349

Oxidative stress and mitochondrial alterations in Kindler Syndrome

S Guerrero-Aspizua,[2] Z Elisabeth,[2] JL García-Giménez,[1] M García,[2] CJ Conti,[2] F Larcher,[2] FV Pallardó[1] and M del Río[2] *1 Physiology, Fundación Investigación Hospital Clínico Universitario de Valencia, Instituto de Investigación INCLIVA, Valencia, Spain and 2 Bioengineering, Unidad Mixta UC3M-CIEMAT. CIBERER, Madrid, Spain*

Kindler Syndrome (KS) (OMIM 173650) is an autosomal recessive skin disorder caused by mutations in FERMT 1 and characterized by skin blistering, photosensitivity, premature aging and skin cancer predisposition. However, the known functions of FERMT1, involved in cell adhesion, do not suffice to fully understand the pleiotropic nature and clinical variability of this genodermatosis. Some of these pathognomonic features of KS not related to cutaneous fragility, such as premature skin aging and cancer predisposition, have been strongly associated with oxidative stress. Mitochondrial oxidative stress has long been implicated in both, aging and cancer. During oxidative phosphorylation, reactive oxygen species (ROS) are generated as by-products and its accumulation results in DNA damage as well as increased cancer susceptibility. We have explored the potential role of mitochondria-derived oxidative stress on the pathogenesis of KS. For that purpose we studied different biomarkers of oxidative stress in cells from KS patients. Patient-derived keratinocytes showed an altered oxidative status as demonstrated by increased glutathione and malondialdehyde levels and by a highly sensitive chimerical redox biosensor system. The ultrastructural analysis of KS skin biopsies and keratinocytes revealed striking abnormalities in mitochondria. In addition, keratinocytes from KS patients showed a reduced and diffuse MitoTracker Red staining and a significant reduction of the membrane potential. Overall, our data indicate that mitochondria in KS are not only altered in structure, but also in their distribution and functionality. This is the first study to demonstrate that mitochondrial dysfunction and oxidative stress contribute to the etiology of KS.

350

Regulation of IL-33 expression by normal human epidermal keratinocyte

H Tsuda,[1] M Komine,[1] T Ohshio,[1] S Tominaga[2] and M Ohtsuki[1] *1 Dermatology, Jichi Medical University, Shimotuke, Japan and 2 Biochemistry, Jichi Medical University, Shimotuke, Japan*

IL-33 is a member of the IL-1 family, and important to adaptive Th2 immunity. We previously reported that IFNγ induced expression of IL-33 in normal human epidermal keratinocytes (NHEKs). On the other hand, it has been reported that IFNγ did not induce IL-33 in monocyte, while TNFα and IL-1β did. Recently mouse IL-33 (mIL33) has been revealed to have two distinct transcripts with different non-coding exon1, both of which code the same protein sequences. We have confirmed that there were three different transcription products of IL-33, each containing one of three distinct exon1s, i.e., exon1a, 1b and 1c. We speculated that each exon1 has its own promoter region, which may be regulated in cell type- and stimulus-dependent manner. We aimed to clarify the differential usage of three distinct promoters containing each exon1 in different types of cells, with various stimuli. We amplified approximately 2.5 kbp region of the 5′ UTR of each three exon1 with PCR and cloned them into upstream of firefly luciferase gene construct. These constructs were transfected to NHEKs, human umbilical endothelial cells (HUVECs) and normal human dermal fibroblasts (NHDFs), stimulated with IFNγ or TNFα, and luciferase assay was performed. The construct containing 5′ UTR of exon1b had high luciferase activity without stimulation in NHEKs, HUVECs, and NHDFs. It strongly responded to IFNγ, but did not respond to other stimuli investigated, such as TNFα, IL-1β and LPS for TLR4 ligand in NHEKs and HUVECs. In NHDFs, exon1b containing promoter construct did not respond to any stimuli investigated. Other constructs containing 5′ UTR of exon1a or exon1c, showed only very weak luciferase activity, which did not respond to any stimuli investigated. We identified an active promoter of hIL33. The regulation of IL-33 transcription in NHEKs, HUVECs, and NHFDs may depend on the same promoter, which may utilize common transcription factors.

351

Reversion-triggered release of confined lethal mutations: an unreported genetic pathogenesis

Y Ogawa,[1] T Takeichi,[1] M Kono,[1] N Hamajima,[2] T Yamamoto,[3] K Sugiura[1] and M Akiyama[1] *1 Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan, 2 Department of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan and 3 Department of Legal Medicine and Bioethics, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Loss of gene functions due to nonsense mutations is a typical pathogenic mechanism of hereditary diseases. They may, however, in certain genetic contexts, confine the effects of other dominant pathogenic mutations. Theoretically, reversion of such disease-confining mutation may elicit a disease, like the opening of Pandora's box, although no actual case have been reported. We report the first instance in the literature where the reversion of a "confining" nonsense mutation in *GJB2* gene released the dominant pathogenic effect of a coexisting gain-of-function mutation, eliciting the lethal keratitis-ichthyosis-deafness (KID) syndrome. We encountered a KID syndrome patient who had a heterozygous missense mutation p.Gly45Glu in *GJB2* gene. Unexpectedly, her healthy mother also had the heterozygous missense mutation p.Gly45Glu, as well as another heterozygous nonsense mutation p.Tyr136X (Ogawa *et al.*, PLoS Genet. 2014). The biological relationship between the parents and the child was confirmed by genotyping of 15 short tandem repeat loci. We performed haplotype analysis using 40 SNPs spanning the >39 kbp region surrounding the *GJB2* gene to find that an allelic recombination event involving the maternal allele carrying the mutations generated the pathogenic allele unique to the patient. Previous reports and our mutation screening support that p.Gly45Glu is in complete linkage disequilibrium with p.Tyr136X in the Japanese population. Estimated from statistics in the literature, more thane 10,000 p.Gly45Glu carriers in the Japanese population are protected from the disease by this second-site mutation. The reversion-triggered onset of the disease shown in this study is a previously unreported genetic pathogenesis based on Mendelian inheritance.

352

Selection of antisense oligonucleotides for trans-splicing enhancement using a fluorescence-based screening system

U Koller, S Hainzl, C Hüttner, T Kocher, A Klausegger, EM Murauer, C Gruber, E Mayr, V Wally and JW Bauer *Department of Dermatology, Division of Experimental Dermatology and EB House Austria, Paracelsus Medical University, Salzburg, Austria*

RNA *trans*-splicing is a promising tool for RNA repair in genetic diseases. The RNA *trans*-splicing process is induced by RNA *trans*-splicing molecules (RTMs) via specific binding of the respective target pre-mRNA region. To accelerate RTM design we have established a reporter-based screening system which allows us to analyse the impact of defined factors on the RTM *trans*-splicing efficiency *in vitro*. Using this screening system we are able to analyse the potential of antisense oligonucleotides (ASOs) to improve the *trans*-splicing capacity of a selected RTM, specific for intron 102 of *COL7A1*. Defects in collagen type VII encoded by *COL7A1* are associated with the dystrophic form of the skin blistering disease epidermolysis bullosa (DEB). A selected ASO, which interferes with the competitive splicing elements on a stable expressed *COL7A1*-minigene (*COL7A1*-MG), was co-transfected with a highly functional RTM into HEK293 cells leading to a significant increase of the RNA *trans*-splicing efficiency. Thereby, accurate *trans*-splicing between the RTM and the *COL7A1*-minigene is represented by the restoration of full-length acGFP, quantified on mRNA and protein level. We assume that this observation can improve the RTM-mediated correction of genes on mRNA level, especially in cases where a high *trans*-splicing efficiency is required.

353

SNEV$^{hPrp19/hPSO4}$ haploinsufficiency accelerates premature skin aging in response to 8-methoxypsoralen/UVA treatment in mice

R Monteforte,[2] G Beilhack,[3] R Grillari-Vogelauer,[4] M Sibilia,[5] E Tschachler,[6] F Gruber[7] and J Grillari[1] *1 Department of Biotechnology, Christian Doppler Laboratory on Biotechnology of Skin Aging, University of Natural Resources and Applied Life Sciences, Vienna, Austria, 2 Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria, 3 Division of Nephrology & Dialysis, Internal Medicine III, Medical University of Vienna, Vienna, Austria, 4 Evercyte GmbH, Vienna, Austria, 5 Institute for Cancer Research, Medical University of Vienna, Vienna, Austria, 6 Department of Dermatology, Medical University of Vienna, Vienna, Austria and 7 Department of Dermatology, Christian Doppler Laboratory on Biotechnology of Skin Aging, Medical University of Vienna, Vienna, Austria*

Senescent cells accumulate during aging in various tissues and contribute to organismal aging. However, factors that are involved in the induction of senescence in vivo are still not well understood. SNEV$^{hPrp19/hPSO4}$ is a multifaceted protein, known to be involved in DNA damage repair, including interstrand cross link (ICL) repair, and senescence, albeit only in vitro. In this study we used heterozygous SNEV$^{+/-}$ mice (SNEV knock-out results in early embryonic lethality) and wild type littermate control mice as a model to elucidate the role of SNEV$^{hPrp19/hPSO4}$ in DNA damage repair and senescence in vivo. We performed PUVA treatment, consisting of 8-methoxypsoralen (8-MOP) in combination with UVA on mouse skin to induce DNA damage - prevalently ICL - and premature skin aging. We show that SNEV$^{hPrp19/hPSO4}$ expression decreases during organismal aging, while p16, as marker of aging in vivo, increases. In response to PUVA treatment on the skin of old SNEV$^{+/-}$ mice, we observe increase of γ-H2AX level, as DNA damage marker, accompanied by reduced epidermis thickening, increase of p16 level as well as loss of structure accompanied by increase in MMP1 levels. Thus, the DNA damage response occurring in the mouse skin upon PUVA treatment is dependent on SNEV$^{hPrp19/hPSO4}$ expression and lower levels of SNEV result in accumulation of DNA damage, increase of cellular senescence and thus in acceleration of premature skin aging.

354

Splicing regulation disturbances in psoriasis pathogenesis

E Szlávicz,[1] K Szabó,[1] Z Bata-Csorgo,[1] A Bebes,[1] L Francziszti,[1] A Dobozy,[1] G Groma,[1] L Kemény[1] and M Széll[2] *1 Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary and 2 Department of Medical Genetics, University of Szeged, Szeged, Hungary*

In a recently performed cDNA microarray experiment we identified three splicing regulators (serine/arginine-rich splicing factor 18 (SFRS18), peptydilpropyl isomerase G (PPIG), luc-7 like3 (LUC7L3) that where differentially expressed in response to T-lymphokines in healthy and psoriatic non-involved epidermis samples. We and others have previously shown that the oncofetal splice variant of fibronectin - the isoform containing the EDA domain (EDA+) - is overexpressed in psoriatic non-involved epidermis. In this study we investigated whether SFRS18, LUC7L3 and PPIG are able to alter the ratio of the normal (EDA-) and psoriasis-associated (EDA+) variant. To this aim the expressions of the splicing regulators were silenced in immortalized keratinocytes (HPV-KER). We found that the EDA+/EDA- ratio was altered upon silencing of LUC7L3 and PPIG: before silencing the expression level of EDA+ fibronectin isoform was higher than the EDA- fibronectin, but as a result of silencing the amount of the two splice variants became comparable. In addition, the expression patterns of the splicing regulators were compared in two different synchronized, immortalized cell lines, HaCaT and HPV-KER cells. Gene and protein expression patterns of the three regulators were very similar in both cell lines during their proliferation and differentiation suggesting that they may share common regulation.