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Photodynamic therapy corrects abnormal cancer-associated gene expression observed in actinic keratosis lesions and induces a remodeling effect in photodamaged skin

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ABSTRACT

Background: Actinic keratoses (AK) are proliferations of neoplastic keratinocytes in the epidermis resulting from cumulative exposure to ultraviolet radiation (UVR), which are liable to transform into squamous cell carcinoma (SCC). Organ Transplant Recipients (OTR) have an increased risk of developing SCC as a consequence of long-term immunosuppressive therapy. The aim of this study was to determine the molecular signature of AKs from OTR prior to treatment with methyl aminolevulinate-photodynamic therapy (MAL-PDT), and to assess what impact the treatment has on promoting remodeling of the photodamaged skin.

Methods: Seven patients were enrolled on a clinical trial to assess the effect of MAL-PDT with biopsies taken at screening prior to the first treatment session (week 1), and six weeks after completion of final treatment (week 18). Whole-genome gene expression analysis was carried out on skin biopsies isolated from an AK lesion, an area surrounding the lesion, and a non-sun exposed region of the body. Quantitative PCR was utilized to confirm the differential expression of key genes.

Results: MAL-PDT treatment corrected abnormal proliferation-related gene profiles, corrected aberrantly expressed cancer-associated genes and induced expression of dermal extracellular matrix genes in photo-exposed skin.

Conclusion: The efficacy of the MAL-PDT on AK lesions was confirmed at whole-genome gene expression level. A transcriptional signature of remodeling, identified through assessing the effect of MAL-PDT on photodamaged skin, supports the use of MAL-PDT for treating photodamaged skin and field cancerized areas.

1. Introduction

Actinic keratoses (AK) are lesions formed from proliferations of transformed neoplastic keratinocytes in the epidermis, resulting from cumulative ultraviolet radiation (UVR) from sun exposure [1,2]. Lesions are found on sites of sun-exposed skin such as the face, balding scalp, chest, forearms and back of the hand. AKs may present on a patient as visible lesions, of which 5–10% have the potential to transform into squamous cell carcinoma (SCC) [3,4]. In Organ Transplant Recipients (OTR) as a consequence of long-term immunosuppressive therapy, the risk of developing cutaneous SCC is much higher, with an almost 250-fold increase compared with immunocompetent individuals [5,6]. Skin cancers are by far the most prevalent neoplasia observed in OTR [7]. The occurrence of skin cancer in OTR varies depending on the grafted organ; the composition, intensity and duration of immunosuppressive treatments; as well as UVR exposure and genetic factors [8]. In addition to multiple visible (clinical) AK lesions present in OTR, invisible

Abbreviations: AK, actinic keratosis; DEG, differentially expressed genes; L, lesional; PL, peri-lesional; ECM, extracellular matrix; MAL, methyl aminolevulinate; NSE, non sun-exposed; NTF, Non-Negative Tensor Factorization; FDR, false discovery rate; OTR, organ transplant recipients; PDT, photodynamic therapy; SCC, squamous cell carcinoma; UVR, ultraviolet radiation.

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(subclinical) lesions are estimated to occur up to 10 times more often [9]. Clinical and subclinical AK lesions are thought to occur in photodamaged skin by the process of field cancerization, a concept that describes the presence of genetic abnormalities in a tissue chronically exposed to a carcinogen [10]. Therefore, normal appearing skin near AK lesions (herein described as peri-lesional) may already have accumulated discrete molecular changes, not sufficient by themselves to promote tumorigenesis, but sensitizing tissue to subsequent transformation.

There is a higher risk of accumulating DNA mutations during the continuous division process of chronically UVR-exposed basal keratinocytes than in non-sun exposed skin. Interestingly, it appears that over a quarter of normal photo-exposed keratinocytes do accumulate driver mutations in cancer-associated genes such as NOTCH1, NOTCH2, NOTCH3, TP53 and FAT1 which are also found in SCC [11]. Such keratinocytes nonetheless behave without apparent alterations in their physiology. The comparison between AK and SCC lesions using large scale expression profiling has been already addressed in several reports [12–15] and is not the topic of this study.

Alongside gene mutations in the keratinocytes, significant alterations in the structure of the dermal extracellular matrix (ECM) and in the composition of the epidermal basement membrane have also been identified [16,17]. The well-recognized ECM alterations that occur in the tissue during progression of the tumor are increased collagen deposition and proteolytic remodeling of the stromal ECM with unusual expression of specific proteinases and their inhibitors such as MMPs or serpins [18,19].

MAL-PDT is a form of phototherapy involving light and a photosensitizing chemical substance, used in conjunction with molecular oxygen to elicit cell death. It has been approved for several years [20] and has proven to be very effective for various skin pathologies such as AK [21–25], with a very good clearance rate of treated lesions. It is commonly used as first line treatment for OTR populations as it can treat multiple lesions at a time and is well suited for field treatment [26,28].

Previous clinical studies involving patients with AK lesions treated with photodynamic therapy have been described through clinical reports, histopathology, immunohistochemistry and quantitative RT-PCR methods [21,27]. While AK pathophysiology has been studied using large scale gene expression profiling [12–15,27], to date, no whole genome transcriptomic data have been available to investigate the molecular basis of MAL-PDT on treated skin areas.

The present study investigated, based on transcriptomics data, the molecular changes that occur in lesional and peri-lesional skin of OTR patients following two cycles of MAL-PDT.

2. Material & methods

2.1. Patients

This study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practices, and in compliance with local regulatory requirements. It was approved by institutional review board, and all subjects provided written informed consen prior to study procedures.

This study was an exploratory, monocentre and open study, involving renal transplant male subjects, aged at least 18 years old, with history of immunosuppression from 5 to 13 years, and with a diagnosis of field actinic keratosis on face, scalp, forearms or chest (details in Table S1).

Renal transplant recipients subjects were included to receive two sessions of MAL cream on a target field (AK field of $5 \times 10\,cm^2$ localized on face, scalp, forearms or chest) after lesion preparation.

The target field ($5 \times 10\,cm^2$) was defined as a skin area on the face, scalp, forearms or the chest, usually sun-exposed, photodamaged. The border of the target field was at least at 5 mm from the clinically visible edge of each lesion included in it.

To examine possible effects of MAL cream on gene expression, five skin punch biopsies of 3 mm each were performed during the study. The target field was treated at baseline and week 12 (regardless of whether lesions were still present) with MAL cream.

2.2. Sample collection and treatment

In total, two treatment sessions were performed on patients at week 1 and again at week 12. During each treatment session, MAL cream was applied for three hours on the whole target field and excess cream was then removed. The target field was exposed to red light (using a large-field LED light Source: Aktilite® 128 lamp) during seven to ten minutes at a dosage of $37\,J/cm^2$.

For each patient, five skin punch biopsies (3 mm) were taken. During the screening visit at week 1 and prior to the first treatment, two biopsies were taken within the target field to include lesional (L1) and peri-lesional (PL1) regions, while an additional biopsy was isolated from the buttock, representing a non-sun-exposed skin area (NSE). At week 18; two further biopsies were isolated, in the same lesional (L18) and peri-lesional (PL18) areas. Lesions were located on the scalp for five patients; for the remaining two patients, the lesions were located on the cheek and on the forehead, respectively. Patients were clinically evaluated for disease regression before treatment, at week 12 and week 18, month 9 and at month 15.

2.3. Clinical evaluation criteria

Clinical AK lesion response was assessed in the target field with a dichotomous scale (complete and not complete) at each evaluation visit (including new and recurrent lesion(s)). Efficacy criterion was global percent reduction in AK lesion count in the target field (including new and recurrent lesions) from baseline at month 15. Photographs of the target field were taken at screening, baseline, week 12, week 18, month 9 and month 15.

2.4. RNA extraction

Biopsies were stored in RNALater (Thermo Fisher Scientific, Waltham, MA, USA) and total RNA was extracted from skin biopsies using RNeasy Micro extraction kits (Qiagen, Valencia, CA) according to the manufacturer's protocol. RNA quality was monitored using a 2100 Bioanalyser (Agilent, Santa Clara, CA, USA), with the RNA Integrity Number (RIN) determined. All samples displayed a RIN > 8.

2.5. Probe synthesis and hybridization

300 ng of total RNA was used as starting material for cRNA synthesis, which was performed using the Affymetrix 3′ IVT Express kit. Labelling and hybridization were carried out according to standard protocols (Affymetrix, Santa Clara, CA, USA). Fragmented RNA was hybridized on Affymetrix U133 Plus 2.0 chips for 16 h at 45 °C, washed and stained on an Affymetrix Fluidic Station 450 and scanned using the Affymetrix GeneChip scanner 3000.

2.6. Bioinformatics analysis of microarray data

2.6.1. Data handling and supervised analysis

Affymetrix U133 Plus 2.0 chips were normalized in ArrayStudio® software version 9.0.8.70 (OmicSoft, Cary, NC) using the robust multichip average (RMA) method [28]. Only Affymetrix identifiers (probesets) with expression levels $\geq 2^6$ in at least 70% of samples in one of the five biopsy conditions were selected for

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