Hot Off the Bench—Late Breaking Abstracts

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HBO1

Human Tumors of the Hair Matrix Contain Activating Mutations in Beta-catenin

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Wnt signaling orchestrates a number of developmental programs in eukaryotic organisms. In response to this stimulus, cytoplasmic β-catenin is stabilized, enabling downstream transcriptional activation by members of the Lef/TCF family. Most colon cancers contain mutations in the adenomatous polyposis coli (APC) gene, which is required for ubiquitin-mediated degradation of β-catenin. However, in a small percentage of cancers with wild type APC, stabilizing mutations occur in β-catenin. Recently we showed that transgenic mice expressing a stabilized β-catenin in the epidermis develop *de novo* hair follicle formation and tumors of the hair matrix, or pilomatricomas. We sought to elucidate the cell origin and etiology of sporadic human pilomatricomas. We found nuclear Lef-1 in dividing tumor cells, providing biochemical evidence that pilomatricomas are derived from hair matrix cells. Remarkably, 12 of 16 pilomatricomas analyzed contained β-catenin missense mutations clustered in the amino-terminal segment, which is involved in ubiquitin-mediated degradation of the protein. This percentage of β-catenin mutations is significantly higher than in all other human tumors examined thus far, and directly implicates β-catenin/Lef misregulation as the major cause of hair matrix cell tumorigenesis in humans.

HBO2

Epinephrine Inhibits Epidermal Cell Antigen Presenting Capability

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Langerhans cells (LC) within the epidermis are situated close to nerves and the murine skin-derived dendritic cell line XS52 expresses the β2 adrenergic receptor. Epinephrine (Epi) and norepinephrine (NE) modulate cytokine expression in this cell line.

We have now examined XS52 cells and freshly-obtained LC from CAF_1 mice enriched to >99% purity utilizing antibodies to I-A conjugated to magnetic beads. By RT-PCR, mRNA for the α1a and β2 adrenergic receptors was observed.

Therefore, we examined the ability of Epi to modulate EC antigen presentation. EC were prepared from CAF_1 mice ($H\text{-}2^{a/d}$) and enriched for LC content by treatment with anti-Thy 1.2 and complement (eEC) and then cultured for 16 hr with or without 10^{-7} M Epi. Epi-treated and untreated cells were pulsed for 3 h with a soluble extract of the S1509a spindle cell tumor ($H\text{-}2^{a}$) as a source of TAA (tumor associated antigens) or with medium alone. After thorough washing, these cells were used to elicit delayed-type hypersensitivity (DTH) in each of several CAF_1 mice previously immunized against S1509a. Cells of each type (7.5×10^5) were injected into a hind footpad of each mouse and 24 h footpad swelling assessed as a measure of DTH response. Mice challenged with Epi-treated, TAA-pulsed cells showed significantly lesser DTH than mice challenged with non-Epi-treated, TAA-pulsed cells [Epi-treated, TAA-pulsed eEC- 0.19×0.01 mm: *versus* non-Epi-treated, TAA-pulsed eEC- 0.284×0.01 ($p<0.004$). Epi-treated, non-TAA-pulsed eEC- 0.106×0.01, non-Epi-treated, TAA-pulsed eEC- 0.146×0.01].

Furthermore, we examined BALB/C eEC ($H\text{-}2^{d}$) treated or not treated with Epi for their ability to present keyhole limpet hemocyanin (KLH) to the KLH specific, $I\text{-}A^{d}$ restricted T-T hybridoma HDK1. Epi significantly inhibited the ability of EC to present antigen in this assay while (+)-isoproterenol, the inactive isomer of the β-adrenergic agonist (-)-isoproterenol, had no effect on antigen presentation.

The findings that Epi inhibits antigen presentation by epidermal cells suggests that this hormone may play a role in regulation of immunity within the skin.

HBO3

Regulation of the Size of Skin Appendages

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Regulation of organ size is a fundamental process in organogenesis, and has practical implications in hair growth. The feather is an ideal model since there are many feathers on one bird and most of them are of different sizes. How do they become different in sizes? One possibility is that initial feather primordia are already different in sizes. The other is that all primordia start from the same size but become different in subsequent development due to different growth rate or different growth period. Here we examine feather sizes from dorsal trunk, wing and caudal regions. In chicken, we found that the sizes are different (up to 20 folds) from the beginning. To catch the difference in the growth phase, we turned to newborn parakeets so we can measure the same feather (in chicken, this phase occurs inside the egg). We found that feathers in the spinal tract stop growth early. Feathers in wing and caudal regions start later but grow faster. For those in more distal limb or caudal region, the growth phase lasts longer. Finally, the size gradient of feather forms along the body of a bird, usually longer in the caudal region and distal wing. Thus the different sizes of feathers can result from initial difference in placode size, difference in growth rate and period of continuous growth. To test whether we can modulate the initial size, we use feather reconstitution assay and found that the size of feathers remains a constant. When mesenchymal cells were transduced with RCAS BMP receptors, then (initial) feather primordia became smaller. This is consistent with the hypothesis that the ratios of activators and inhibitors are involved in regulating the size of skin appendages.

HBO4

A Novel Approach To Gene Therapy Of Pigment Disorders With A Retroviral *Streptomyces* Tyrosinase Vector

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In order to induce melanin production in mammalian cells with pigment disorders, a recombinant retrovirus containing the *mel* operon of *Streptomyces antibioticus* was constructed. The *S. antibioticus mel* locus, which consists of the ORF-438 and the tyrosinase gene, was specifically derived by PCR from *Streptomyces* plasmid pIJ702. The ORF-438 is required for transfer of copper to apotyrosinase, which is required for its enzymatic activity. The tyrosinase gene was inserted into the XhoI/BamH I cloning site of the pLXSN retroviral vector to obtain pL*tyr*SN. An internal ribosome entry site (IRES) suitable for mammalian-cell expression was obtained from the pLXIN retroviral vector by PCR. The ORF438 and IRES DNA fragments were ligated at the BclI site, and then inserted into the EcoR I/Xho I cloning site of the pL*tyr*SN vector to obtain the therapeutic retroviral vector pL*mel*SN. This vector was amplified in mouse PT67 packaging cells, where the ORF-438 and tyrosinase genes were also co-expressed as determined by RT-PCR. In order to evaluate the vector in a model pigment disorder, albino mouse hair follicles were isolated, cultured and then infected with the retrovirus pL*mel*SN. Six days after retroviral infection, melanin pigments were observed in the albino mouse hair follicles. These results demonstrate that the *S. antibioticus mel* operon could express an active tyrosinase and produce melanin in the albino mouse hair follicles. This novel gene therapy approach, using a small and simple tyrosinase operon in a high expression vector, has potential wide application for pigment disorders.

HBO5

Classical and Variant Darier Disease due to Mutation in ATP2A2

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Darier disease (DD) is an autosomal dominant disorder causing focal acantholytic and dyskeratotic hyperkeratosis. Clinical variants include cases with erosive, cornifying, comedonal and acral hemorrhagic lesions. The disorder has also been associated with mental disorder and epilepsy. The DD gene maps to 12q24.1, and causative mutations have recently been identified in ATP2A2 (SERCA2; Sakuntabhai *et al.*, *Nature Genet* 21:271-277, 1999), a widely expressed ATPase which pumps cytoplasmic calcium into the endoplasmic reticulum for use in calcium signalling.

In screening ATP2A2 for new mutations, we have sought to establish whether clinical variants are allelic with classical DD, and whether specific mutations cause certain phenotypes. ATP2A2 has 21 exons and 2 alternatively spliced products, both expressed in skin. Using mutation screening of all exons with SSCP and/or direct sequencing, to date we have identified mutations in 37 European pedigrees of DD, with 31 distinct mutations (16 missense, three nonsense, nine deletion, three insertion) in 20 exons. Examples of all the above clinical variants are allelic with classical DD. We have identified consistent mutations only in acral hemorrhagic DD, in which we found an identical missense mutation in three unrelated families from Scotland & Italy. However a distinct mutation in a different exon was found in a Swedish family.

It remains to be established whether other mutations in ATP2A2 have specific consequences, and indeed how all mutations contribute to the pathogenesis of DD.