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Topical application of a film-forming medical device containing photolyase and UV filters in actinic keratoses: role of CPI-17 gene

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Actinic keratoses (AK) are precancerigenous lesions which are caused in part by the carcinogenic effect of the UV genotoxic photoproducts cyclobutane pyrimidine dimmers CPD and 6-4 photoproducts (6-4PPs). Photoreactivation is a repair mechanism carried out by photolyases which specifically recognize and repair either CPDs or 6-4PPs. Beneficial effect of such enzyme into AK treatment has been recenty postulated. The aim of the study was to analyze the molecular effect of photolyase in 7 AK patients by expression array approach and bioinformatics methods. Skin recovery after treatment was confirmed in all patients by histopathological and molecular data which found overrepresentation of genes with biological functions involved in cell-cell communication, cell adhesion and homeostasis. The AK response was associated to overexpression of CPI-17 gene and determined by the initial expression level of the gene (P-value=0.001). Low levels of CPI-17 were directly associated to proinflamatory genes such as TNF (P-value=0.012) and IL-1B (P-value=0.07). Gene set analysis found that skin recovery was associated to biological process involved in tissue homeostasis and cell maintenance. This study suggests a role of CPI-17 gene in reconstitution of skin homeostasis in AK lesions.

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TLR4 as a negative regulator of keratinocyte proliferation

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Proliferation and differentiation are two sequential processes which drive the maturation of normal keratinocytes till they reach their full differentiation status. However, abnormal proliferation of keratinocyte is also a characteristic feature of tumor cells including squamous cell carcinoma (SCC) cells. In this study, we want to delineate the inverse relationship between the proliferation of keratinocytes and the pathogen-associated molecular pattern receptor TLR4. We observed that TLR4 surface expression in normal primary keratinocytes increases with subsequent passages in cell culture. In contrast, the expression of TLR4 in established highly proliferative squamous cell carcinoma (SCC) cell lines was low, similar to the expression in normal keratinocytes at low passage numbers. We found a stimulating effect of TLR4 blockade by a specific antibody on the proliferation rate of keratinocytes. In response to antibody-mediated blockade of TLR4, keratinocyte proliferation increased greatly as assessed by the incorporation of BrDU. As an alternative blocking method, we abrogated the interaction between TLR4 and its accessory protein MDII using a specific blocking peptide. Keratinocytes reacted with increased proliferation, though not to the same degree as achieved by direct blocking of TLR4. Based on our results we hypothesize that TLR4 is a negative regulator of keratinocyte proliferation and may be associated with the progression of SCC of the skin. Such a regulatory function of TLR4 in keratinocyte proliferation has not been investigated yet. This study delineates the regulatory role of TLR4 in keratinocyte proliferation and thus provide important insight into the homeostasis of normal and tumor keratinocytes. Better understanding of a regulatory role for TLR4 is the basis for a later use in a therapeutic setting to stop keratinocyte proliferation such as in squamous cell carcinoma of the skin and to induce keratinocyte proliferation such as in wound healing.

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Mitotic catastrophe is implicated in the resistance of basal carcinoma cells to photodynamic therapy

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Basal cell carcinomas (BCC) are frequently observed in white population. Many of them arise as a consequence of a constitutive activation of Sonic Hedgehog pathway due to mutations in the PTCH gene, although other DNA alterations should be also considered. Photodynamic Therapy (PDT) with methyl-aminolevulinic acid (MAL) offers excellent results in the treatment of BCC. MAL, acts as a precursor of protoporphyrin IX (PpIX) and accumulates, in tumors tissues. Subsequent irradiation with visible light generates reactive oxygen species, which are responsible of tumoral cell death. However, as it happens in other cancer therapies, PTD is not always effective. Therefore, it is necessary to characterize the BCC response to MAL-PDT in order to optimize it or, eventually, to combine it with other therapies to completely remove the tumour. We have compared the response to MAL-PDT on a human BCC cell line obtained from a tumor located in the face and a cell line obtained from BCC induced by chronic UV-irradiation in a ptch+/- mice (AJ57); the histology performed before the stablisment of the cell culture confirmed that the induced tumors were BCC . We demonstrate that murine cell lines are more sensitive to PDT than the human BCC cells. Lethal conditions for AJ57 (85%) caused death mainly by necrosis. On the opposite, the same conditions induced 50% cell death in the human line. In this case, we identified disorganization of microtubules, increased number of cells in division with altered spindles, heterogeneity both in cell and nucleus size and multinucleated cells. Cell cycle analysis by flow cytometry confirmed the increased number of cells in G2-M and polyploidy 24 and 48 hours after PDT. In parallel, TUNEL assay revealed a high number of cells in apoptosis. These results indicate that PDT caused mitotic catastrophe in BCC human cell line followed, mainly, by apoptosis with DNA disposed in the metaphase plate.

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Changes in survivin subcellular localization correlates with different stages of differentiation in normal and cancerous skin

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The IAP (Inhibitor of Apoptosis Protein) protein survivin is detected in few adult cells, while it is highly expressed in hyperproliferative conditions, including cancer. Nuclear survivin facilitates cell cycle entry, whereas the cytoplasmic pool protects cells from apoptosis. Survivin is overexpressed in cultured keratinocyte stem cells (KSC), and protects them from anoikis and UVB-induced apoptosis. As stem keratinocytes appear to be at the origin of squamous cell carcinoma (SCC), we evaluated survivin expression in normal and cancerous skin *in vivo*, by using a highly sensitive immunohystochemistry (IHC) assay. We first demonstrated that survivin is not only localized in the cytoplasm but also in the nucleus of normal adult keratinocytes, as also shown by western blotting. Nuclear survivin is detected in basal and in few suprabasal keratinocytes. Nuclear survivin positive cells were detected one every 10/11 basal keratinocytes. Suprabasal keratinocytes displaying nuclear survivin also express CK10. Nuclear, but not cytoplasmic survivin expression dramatically increases in actinic keratosis and in SCC *in situ*, as compared to normal epidermis, and further increases in SCC lesions. In addition, nuclear survivin is highest in poorly differentiated SCC and mostly localizes in deep, invasive lesions. Finally, in SCC cells, nuclear survivin positive cells seems to express less CK10 as compared to normal skin. Alltogether, these data suggest that survivin subcellular localization correlates with keratinocyte differentiation and is associated with undifferentiated and more invasive SCC phenotype.

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Genetic Susceptibility In Familial And Multiple Melanoma Patients In Central Italy

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Inherited melanoma susceptibility genes can be stratified by risk profile into high-penetrance genes and low-penetrance alleles. High-penetrance factors are expressed in familial clustering of melanoma and include mutations in CDKN2A andCDK4. In addition to high-penetrance genes, variants in the MC1R gene, one of the major determinants of hair and skin pigmentation, have been classified as 'low-risk' melanoma susceptibility alleles. Recently, the MITF gene has been identified as a novel melanoma-predisposing gene with the E318K variant defined as an intermediate risk variant. To determine the contribution of these candidate genes to melanoma genetic predisposition in patients from central Italy, we recruited 52 familial melanoma (FM) patients from 34 kindreds with at least two documented cases and 20 sporadic multiple primary melanoma (MPM) patients. Genetic analysis of CDKN2A (exons 1 alpha, 1 beta, 2 and 3), CDK4 (exon 2), MC1R (entire coding region) and MITF (exon 9) was performed by direct sequencing in all patients. Five germline missense mutations in the CDKN2A gene were identified in 6/52 (11%) FM patients from 5/34 (15%) families and 1/20 (5%) sporadic MPM patient: V59A, N71I, H83Q and P114L, mapping in exon 2 and T31M mapping in exon 1 beta. In addition to disease-associated mutations, 3 polymorphisms in the CDKN2A gene were detected: A148T in exon 2 in 7/52 (13%) FM patients and 1/20 (5%) sporadic MPM patient, IVS3+29 3'UTR in 17/52 (33%) FM patients and 4/20 (20%) sporadic MPM patient and IVS+69 in 3/52 (6%) FM patients and 5/20 (25%) sporadic MPM patients. MC1R variants were identified in 30% of patients with the most frequently detected variants being V60L, R151C, Y152X, R160W, and P268R. No germline mutations were identified in the specific hot spot of CDK4 or in MITF exon 9. In conclusions, our results indicate that CDKN2A mutations account for a small proportion of FM and sporadic MPM cases in our population, in line with other Italian studies, suggesting a major role for low-penetrance genes and environmental factors.

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miRNA-205 in benign, premalignant and malignant skin

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MicroRNAs (miRNAs) are a group of short non-coding RNA molecules that control gene expression posttranscriptional. They have emerged as regulators in oncogenesis. The role of MiR-205 in cancer is not well established. MiR-205 has recently been shown to be downregulated in malignant melanoma and partly effecting it's posttranscriptional role by targeting the 3'URT part of E2F, an important regulator of cell cycle. Moreover miR-205 seemed to be involved in senescence of melanoma cells. The expression of miR-205 in epithelial skin has recently been found to be broadly expressed in nearly all stratified squamous epithelia. The same group found MiR-205 highly expressed in aggressive oral squamous cell carcinoma (SCC)-lines with lower levels in minimally invasive SCC, suggesting miR-205 acting as a tumor promoter in aggressive SCC. The purpose of our study was to visualize the expression of miR205 in benign, premalignant and malignant skin by in situ hybridization (ISH). The material constituted of formalin-fixed paraffin-embedded tissue samples from normal skin (6), keratoacanthomas (6), basal cell carcinomas (6), actinic keratoses (6), Bowen's disease (6) and squamous cell carcinomas (12): 6 well differentiated and 6 undifferentiated. We investigated the level of miRNA-205 expression by ISH using a robust one-day protocol based on the use of double digoxigenin-labeled LNA-DNA chimeric probe developed by Exiqon (Vedbæk, Denmark). We used a concentration of has-miR-205 of 50nM. We found a broad expression of miRNA in all stratified squamous epithelia. There was a tendency to upregulation of miR-205 in pre-malignant and malignant epithelia compared to normal epithelia, evaluated as more intense blue staining after ISH. The expression was rather homogenous and we were not able to identify specific cells with higher expression of miRNA-205. Our findings correlate well with previous findings of up-regulation of miRNA-205 in aggressive oral SCC. To further evaluate and quantify the levels of miRNA-205 in malignant skin conditions real-time q-PCR will be performed.