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Following are poster abstracts submitted by IPCC members related to PC. The full abstracts are included in the following pages.

SID Poster #307 (2007)

Development and validation of an allele-specific quantitative RT-PCR assay for pachyonychia congenita

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Pachyonychia congenita (PC) is a rare autosomal dominant disorder caused by mutations in K6a, K6b, K16, or K17. We are developing a mutation-specific siRNA that targets the K6a N171K (MX) allele over the K6a wild type (WT) allele in tissue culture and animal models. In order to establish endpoints for this siRNA in future clinical trials, we have developed and validated a quantitative RT-PCR assay that discriminates between the WT and MX alleles. WTand MX-specific primer/probe oligonucleotides were developed to assess absolute copy numbers of each allele in a real-time PCR assay. The assay was validated with plasmid constructs containing either the WT or MX sequence, and with total RNA from a participant carrying a K6a N171K mutation. After 40 cycles, "no template" controls demonstrated no amplification. Eight independent extractions of 100 mg of stratum corneum from the affected plantar surface resulted in a range of 0.61-2.7 µg total RNA recovery. In two independent experiments, the average allelic copy number in 20 ng of total RNA was determined to be 48.3 ± 16.6 (WT) and 140.2 ± 25.7 (MX); and 6.3 ± 1.6 (WT) and 28.0 ± 3.8 (MX) copies, respectively. The coefficient of variation in these experiments was 16%-30% with 20 ng of total RNA, demonstrating functional sensitivity at that concentration of template. Specificity was assessed through mixing experiments of WT and MX plasmid DNA. The WT probe demonstrates an average bias of 17% and the bias does not appear to change with the proportion of MX-containing plasmid. The average MX probe bias was 3.5% and increased to a high of 16% with increasing WT probe. These values demonstrate significant specificity of each probe for the corresponding template. This assay will permit a mutation-specific, sensitive evaluation of the molecular effects of our siRNA candidate in upcoming clinical trials.

SID Poster #563 (2007)

Therapeutic siRNAs for treatment of pachyonychia congenita

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Pachyonychia congenita (PC) is a rare autosomal dominant keratin disorder characterised by hypertrophic nail dystrophy, palmoplantar keratoderma, and oral leukokeratosis. PC is divided into two main types, PC-1 due to mutations in keratin K6a or K16 and PC-2 by mutations in K6b or K17. Currently there are no therapies for PC or the most painful aspect of the disorder, plantar keratoderma, which can lead to wheelchair confinement. The soles of the feet are therefore the initial tissue target for therapy. Here we present studies aimed at using RNAi for treating PC and demonstrate its utility for other autosomal dominant disorders of the skin. The goal is local reduction of expression from both K6a alleles (mutant and wildtype) by treatment with siRNAs to locally alleviate the symptoms in PC patients with K6a mutations. Four siRNAs were designed against the unique 3'UTR region of K6a and tested both in a cell culture model and in a mouse model. In culture, we demonstrated knockdown of endogenous K6a in two keratinocyte cell lines, HaCaT and NEB1 by transient transfection of the four K6a siRNAs independently, resulting in almost 100% reduction of K6a as visualised by protein gels and immunoblotting. Other keratins present in HaCaT and NEB1 cells were virtually unaffected, and specificity for K6a was further demonstrated in a cell culture model of K6a and K6b expression. The lead K6a-3'UTR siRNA was then tested in a mouse model using luciferase activity as an *in vivo* readout. In the mouse model, imaging using an IVIS camera (Xenogen Corp.), demonstrated that the specific siRNA (but not non-specific controls) strongly inhibited bicistronic (a single mRNA encoding both K6a and the firefly luciferase) gene expression in mouse skin (intradermal injection into footpads). These data suggest that with siRNA we can specifically target mutated genes in PC as a potential therapy, and that loss of K6a would be compensated for by other keratins including K6b.

SID Poster #564 (2007)

Small molecule library screening to identify compounds that regulate keratin 6a expression

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Dominant-negative mutations in keratin K6a are the most common cause of pachyonychia congenita (PC), a genodermatosis that is characterized by particularly painful and debilitating plantar keratoderma, accompanied by nail dystrophy and a range of other ectodermal features. Transgenic mouse models imply that complete loss of K6a expression may be less detrimental than expression of a heterozygous dominant mutant K6a, due to compensatory expression of the K6b isogene. Thus, down-regulating K6a expression in PC patients carrying K6a mutations is predicted to be therapeutic. Here, we took a chemical biology approach to identify new classes of small molecules that might up- or down-regulate the human K6a gene. A ~6 kb genomic DNA fragment containing the human K6a promoter was isolated and used to drive a firefly luciferase reporter gene. Both transient and stable transfection experiments showed that the isolated promoter is regulated exactly as expected with agents known to modulate K6a expression, such as retinoic acid. A clone of the keratinocyte cell line HaCaT stably expressing the K6a-f.Luc construct was used to screen a small-scale chemical library of 2522 small molecules. Renilla luciferase expression was used as a cell viability control. Following live cell screening in triplicate, 110 compounds consistently decreased K6a expression by >40%, with 59 of these decreasing activity by >50%, which was comparable to or more potent than retinoic acid. In addition, 61 compounds increased K6a expression by >40%, comparable to the K6a inducer sulforaphane. These compounds had no obvious detrimental effects on cell viability. An additional 146 compounds appeared to modulate K6a expression but were discarded as false positives due to non-specific effects on cell viability or on luciferase expression. This initial set of library hits will form the basis of further more stringent screening procedures, including off-target effects on other keratin genes, to hopefully identify novel chemical entities that might regulate K6a expression to therapeutic effect.

SID Poster #567 (2007)

Paternal germline mosaicism in pachyonychia congenita

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Pachyonychia congenita (PC) is a rare autosomal dominant keratin disorder that is associated with mutations in keratins K6a, K6b, K16, or K17. We describe the first case of germline mosaicism in PC, verified by mutation testing. Although the disorder appears to be completely penetrant, the spectrum and severity of disease manifestations in PC can vary considerably. The most prominent manifestations of the condition include a painful, usually focal palmoplantar keratoderma, thickened nails, pilosebaceous cysts (including steatocystomas), oral leukokeratosis, follicular keratoses, and sometimes natal or pre-natal teeth. This case began with the birth of an affected female infant to non-consanguineous parents. Sequence analyses revealed a K6a, N171del mutation. Family history and clinical examinations of her parents revealed no PC-related features. Their next child, a son, was born with similar phenotypic features and was found to carry the same K6a N171del mutation. The parents consented to genetic testing of a buccal swab sample, which revealed homozygous wildtype alleles. However, sperm collected from the father revealed carriage of the K6a N171del mutation. Thus, the father possessed a germ cell mutational mosaicism, but not a somatic mutation, that was transmitted to both of his children. This case demonstrates the need for caution when counseling clinically unaffected parents without a family history about PC recurrence risk in additional children.

SID Poster #568 (2007)

Rapamycin selectively inhibits expression of an inducible keratin (K6a) in human keratinocytes and alters clinical symptoms in an off-label study in pachyonychia congenita patients

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Pachyonychia congenita (PC) results from mutations in keratin 6a (K6a), K6b, K16, or K17 and is a nail and skin disorder with painful palmoplantar keratoderma. The macrolide sirolimus (rapamycin) has been shown to selectively block translation of mRNAs containing a terminal 5' oligopyrimidine (TOP) tract (Hay and Sonenberg, Genes & Dev 18:1926, 2004). Analysis of the 5' untranslated regions of the inducible keratins K6a, K6b, K16, and K17 revealed the presence of putative TOP regulatory elements. Treatment of human HaCaT keratinocytes treated with various rapamycin concentrations (1-30 nM) resulted in decreased K6a expression as assayed by western blot. For example, a concentration of 3 nM rapamycin led to a 73% decrease of K6a expression as compared to untreated cells. Under these conditions, there was little or no change in K5, K14, or the non-keratin control Lamin A/C protein levels. In parallel, rapamycin has been used in an off-label application in three PC patients. These three patients have tolerated therapeutic doses of rapamycin (10-12 ng/ml) for greater than one month without serious side effects. Well tolerated, previously described side effects include folliculitis and aphthous ulcers. Clinical responses of PC patients to rapamycin have been measured with a pain and activity diary, the validated Dermatology Quality of Life (DQOL) scale, and digital photography. Preliminary data at one month suggest a therapeutic response in callus character and subjective improvement. Follow-up data will be available.

SID Poster #571 (2007)

Hair abnormalities are rare in pachyonychia congenita

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Pachyonychia congenita (PC) is a rare autosomal dominant keratoderma associated with mutations in keratins (K) 6a, 6b, 16, and 17. PC is sub-classified into two major subtypes: PC1 (K6a and K16) and PC2 (K6b and K17). Both subtypes are associated with thickened nails, palmoplantar keratoderma, pilosebaceous cysts, follicular keratoses, and oral leukokeratosis. An additional feature observed in PC2 but not in PC1 includes hair abnormalities (including protuberant eyebrows and pili torti), but a systematic examination of hair from multiple PC patients has not previously been performed. We analyzed 47 PC individuals from 34 families to determine the frequency of hair abnormalities in PC. Mutation status was known on 68% (N=32). We examined scalp hair samples from 18 subjects (16 families) with K6a mutations, 10 subjects (10 families) with K16 mutations, 1 subject with a K6b mutation and 3 subjects (2 families) with a K17 mutation (PC-2). Microscopic hair shaft analysis revealed one subject with a K17, N92D mutation that showed microscopic features suggestive of pili torti (versus mechanical trauma), but a family member with the same mutation did not share this feature. The remaining hair samples with unknown mutation status also showed no features of pili torti or other hair abnormalities (32%, N=15). Interestingly, despite the fact that K6a/K16 mutation carriers manifest follicular keratoses, there is apparently no hair abnomalities in PC-1 scalp hair. Unfortunately too few cases of PC-2 were available to permit confirmation of previous reports of scalp hair involvement. Additional cases of mutation tested PC-2 patients and family members will be analyzed in our ongoing investigation of etiology of hair abnormalities in PC.

SID Poster #572 (2007)

Stability study of unmodified siRNA and shRNA and relevance to clinical use

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RNA interference offers a novel approach for developing therapeutics for dominant genetic disorders by selectively inhibiting mutant gene expression over wild-type. We have developed a siRNA (K6a.12) that selectively targets the mutant form of keratin 6a (K6a) responsible for the rare monogenic skin disorder pachyonychia congenita (PC). A number of chemical modifications designed to provide nuclease resistance or enhance delivery were tested in tissue culture and mouse models and were found to have similar potencies when compared to the unmodified version. However, in some instances, these chemical modifications resulted in loss of single nucleotide specificity. Here we present a stability study of unmodified K6a.12 and EGFP siRNAs and their shRNA counterparts under conditions relevant to clinical use. Our results indicate that neither repeated freeze/thaw cycles nor extended incubations (up to 28 days at 4 or 21°C) have any effect on si/shRNA integrity as measured by PAGE and functional activity assays. Furthermore, no degradation was observed at high temperatures (65 and 85°C for 2 hr) nor following contact with hair/skin. Incubation at 95°C (2 h) showed minor siRNA degradation but no change in functional activity; however, the shRNA counterparts showed degradation and loss of activity after only 30 min at 95°C. Incubation in fetal bovine serum for 5 h at 37°C showed near complete degradation and loss of activity, whereas incubation in human serum showed only partial degradation, even after 48 h. Interestingly, partial degradation observed by gel electrophoresis did not always correlate with loss of activity, suggesting that partially degraded si/shRNAs can retain full functional activity. Additionally, we demonstrate the ability to knockdown EGFP expression in a transgenic EGFP mouse model by intradermal injection of unmodified EGFP-specific siRNAs. Taken together, these data suggest that unmodified siRNAs and shRNAs are viable candidates for drug development