Dermatology Practical & Conceptual

Melanoma on Chronically Sun-Damaged Skin: Deciphering Gene Expression Signatures

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ABSTRACT

Introduction: Melanoma of the skin is responsible for most skin cancer-related deaths. It is well known that exposure to ultraviolet radiation is the most common and modifiable risk factor for melanoma. Melanomas arising on chronically sun-damaged skin (CSDS) have shown a higher mutational burden.

Objectives: To analyze skin samples of patients with melanoma on CSDS to identify possible gene expression signatures that may contribute to melanomagenesis.

Methods: This experimental observational analysis, conducted at the Dermatology Melanoma and Pigmented Lesion Clinic at University of Miami Hospitals/Sylvester Comprehensive Cancer Center, Miami, Florida, included a total of 10 patients over 18 years of age with a recent diagnosis of melanoma on CSDS. For each patient, two skin samples were obtained using a 2-mm punch (one from CSDS within 2 cm of the primary melanoma, another from sun-protected skin). Skin samples were sent to the Sylvester Onco-genomics Shared Resource (OGSR) for library preparation and RNA sequencing. Main outcome was the identification of differentially expressed genes between CSDS and non-CSDS of patients with a recent diagnosis of melanoma.

Results: A total of four skin samples met the necessary quality standards for molecular analyses. Significant differences were observed between the CSDS and non-CSDS samples. Pathways involved in inflammation (e.g., IL-17 signaling), immune responses (e.g., ABC transporters), and oxidative phosphorylation were overexpressed in CSDS.

Conclusions: CSDS can be an adequate milieu for the development and progression of melanoma. CSDS reveals overexpression of pathways involved in inflammation, immune responses, and oxidative phosphorylation, all of which may facilitate interactions between the skin microenvironment and melanocytes/melanoma cells, predisposing to melanoma development and progression.

Introduction

It is well known that exposure to ultraviolet radiation (UVR) is the most common, modifiable, environmental risk factor for melanoma. UVR exposure causes oxidative stress, direct and indirect damage to the DNA, and alteration to DNA-repair proteins [1]. From a biological and genetic standpoint, melanomas arising on chronically sun-damaged skin (CSDS) demonstrate a higher mutational burden [2], including increased frequency of mutations such as BRAF-V600K, NF1, TP53, and KIT as well as increased levels of PD-L. [3] Studies show that the skin surrounding a melanoma on CSDS can harbor an increased mutational load (similar to that seen within the melanoma), suggesting that the high number of oncogenic mutations within the melanoma may be acquired at an earlier stage, and therefore additional mutations may not be required for its progression [3].

Objectives

To identify possible gene expression signatures in the skin of patients with melanoma on CSDS that may contribute to melanomagenesis.

Methods

This observational experimental research study included a total of 10 patients over 18 years of age with a recent diagnosis of melanoma on CSDS (i.e., diagnosis no more than 6 months before the day of enrollment). CSDS was defined by the clinical presence of multiple solar lentigines involving at least three anatomical areas, and pigmentary changes including dyschromia, hypo- and/or hyperpigmentation. For each patient, two skin samples were obtained using a 2-mm punch biopsy, and specifically, one sample from CSDS within 2 cm of the primary melanoma and a second sample from sun-protected skin (i.e, retroauricular, inner buttock/gluteal fold). Each skin sample was embedded in RNA-later buffer. RNA was isolated utilizing the RNA extraction kit (Qiagen Cat#74134) and sent to the Sylvester Onco-genomics Shared Resource for library preparation and RNA sequencing. This study was approved by the Institutional Review Board at the University of Miami. The RNA-seq raw expression data was processed in the R-programming environment to perform statistical calculations and differential expression analysis. To evaluate the different signaling pathways between CSDS and non-CSDS, the gene set enrichment analysis (GSEA) was conducted using the cluster Profiler R-package [4], and MultiRankSeq [5] was used to identify differentially expressed genes (DEG). Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analysis were performed to understand the gene functional annotation and functional enrichment for DEG, respectively. False discovery rate $P < 0.05$ was set as the significance criterion.

Conclusions

Over the course of one year, a total of 10 patients met the inclusion criteria. Due to the small skin sample size (2 mm punch) and fragile nature of RNA, which requires optimal processing to prevent degradation, only the skin samples from four patients met the necessary quality standards for molecular analyses. The patients' average age at diagnosis was 47.7 years; 75% (3/4) were females. Two patients had an invasive melanoma (average thickness 0.65 mm; range 0.3–1 mm), and two, in-situ disease. Superficial spreading melanoma (50%) and lentigo maligna (50%) were the histological subtypes. Tumors were found on the scalp (50%, N=2), neck and chest. Patients' average body mass index was 32.35 (obesity range) (Figure 1). To screen out the hub CSDS-related genes that may have contributed to the development of melanoma on CSDS, the principal component analysis (PCA) was performed to classify the relationship between the eight samples. The PCA showed that three pairs of the analyzed samples clustered together. Significant differences were observed between the CSDS and non-CSDS samples (Figure 2). Applying the defined cutoff values (log2FoldChange >2.0 and p.Adj 0.05), six genes of interest were identified and associated with CSDS compared to non-CSDS, including four upregulated (i.e., SERPINB4, RHCG, CHST2, KRT16) and one downregulated gene on CSDS (i.e., CACNA1H). To investigate the potential regulatory mechanism of the DEGs in CSDS, we performed gene ontology and KEGG enrichment analysis. The biological functions of these skin samples were detected by KEGG pathways analysis, which revealed that the main pathways were IL-17 signaling pathway, ABC transporters, and oxidative phosphorylation (activated pathways), proliferator-activated receptor (PPAR) as well as

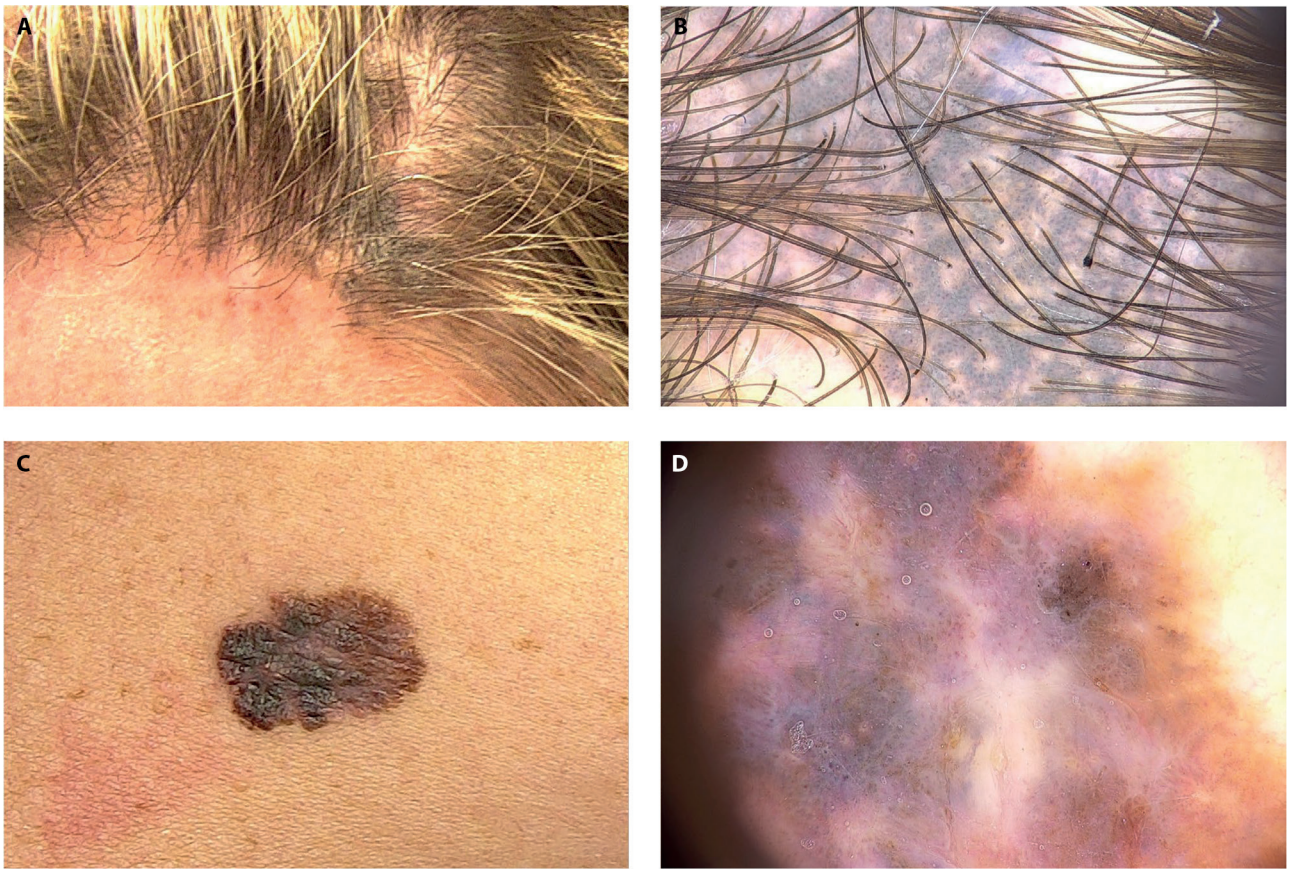

Figure 1. Primary cutaneous melanoma of two patients. **A-B:** Clinical and dermoscopic images of lentigo maligna on the frontal scalp. **C-D:** Clinical and dermoscopic images of superficial spreading melanoma 0.7mm on the chest.

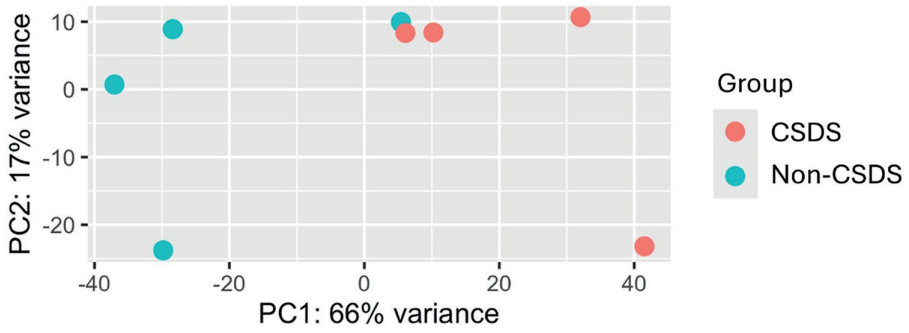

Figure 2. Principal component analysis of the gene expression profiles of skin samples from chronically sun-damaged skin (CSDS) and non-CSDS of patients with melanoma on CSDS. Each dot represents an RNA-Seq sample, with red dots indicating CSDS and blue dots indicating non-CSDS. Samples with similar gene expression profiles are clustered together.

endocytosis and Notch signaling pathway (suppressed pathways). The significantly enriched pathways in the two groups were selected by adjusted p-value <0.05. We found that keratinization, epidermal cell differentiation, and keratinocyte differentiation genes were significantly enriched in the CSDS group (Figure 3). Melanoma development and progression is a complex and dynamic process involving melanoma cells and their interaction with the skin microenvironment. Evidence demonstrates the role of the microenvironment in the malignant transformation of melanocytes, with some niches (e.g., hair follicle bulge) protecting against their malignant transformation and tumor initiation and other interactions supporting proliferation, angiogenesis, and metastasis. [6]. Depending on specific factors, the skin can be a favorable microenvironment for tumor growth, hosting initial mutations that may maintain their normal function until further mutations lead to melanoma formation. Moreover, depending on specific genetic drivers as well as internal and/or external stimuli (e.g., UVR), some microenvironments may or may not be permissive to melanocyte transformation. For

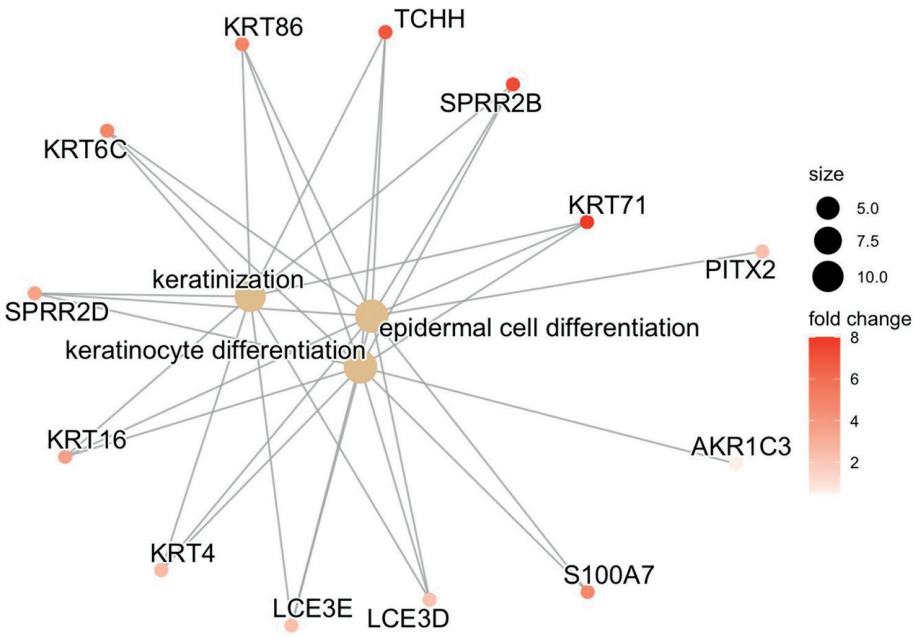

Figure 3. The network diagram illustrates the enriched pathways identified between the two groups (CSDS and non-CSDS) based on an adjusted p-value threshold of <0.05. Key pathways identified include keratinization and epidermal cell differentiation.

example, it is suggested that melanocyte stem cells, which are usually quiescent on the hair follicle bulge, can only proliferate and migrate to the epidermis in response to UVR-induced inflammation or by paracrine signals secreted by the epidermal microenvironment [7]. Indeed, it is well known that UVR promotes skin inflammation, decreases immune surveillance, induces cellular stress (e.g., oxidative stress, accumulation of reactive oxygen species), and induces changes in gene expression and protein production as well as nuclear and mitochondrial DNA damage. In our study, we found pathways and genes that were overexpressed on CSDS but not on sun-protected skin of patients with melanoma on CSDS. Interestingly, these overexpressed pathways (i.e., IL-17, ABC transporters, oxidative phosphorylation, PPAR signaling pathway) are all involved in inflammatory conditions. For example, the oxidative phosphorylation pathway, which is crucial to cell energy homeostasis and metabolic demands, may induce a hypoxic, glucose-deficient, and acidic tumor microenvironment that may inhibit the function of immune cells [8]. Furthermore, the IL-17 and ABC transporters are involved in immune cell responses. Specifically, the IL-17 pathway is involved in the activation and recruitment of immune cells to sites of damaged tissue as well as in the release of pro-inflammatory cytokines. Interestingly, PPAR-γ exerts inhibitory effects on T-helper cells, specifically in those secreting IL-17 [9]. Similarly, some ABC transporters are implicated in immune responses by regulating the transport of lipids and peptides needed for the function of membrane proteins, including innate immune system receptors [8]. On the other hand, the Notch signaling pathway, which plays a role in cell renewal, differentiation, homeostasis, and repair, was found to be suppressed in CSDS, suggesting an imbalance between pro-inflammatory and repair responses. This pathway has anti-apoptotic functions in keratinocytes UVB-response through down-modulation of FoxO3a expression [10].

The limitations of our study include the limited number of cases and their heterogeneity.

Our findings support the pivotal role of the skin microenvironment, which under the influence of UVR facilitates inflammatory and immune responses that can lead to melanomagenesis. CSDS reveals overexpression of pathways involved in inflammation, immune response, and oxidative phosphorylation, which allow interactions between the skin microenvironment and melanocytes/melanoma cells, predisposing to melanoma development/progression. These findings may serve as a valuable baseline for future studies and therapeutic strategies targeting the microenvironmental factors that may contribute to melanoma progression.

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