Benjamin E. Rich and Philip Leder, Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115

A growing body of experimental data describes the biology of interleukin-7 (IL-7). Several observations have linked IL-7 specifically to the skin. IL-7 is expressed by skin keratinocytes in a regulated fashion and this expression supports the viability of dendritic epidermal T cells. Three different lines of IL-7 transgenic mice have been generated in which lymphoid or epidermal expression of IL-7 leads to cutaneous infiltrates of lymphocytes. Finally, many cutaneous T cell lymphomas express and respond to IL-7. These observations, while not conclusive, suggest that IL-7 may be an especially significant factor for cutaneous lymphocytes. Therefore IL-7 is likely to be important for normal cutaneous immunity and to be involved in certain skin diseases.

To examine the biologic role of IL-7 in living animals, a defective allele of the IL-7 gene was introduced into the germ line of mice. Mice homozygous for this allele have sharply curtailed expansion of lymphoid cells. This is evident in a significant reduction but not elimination of lymphoid cells in the spleen, thymus and bone marrow of these mice. Small numbers of B cells are detected in the bone marrow. Similarly, the few thymocytes present in IL-7 deficient mice express CD4 and/or CD8 markers associated with T cell maturation. Thus the signal transmitted by IL-7 plays a central role in the expansion of lymphoid populations during maturation while it may not be absolutely required for maturation. A transgene containing an IL-7 cDNA under the control of immunoglobulin heavy chain gene promoter and enhancer sequences directs expression of IL-7 to lymphoid cells. This transgene was found to restore T and B cells of IL-7 deficient mice to approximately normal levels. This complementation confirms that the lymphoid defect is specifically due to disruption of the IL-7 gene and suggests that the number of lymphoid cells may be regulated by the level of IL-7 expression.