LB798

Latrogenic 'genodermatoses' induced by targeted therapy

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Recent advances in targeted anticancer therapies have substantially improved the prognosis of several cancers. Such targeted therapies are not, however, free of side effects, but these side effects are clearly distinct from those induced by classical cytotoxic chemotherapies. This is likely so because targeted therapies are designed to interfere with specific oncogenic signaling pathways rather than to inhibit cell proliferation in general. In fact, interference with specific signaling pathways may lead to effects that mimic those associated with genetic disorders due to alterations in the corresponding signaling pathways. Here, based on clinical pictures of patients and a review of the litterature, we compare the clinical effects of treatment with BRAF-inhibitors with those of genetic RASopathies. We find a striking overlap between the inhibitor-induced, iatrogenic dermatoses with the genodermatoses seen in patients with corresponding congenital RASopathies, including keratosis pilaris, palmo-plantar hyperkeratosis of areas of pressure, verrucous papillomas, nevi efflorescence, wavy hair, sparse eyelashes/eyebrows, poor hair growth, and increased cancer risk. Interestingly, several cutaneous side effects induced by BRAF-inhibitors that are not typically found in RASopathies, such as acneiform dermatitis and vemurafenib-specific phototoxicity, persist/increase under co-treatment with a MEK inhibitor. This may account for an off-target effect, independent from the well described paradoxical activation of the MAPK pathway by BRAF-inhibitors in BRAFwt cells. We hope that such comparisons lead to a better understanding of the side effects of targeted therapies and perhaps a reassessment of their validity in our current therapeutic arsenal.

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BAC clone modification strategy to generate a new mouse model for RDEB suitable for gene-editing

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To date, existing murine models for recessive dystrophic epidermolysis bullosa (RDEB) are not applicable to test the feasibility of gene editing in *vivo*. To develop such model, we have modified an existing bacterial artificial chromosome (BAC) carrying the full genomic human *COL7A1* sequence, which preserves key features of the introduced mutations and allows for gene-editing strategies targeting the human sequence to specifically correct *COL7A1*mutations *in vivo*. We have introduced a recurrent hotspot mutation in exon 3 (c.425A>G) in *COL7A1* based on homologous recombination (HR) using Red/ET recombination in *E. coli*. This two steps recombination method is based on highly efficient positive/negative *neo/rpsL* selection which allows for scar-less modification of the DNA. The efficiency of this strategy could be demonstrated by high number of positive clones after the second step of HR. Finally, we provide evidence for the integrity of the genomic sequence near exon 3 by sequence analysis. A linearized fragment containing the *COL7A1* locus was isolated from the BAC, purified and injected into fertilized oocytes of the mouse. Here we have established a powerful approach to introduce specific mutations in a large locus such as *COL7A1* and have recently obtained a first positive mouse founder. This mouse model will be used for the development of new therapeutic strategies such as gene editing using CRISPR/Cas9 technology. Moreover, this BAC clone modification strategy could be applied to generate other *COL7A1* genomic variants or mutations in other genes.

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Comparison of gene expression changes associated with intrinsic aging in healthy gingiva and sun-protected skin

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A cross-study comparison was conducted of transcriptomic changes in aging gingiva and buttock skin to better understand epithelial tissue aging. The gingival study involved collecting gingival biopsies from 90 female and male Caucasian subjects distributed across age cohorts from the early 20s to 70s. All participants received dental and periodontal exams and had healthy gingiva. In the skin aging study biopsies were collected from 10 young (aged 19-20 years) and 10 older (aged 63-67 years) Caucasian women. RNA samples isolated from biopsies were analyzed on Affymetrix HG-U219 arrays. Of the 1233 probe sets meeting statistical filtering criteria for significance in both studies, 335 showed consistent directional regulation in gingiva and buttock skin with aging. Gene Ontology term enrichment analysis revealed that responses to DNA damage and apoptosis were up-regulated with age in both tissues. CDKN2A ($p16^{INK4A}$), a key senescence marker, was among the genes positively associated with aging in gingiva (p=3.5 E-4) and skin (p=3.9 E-3). Processes associated with the consistently down-regulated genes were melanin biosynthesis and collagen fibril organization. The major structural collagens, COL1A1, COL1A2 and COL3A1, and the low abundance collagen, COL11A1, each showed significantly decreased expression in both tissues with aging. In contrast, many genes involved in energy metabolism, including components of the mitochondrial respiratory chain, were up-regulated with aging in gingiva, but down-regulated in skin. Also, in aging gingiva there was up-regulation of expression of late epidermal differentiation genes (e.g., LCE1F, LCE2D, LCE3C, CST6), whereas the pattern was generally opposite in skin with decreased expression of differentiation markers with aging. This analysis provides evidence of similarities in skin and gingival aging as well as tissue-specific changes.

LB801

GJB2 as a candidate gene involved in the pathogenesis of psoriatic hearing loss

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Psoriasis is a common chronic inflammatory skin disorder and could accompany with a serious systemic disease that affect people's life seriously. Recent study found that people with psoriasis is more likely to develop a mild form of sudden sensorineural hearing loss. However, the common biological mechanism of these two diseases is unknown. To uncover the underlying predictor regulators on psoriasis accompanied with hearing loss, in the present study we use network biology approach coupled with experimental data to identify potential mechanisms. miRNAs is a key transcriptional regulator of coding-protein mRNAs that regulate biological process. In this study, firstly through online database and literature review, we found 9 miRNAs based on previous study involved in both psoriasis and hearing system. And using bioinformatics techniques, 12 common target genes were screened out and were predictively involved majorly in translational process. With further gene ontology annotation analysis, GJB2 gene was found relevant with both psoriasis and hearing loss diseases. In addition, the results of comparing Cx26 protein (encoded by GJB2 gene) expression of psoriasis and health control plasma shown that Cx26 protein expression significantly down regulated in patients plasma ($p<0.0001$) and was negatively correlated with PASI clinical score (r, -0.286; $p=0.036$), but not with other clinical characteristics, such as sex, onset age, smoking, drinking, family history. In conlusion, our study revealed a potential critical gene GJB2 in psoriasis accompanied with hearing loss, and warrants a further experimental study on the pathogenesis mechanism on psoriasis accompanied with hearing loss.

LB802

Whole exome sequencing of 16 psoriasis high-risk pedigrees

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Psoriasis (Ps) is a complex multigenic disorder, with heritability estimated to be 60-90%. Although several large-scale genome-wide association studies (GWAS) and linkage studies have been performed, there is still a large proportion of the familial relative risk not explained by the known loci. To search for the missing heritability of psoriasis, focusing on low-frequency variants conferring an intermediate to high risk, we performed a whole exome sequencing (WES) of 16 high-risk pedigrees. We analyzed the WES data by a novel variant interpretation framework that quantitatively integrates the deleteriousness of genetic variants, quality of variant calls, co-segregation of variants with Ps within pedigrees, association of variants with Ps among case-controls, gene-gene interaction networks, and the differential expression of genes between lesional and normal skin samples. The results demonstrated several candidate genes for psoriasis. Published data showed that among the top genes, CRNN (LOD=1.92) may play a role in the mucosal/epithelial immune response and was down-regulated in eczema. Product of AGER (LOD=1.75) binds to psoriasin and koebnerisin, a link between the epidermis and innate immune system in inflammation priming of psoriasis. DRG2 (LOD=1.62) appears to be involved in the inhibition of NF-κB activity, IL-6 production, and the development of T(H)17 cells. These results are consistent with the possibility that new susceptibility loci and therapeutic targets for psoriasis are discoverable using our approach. Whole exome sequencing of thousands of cases and controls for the confirmation of the findings is currently ongoing.

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Overexpression of ezrin in melanoma does not alter cellular proliferation and migration *in vitro*

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Progression of cutaneous melanoma has been associated with ezrin expression. Ezrin is a member of the MERM (merlin, ezrin, radixin, moesin) family of proteins that link the actin cytoskeleton to the plasma membrane. MERM protein family play a key role in mediating cell to cell adhesion, migration, proliferation, and maintenance of polarity. The main objective of this study is to determine if over-expression of ezrin induces different activity in primary melanoma and metastatic melanoma cell lines. Viral transduction was done to the WM-115 primary melanoma cells and WM-266-4 metastatic melanoma cells to boost the ezrin expression level in the cells. Then we compared cell attachment, viability, proliferation and migration in MEM medium with various concentrations of fetal bovine serum (FBS), and 1% penicillin/streptomycin solution at 37°C and 5% CO2. Ezrin plasmid was amplified and inserted into pLVX-AcGFP1-C1 vector to generate pLVX-AcGFP1-C1-Ezrin plasmid. The pLVX vector expresses GFP and a puromycin selection marker. Using Lenti-XTM Lentiviral Expression Systems, both pLVX-AcGFP1-C1 empty vector and pLVX-AcGFP1-C1-Ezrin plasmid were transferred to WM-115 primary melanoma cells and WM-266-4 metastatic melanoma cells. Stable cell lines were achieved by puromycin selection. GFP signal was detected in the stably transduced cells, and the expression of the Ezrin protein was confirmed by Western Blot. In-vitro cell attachment, viability, proliferation and migration assays were carried out to test the effect of the transduced ezrin viral constructs. We found no significant differences in in-vitro cell attachment, viability, proliferation and migration activity using wild type and ezrin transfected melanoma cells. These in vitro results suggest that ezrin over-expression alone is not sufficient to alter the viability, attachment, migration, and proliferative activity of melanoma cells and, most likely, their capacity to metastasize.