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The presence of red hair MC1R variants enhance melanoma sun-induced susceptibility in a CDKN2A skin-humanized mice model of cutaneous familiar melanoma

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Melanoma is the leading world cause of death from skin cancer being a polygenic multifactorial disease involving environmental factors and genetic susceptibility. High susceptibility germline mutations in CDKN2A and CDK4 genes combined with low-medium penetrance MC1R gene variants have been associated to melanoma-prone families. The p.G101W, the most common CDKN2A mutation in Mediterranean European Countries is also frequent in USA. Additionally, MC1R variants responsible for red-hair increase melanoma risk in p.G101W melanoma families. We have developed a skin-humanized mouse model by orthotopic transplantation of a bioengineered skin containing keratinocytes/melanocytes and fibroblasts from members of two melanoma-prone families: two were p.G101W carriers and two MC1R red-hair variant carriers. The model faithfully reproduced the physiopathological characteristics of the donor's skin in terms of photo-type, DNA damage (dimmers) and UV-response (p53) induction. Consequently, double mutant skin with CDKN2A p.G101W mutation and two red-hair polymorphisms was more susceptible to sun-induced melanoma through the production of pyrimidine dimmers and reduction of p53 dependent DNA repair.

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Differential sensitivity of pigmented and non pigmented melanoma cell lines to vitamin D3 analogs Wasiewicz T, Slominski AT, Kutner A, Zmijewski MA Department of Histology, Medical University of Gdansk, Gdansk, Poland Department of Pathology and Laboratory Medicine, UTHSC, Memphis, TN, USA; Pharmaceutical Research Institute, Warsaw, Poland

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Melanoma cause majority of skin cancer related deaths, but still surgical intervention is preferred method of treatment. Vitamin D was shown to inhibit growth and induce differentiation of melanoma cancer cells, but excessive vitamin D intake leads to hypercalcaemia. Vitamin D analogues with modified side-chain show potentially low calcemic activity, but their antiproliferative properties need to be evaluated. Experiments were performed using mouse melanoma cell line (B16F10) and human melanoma cell lines (SKMEl 188, A 375 and WM 98) and antiproliferative properties of vitamin D analogs: calcipotriol, calcifediol, pD3, 21-OHpD3 viability tests (MTT, SRB) were tested. Expressional analysis of the genes coding proteins responsible for vitamin D metabolism (CYP24A1, CYP2R1, CYP3A4, CYP27B1, CYP27A1) and function (VDR, RXR, Pdia3) was performed using PCR or qPCR and selectively confirmed by Western-blot. It seems that activity of vitamin D analogs depends on the level of expression of vitamin D receptor and co-receptor. In case of human melanoma cell lines, WM 98 and A375 show much higher sensitivity to vitamin D analogs treatment when compared to SKMEL 188. Explanation of this phenomenon could be different pattern of VDR isoforms expressed in those cell lines. There are also indication that short side-chained vitamin D analogues treatment did not resulted in efficient translocation of VDR receptor despite significant antiproliferative activity of those compounds. Summarizing differential gene expression may influence sensitivity of melanoma cells to vitamin D analogues and their activity may not be exclusively dependent on VDR receptor.

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Detection of prohormone convertase PC5/6 in normal and transformed human melanocytes

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Prohormone convertases (PCs) are a family of Ca2+dependent serine proteases which include PC1/PC3, PC2, PC4, paired basic amino acid-cleaving enzyme 4, PC5, PC7, subtilisin kexin isoenzyme-1 (SKI-1), and neural apoptosis-regulated convertase-1. Until now, the role of PCs, especially PC1/3 and PC2, in pigment cells has been mainly investigated in the context of processing of proopiomelanocortin, the precursor for alpha-melanocyte-stimulating hormone. However, there is increasing evidence that PCs may play an important role in cellular transformation, acquisition of a tumorigenic phenotype and metastasis in a number of solid tumors. We recently identified SKI-1 as a possible novel target for melanoma therapy. To check if additional members of the PC family could play a role in melanoma biology we performed an in vitro expression analysis of PC5/6 in normal human melanocytes (NHM) and a panel of 8 human melanoma cells lines derived from different stage of disease progression. Expression of PC5/6 was examined by semi-quantitative RT-PCR, real-time RT-PCR, Western immunoblotting and immunofluorescence analysis. PC5/6 expression was significantly increased at mRNA level in 6 out of 8 melanoma cell lines compared to NHM. This could be confirmed at protein level. Immunofluorescence analysis disclosed cytoplasmic localization of PC5/6 in melanoma cells. Via in silico promoter analysis we detected several putative transcription factor binding sites for transcription factors that are typically activated by melanocyte mitogens as well as by extracellular and intracellular stressors. However, only tunicamycin, an endoplasmic reticulum stressor, increased PC5/6 expression in NHM. Our preliminary data indicate that PC5/6 is expressed by human pigment cells in vitro with more abundance of PC5/6 mRNA and protein in melanoma cells. At present we focus on immunohistochemical studies of PC5/6 in melanoma specimens as well as on functional analysis of PC5/6 using gene knock-down. These data should clarify if PC5/6 is a novel target for melanoma therapy.

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IL-2 therapy is accompanied with increased number of activated T regulatory lymphocytes in peripheral blood of melanoma patients

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Melanoma is the most malignant skin cancer. In opposite to targeted therapies immunotherapies are accompanied with less significant but long lasting clinical responses. Here, regulatory T lymphocytes (Tregs) are shown to be of significant importance deciding for the outcome of clinical responses. The aim of our study was to evaluate the frequency of Tregs in human melanoma patient's after IL-2, interferon, conventional chemotherapies and CpG vaccine treated as compared to untreated patients and healthy controls. Tregs are considered to be CD4+/CD25high/FoxP3+. CD69 is known as activation and CD62L as LN homing marker. In healthy controls, of PBMCs were 1% CD4+/CD25high. 87% of CD4+/CD25high were FoxP3+ (Tregs). 73% of CD4+/CD25high/FoxP3+ cells were expressing CD62L where expression of the CD69 was 0,2%. The frequency of Tregs in peripheral blood of melanoma patients was comparable to healthy controls. Interestingly, the expression of CD69 by Tregs was significantly higher in melanoma patients as compared to healthy controls, however no difference in the expression of CD62L was observed. IL-2 therapy was accompanied with increased number as well as expression of CD69 by Tregs as compared to untreated as well as interferon or CpG vaccine but not chemotherapy treated patients. In opposite to our expectation, increased frequency of Tregs in IL-2 treated patients was correlated with better clinical response. Our results demonstrate that: a) the frequency of activated Tregs is significantly higher in melanoma patients as compared to healthy controls, b) IL-2 therapy is accompanied with increased frequency of activated Tregs as compared to untreated, interferon or CpG vaccine treated patients and finally, c) increased frequency of activated Tregs in IL-2 treated patients was accompanied with better clinical response and overall survival. Our results demand further exploration of the role of Tregs in melanoma patients treated with different immunotherapeutic strategies.

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The rs2910164 G>C polymorphism in microRNA-146a is associated with the incidence of malignant melanoma

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MicroRNA-146a (miR-146a) is one of the microRNAs (miRNAs) implicated in the pathogenesis of various cancers. Recently, single nucleotide polymorphisms (SNPs) located in miRNAs themselves, so-called MIRSNPs, have attracted attention for their possible involvement in the pathogenesis of various diseases. Such MIRSNPs may have functional roles, due to the alteration of miRNAs. In this study, we investigated whether MIRSNP rs2910164 in miR-146a is involved in the pathogenesis of malignant melanoma (MM). DNAs were collected from 50 patients with MM and 107 controls and genotyped by polymerase chain reaction-restriction fragment. We found significant differences in the frequency of genotype distribution between controls and patients with MM. On the other hand, we could not find any significant difference in relationship between genotype distribution and clinical manifestation. In addition, the minimum free energy between miR-146a and its complementary strand with G allele is estimated at -26.8 kcal/mol while that of C allele is at -24.0 kcal/mol, suggesting that the MIRSNP rs2910164 is functional. Taken together, miR-146a may be involved in the morbidity of MM, and patients with CG genotype have a higher risk to develop MM.

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Protein-protein interaction networks in cocultured melanocytes and keratinocytes regarding the genomic status of CDKN2A and MC1R genes

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To identify molecular networks associated to presence of either germline mutations in CDKN2A or variants in MC1R genes which may be related with the biological impact of both genes into melanoma susceptibility. Expression data from cocultured keratinocytes and melanocytes from CDKN2A mutant melanoma families was extracted and analyzed using Whole Human Genome Microarray 4x44K (Agilent). Two patients were CDKN2A mutation carriers and two were MC1R red hair colour variants carriers. Differential gene expression data (1535 transcripts deregulated in CDKN2A mutated cells and 3570 in MC1R variants carriers) were analyzed by SNOW (a statistical analysis of protein–protein interaction networks). Statistically significant networks were identified among down regulated genes. Overall, 24.7% of them in CDKN2A mutants and 27.8% in MC1R variants carriers were localized in networks. The network cores were genes involved in autophagy process (e.g.GABARAPL2,SQSTM1 and LC3). Moreover, a network formed by 28.5% of upregulated transcripts in MC1R variants cells also reaches the significance. In this case, the network core was composed by genes playing a role in oxidative phosphorylation and mithocondrial ribosome (e.g., GBAS and ICT1). Our results suggest that variants in both MC1R and CDKN2A gene promote autophagy deregulation in skin cell types. Also, we have identified genes involved in the elevated cellular levels of reactive oxygen species in MC1R variant carriers.