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Vitamin C–Induced Gene and miRNA Crosstalk Orchestrates Regeneration, Antioxidant Defense, and Pigmentation Modulation in Human Skin Cells

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Abstract

Vitamin C is widely recognized in cosmetic medicine and dermatology for its significant physiological effects on the skin, including promoting collagen biosynthesis, inhibiting melanogenesis, preventing radiation-induced damage, and accelerating wound healing. Despite these benefits, the detailed molecular mechanisms by which Vitamin C facilitates skin regeneration and modulates pigmentation remain poorly understood. In this study, we applied high-throughput RNA sequencing to profile both coding and non-coding transcripts in primary human keratinocytes, fibroblasts and melanocytes treated with a novel Vitamin C. We found that Vitamin C orchestrates a coordinated modulation of pathways governing collagen biosynthesis, melanin production, keratinocyte differentiation, oxidative stress responses and tissue repair, activities that are mediated in part through fine-tuning of microRNA networks. These results highlight underexplored regulatory layers through which Vitamin C exerts its dermo-active effects, underscoring its promise as a key ingredient in next-generation skincare formulations.

Key words: Vitamin C, miRNA, skin, biological pathways

Introduction

The skin, the body's largest organ, is a dynamic barrier against mechanical, chemical, microbial insults and ultraviolet (UV) radiation [1]. Its protective functions depend on coordinated interactions among epidermal keratinocytes, which renew and fortify the stratum corneum [2]; dermal fibroblasts, which synthesize collagen and extracellular matrix [3], and melanocytes, which regulate pigmentation to absorb UV energy [4]. Effective homeostasis and wound healing thus require precise crosstalk mediated by growth factors, cytokines and redox signaling.

Vitamin C (ascorbic acid) is abundant in healthy skin and underpins key processes—collagen biosynthesis, reactive oxygen species scavenging, and melanogenesis modulation [5], leading to its widespread therapeutic and cosmetic use. However, despite well-documented benefits, the detailed post-transcriptional mechanisms, especially the roles of microRNAs in concert with mRNA targets, remain largely unexplored. Here, we evaluated the effects of a novel vitamin C on the global mRNA and microRNA expression landscapes of primary human keratinocytes, fibroblasts and melanocytes. Our aim was to delineate the molecular circuits through which Vitamin C coordinates antioxidant defense, tissue repair and pigmentation control in skin cells.

Materials and Methods

Cell culture : Human fibroblasts and keratinocytes were obtained from skin of human healthy volunteers undergoing plastic surgery. Keratinocytes and melanocytes were isolated from epidermis while fibroblasts were isolated from dermis. Fibroblasts were cultured in RPMI medium with 10% FBS. Keratinocytes are initially cultured in Green's medium on a feeder layer of mitotically inactivated 3T3 fibroblasts. After one to two passages on the feeder layer, they are re-selected and seeded directly into collagen I-precoated flasks in Epilife medium with HKS supplement. Melanocytes were cultures in CNT40 (CELLnTECH). Cells were grown as a monolayer culture in culture flask at 37°C in a humidified atmosphere containing 5% CO2.

Cell viability assay : Human keratinocytes, fibroblasts and melanocytes were trypsinized before confluence and seeded in 48 well plate. Each type of cells was culture in the appropriate medium and was stimulated with increasing doses of vitamin C (0-1000 µg/mL). After 5 days of culture, cell viability was measured by analysis of 7-AAD uptake and cell size by flow cytometry, as described [6].

Gene expression profiling : Human keratinocytes, fibroblasts and melanocytes were cultured in presence of 100 µg/mL of vitamin C during 24h and 48h. After incubation, total RNA (including small and long transcripts) was extracted using the RNeasy Plus Universal Kit (Qiagen) according to the manufacturer's instructions, which includes genomic DNA elimination. RNA integrity was verified (RIN ≥ 8) on an Agilent 2100 Bioanalyzer.
Pooled small RNA and total RNA libraries were each sequenced on an Illumina NovaSeq X plus 10B Reagent Kit (illumina) (paired-end 2×100 bp for total RNA; single-end 50 bp for small RNA), targeting ~20 million reads per miRNA library and ~50 million read pairs per total RNA library. Sequencing reads were quality-checked (FastQC) and adapter-trimmed (Trimmomatic). For small RNA-seq, reads were aligned to GRCh38 with Bowtie1 and miRNAs quantified using miRDeep2 against miRBase v22. For total RNA-seq, reads were

mapped with STAR v2.7.3a and gene counts obtained via featureCounts v2.0.1 using GENCODE v32 annotations. Library-size normalization to counts per million (CPM) was performed with the cpm() function in edgeR v3.32.1. CPM matrices were used for exploratory analyses (variance ranking, heatmaps) and targeted gene-panel heatmaps.

Immunofluorescence: Primary human keratinocytes and dermal fibroblasts were each seeded in µ-Slide 8-well IBIDI chambers and allowed to adhere for 24 hours in their respective growth media. Cells were then treated with 30 µg/mL vitamin C. To serve as positive controls for differentiation, keratinocytes were exposed to 1.5 mM $CaCl_2$, whereas fibroblasts were incubated with 5 ng/mL recombinant TGFβ. After 3 days of culture, all wells were gently rinsed with PBS and fixed. Following fixation, cells were permeabilized and then blocked. Primary antibody staining was carried out overnight at 4 °C in blocking buffer: keratinocytes received rabbit anti-KRT10 (1:150, Abcam ab76318) and mouse anti-KRT14 (1 microg/ml, Abcam ab7800), while fibroblasts were incubated with rabbit anti-Collagen 1 A1 (0.4 µg /ml, Abcam ab138492) and mouse anti-α-SMA (1 µg /ml, Abcam ab7817). The following morning, samples were washed three times in PBS and incubated for one hour at room temperature with Alexa Fluor-conjugated secondary antibodies. Nuclear counterstaining was performed by incubating with DAPI (1 µg/mL in PBS) for five minutes. Finally, slides were mounted in Fluoromount-G and imaged on a confocal laser scanning microscope (Zeiss LSM 800) using identical acquisition settings across all conditions.

Results

Selection of Non-Cytotoxic Vitamin C Concentration

Primary human keratinocytes, fibroblasts and melanocytes were exposed to increasing concentrations of Vitamin C for five days, and cell viability was assessed by 7-AAD staining and flow cytometry. All three cell types retained >90 % viability up to 100 µg/mL (Fig. 1). We therefore chose 100 µg/mL as the maximal non-cytotoxic dose for all subsequent transcriptomic and immunofluorescence experiments.

Vitamin C–Induced Antioxidant, Anti-Inflammatory and Pro-Differentiation Effects in Keratinocytes

Keratinocytes treated with 100 µg/mL Vitamin C underwent extensive mRNA and miRNA reprogramming (Fig. 2a–b).

Antioxidant genes (*NQO1, HMOX1, GPX2*) and ferroptosis-resistance factors (*FTH1, FTL, SLC7A11, TXNRD1*) were strongly upregulated, while pro-inflammatory molecules (*CXCL1, CXCL14, IL7R*) were suppressed. Concurrent downregulation of *TAGLN* and *TGFB2* indicates cytoskeletal reorganization and TGF-β signaling attenuation, which likely promote balanced wound repair and limit fibrosis (Fig.2c). Immunofluorescence confirmed enhanced differentiation, with increased KRT10 and decreased KRT14 staining in Vitamin C–treated cells (Fig. 2d).

At the post-transcriptional level, a targeted set of miRNAs (miR-590-5p, miR-4458, miR-148-3p) was upregulated, each inversely correlating with *TAGLN* or *TGFB2* levels, demonstrating fine-tuned post-transcriptional control of cytoskeletal and growth-factor pathways

Vitamin C Orchestrates Cell-Cycle, DNA-Repair and Myofibroblast Differentiation via mRNA and miRNA Networks in human primary Fibroblast

In dermal fibroblasts, 100 µg/mL Vitamin C induced a coordinated transcriptional and miRNA response ((Fig. 3a–b) consistent with a survival-promoting, pro-regenerative phenotype
Key cell-cycle drivers (*CCND1, CDC25B, CDK6, E2F1, POLA2, PRIM1, PLK1, MCM10*) were sharply upregulated, while the apoptotic mediator *BCL2L11* was repressed. DNA-repair genes (*MYC, CHEK1, FANCD2, SMC1A, TDP1, BRCA1*) and antioxidant enzymes (*GPX3, TXNRD1*) also increased. Metabolism shifted toward both anabolic and antioxidant defense pathways, with robust induction of cyclins CCNA2 and CCNB1 and nucleotide-biosynthesis enzymes DTYMK and TYMS. Concurrent downregulation of growth-factor responders IGF1 and EPAS1, together with upregulation of PIK3R1 and UCP2, further supports enhanced PI3K-mediated signaling, mitochondrial uncoupling, and an overall energetic and redox-protective boost (Fig.3c)
Matrix regulators (*AURKB, STMN1, ITGA2*) rose, while angiogenic *VEGFA* fell (Fig.3c) Immunofluorescence demonstrated increased α-SMA fibers and Collagen I A1 deposition, hallmarks of myofibroblast differentiation (Fig. 4).
A suite of validated miRNAs was modulated at 24 h, each inversely tracking its mRNA target to refine cell-cycle, DNA-repair, cytoskeletal and matrix pathways. Additional miRNAs emerged at 48 h, indicating a dynamic, time-dependent regulatory network.

Vitamin C–Induced Antioxidant Activation and Melanogenesis Suppression in Melanocytes

Adult melanocytes treated with 100 µg/mL Vitamin C exhibited a balanced transcriptional and miRNA reprogramming (Fig. 4a–b) that enhanced oxidative resilience while suppressing pigment synthesis (Fig. 4c). Cell-cycle regulators were differentially modulated: E2F2 transcript levels rose, whereas CDK6, MCM2 and OSMR were downregulated. BRCA1 remained stable to slightly reduced, and JAK2 was suppressed, indicating a shift toward cell-cycle restraint rather than overt proliferation.
DNA-damage response showed a biphasic pattern: APAF1 fell at 24 h before rebounding by 48 h, while E2F1 was consistently upregulated, suggesting enhanced checkpoint activity without triggering apoptosis.
Mitochondrial and redox pathways were also rebalanced. The mitochondrial subunit NDUFA4 was downregulated, yet UCP2 increased modestly, reflecting an early stress adaptation followed by improved bioenergetic flexibility. HMOX1 levels surged, underscoring a strengthened antioxidant response.
Crucially, master melanogenic effectors—MITF, TYRP1, PMEL, SLC24A5, RAB27A and MLPH—were uniformly downregulated. In addition, Vitamin C–treated melanocytes exhibited marked dendrite retraction, which may suggest reduced melanosome transfer capacity and a stabilized, less active pigmentation phenotype (data not shown)
All these changes in gene expression are accompanied by modulation of a precisely defined miRNA network at the post-transcriptional level. (Fig. 4d).

Discussion

In this study, we demonstrate that Vitamin C exerts multifaceted effects on skin cell biology by orchestrating coordinated changes in both coding and non-coding RNA networks. In keratinocytes, Vitamin C robustly activated Nrf2-dependent antioxidant and ferroptosis-resistance pathways while simultaneously attenuating pro-inflammatory and TGF-β signaling (via downregulation of TGFB2 and cytoskeletal regulator TAGLN). These

transcriptional shifts, confirmed at the protein level by increased KRT10/KRT14 ratio, likely underpin the enhanced barrier function and accelerated differentiation known to improve wound healing and photo-protection.

Dermal fibroblasts likewise responded to Vitamin C with a pro-regenerative transcriptional program: key cell-cycle drivers, DNA-repair genes and extracellular matrix regulators were upregulated, whereas apoptotic and angiogenic signals were dampened. Immunofluorescence–detected increases in α-SMA and Collagen I A1 further validate Vitamin C's promotion of myofibroblast differentiation—a critical step in tissue repair. Importantly, this anabolic, matrix-building phenotype did not come at the expense of unchecked fibrosis, as TGFβ pathway components were fine-tuned rather than broadly activated.

Melanocytes treated with Vitamin C exhibited enhanced oxidative resilience (via induction of HMOX1 and SLC7A11), subtle modulation of cell-cycle and mitochondrial genes, and striking suppression of the melanogenic circuit [7] (MITF, TYRP1, PMEL, etc.). The observed dendrite retraction provides a morphological correlate to the transcriptional whitening signature, suggesting that Vitamin C not only reduces melanin synthesis but also limits melanosome transfer to keratinocytes.

Across all three cell types, we observed parallel modulation of specific miRNA networks whose experimentally validated mRNA targets align with the major transcriptomic changes. By restricting our analysis to literature-supported miRNA–mRNA pairs, we delineate a high-precision post-transcriptional layer that fine-tunes Vitamin C's effects. Integrating RNA-seq with microRNA profiling thus characterizes regulatory circuits through which Vitamin C delivers targeted benefits—enhanced antioxidant defenses, controlled proliferation, regenerative extracellular matrix synthesis, and pigmentation control.

Conclusion

Our integrative analysis reveals that Vitamin C modulates skin cell function through a concert of transcriptional and post-transcriptional mechanisms, mediated in part by fine-tuned miRNA networks. By enhancing antioxidant defenses, promoting balanced proliferation and differentiation, and suppressing pigmentation, Vitamin C emerges as a versatile dermo-active agent. These insights provide a molecular rationale for its widespread use in cosmetic and therapeutic formulations and support the development of next-generation skincare products that leverage its multifaceted regulatory effects.

Figures

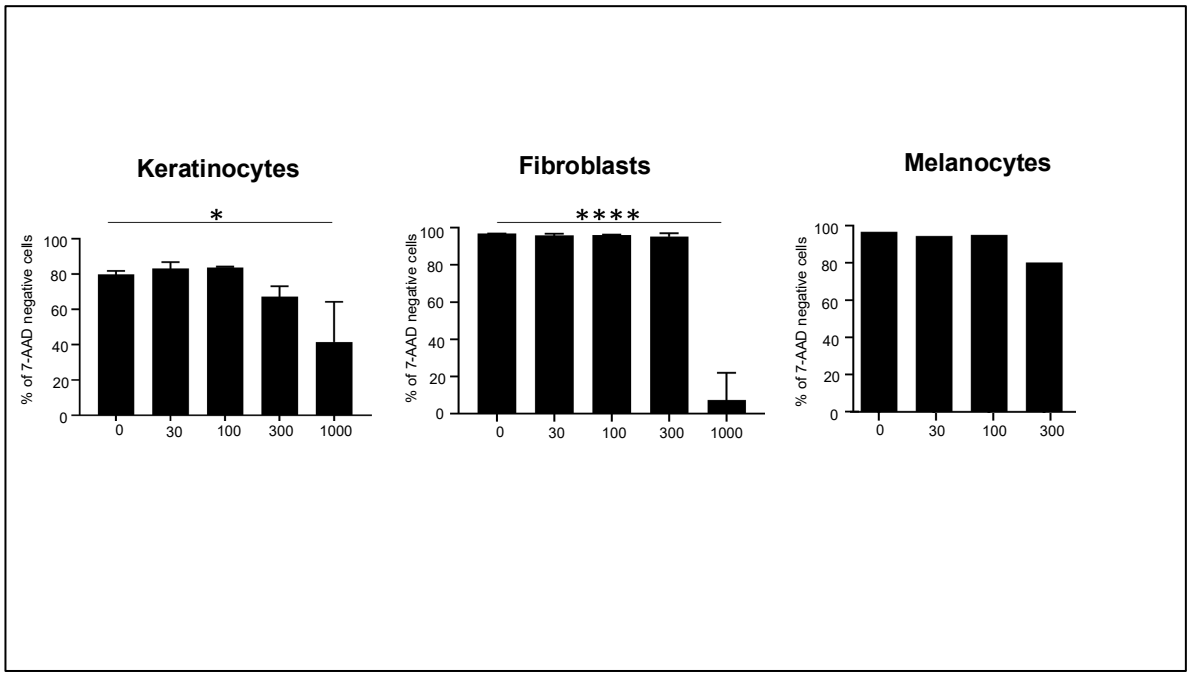

Figure 1: Selection of Non-Cytotoxic Vitamin C Concentration Histograms showing dose–effect of vitamin C on the frequency of 7-AAD- cells (n = 3 for keratinocytes and fibroblasts; n = 1 for melanocytes).

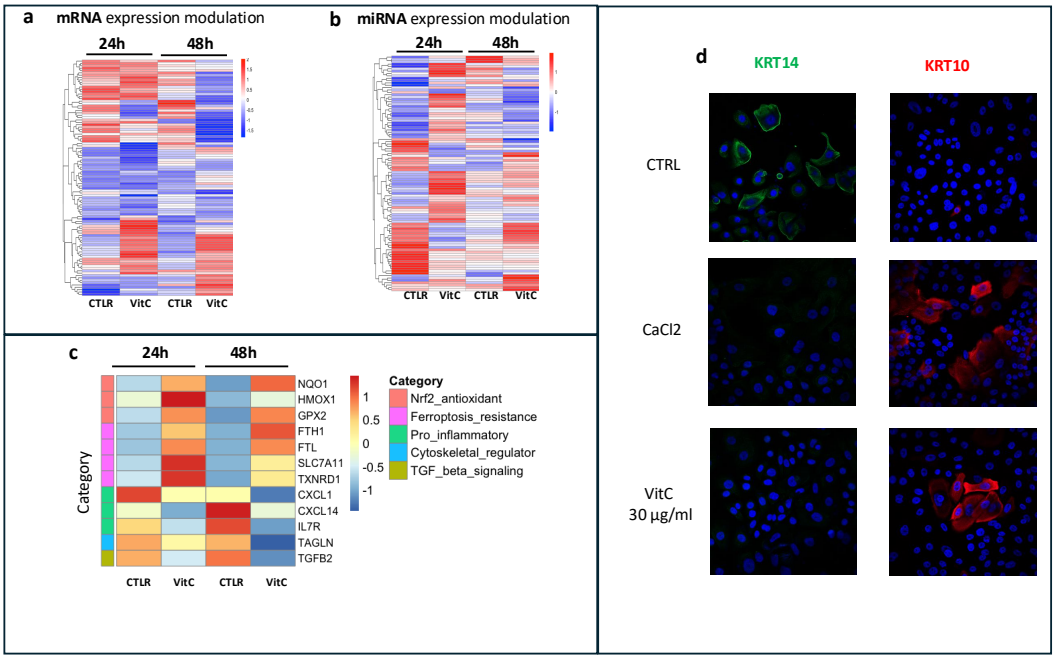

Figure 2: Vitamin C–Induced Redox Homeostasis, Anti-Inflammatory Response and Pro-Regenerative Differentiation in Human Keratinocytes.

Heatmaps of the 100 most variable transcripts across all Vitamin C treatment conditions (0 vs. 100 µg/mL at 24 h and 48 h), showing mRNAs (**a**) and miRNAs (**b**). Rows represent individual transcripts; columns represent samples. Data are row-wise Z-score normalized (blue = low, red = high) and clustered by Euclidean distance. (**c**) Heatmap of selected Vitamin C–responsive genes, grouped by functional category. Expression is row-scaled (Z-score) across the same treatment conditions; red and blue indicate above- or below-average expression, respectively. (**d**) Representative immunofluorescence of primary human keratinocytes after 3 days of culture under three conditions: untreated control, 1.5 mM $CaCl_2$ (positive control), or 30 µg/mL Vitamin C. Keratin 14 (KRT14, red) and Keratin 10 (KRT10, green) staining are shown; nuclei are counterstained with DAPI (blue).

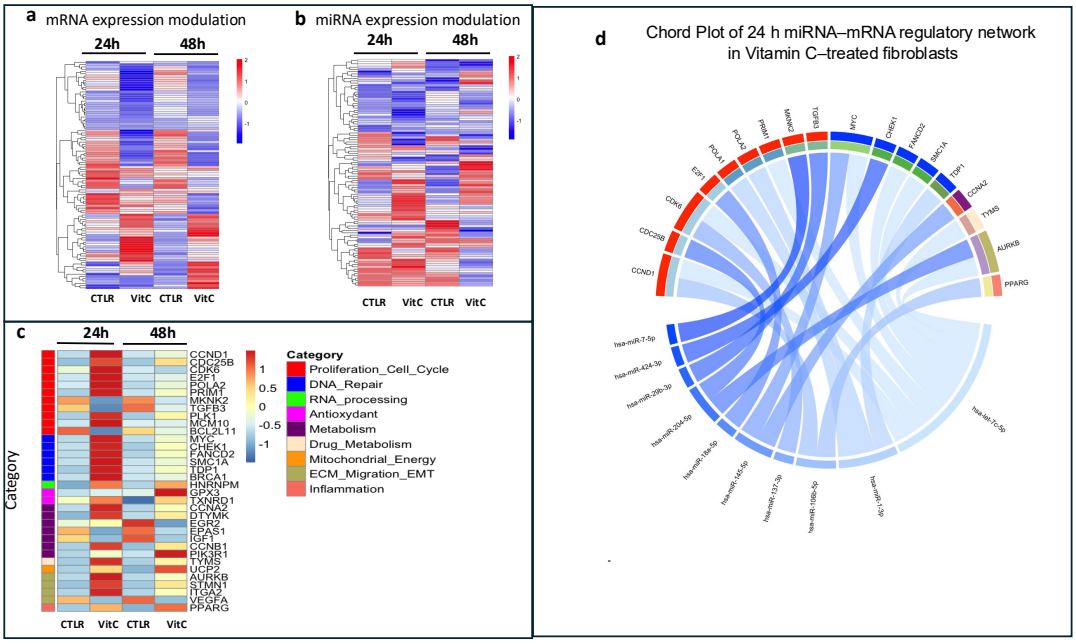

Figure 3: **Vitamin C Orchestrates Cell-Cycle, DNA-Repair and Myofibroblast Differentiation via mRNA and miRNA Networks.** Heatmaps of the 100 most variable transcripts across all Vitamin C treatment conditions (0 vs. 100 µg/mL at 24 h and 48 h), showing mRNAs (**a**) and miRNAs (**b**). Rows represent individual transcripts; columns represent samples. Data are row-wise Z-score normalized (blue = low, red = high) and clustered by Euclidean distance. (**c**) Heatmap of selected Vitamin C–responsive genes, grouped by functional category—Expression is row-scaled (Z-score) across the same treatment conditions; red and blue indicate above- or below-average expression, respectively. (**d**) Chord plot depicting the network of miRNAs modulated at 24 h following 100 µg/mL Vitamin C treatment and their experimentally validated mRNA targets. Outer arcs on the upper semicircle are color-coded by functional category, and inner arc labels indicate each gene's pathway affiliation. Ribbons connect each miRNA to its confirmed mRNA targets.

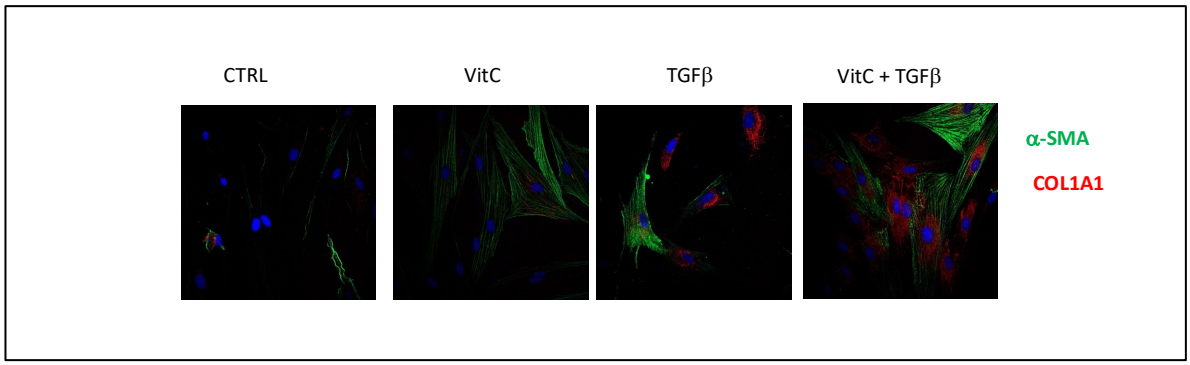

Figure 4: Vitamin C Enhances α-SMA and Collagen I A1 Expression in Human Dermal Fibroblasts. Representative immunofluorescence of primary human dermal fibroblasts after 3 days of culture under four conditions: untreated control, 30 µg/mL Vitamin C, 5 ng/mL TGFβ, and combined Vitamin C +TGFβ, α-SMA is shown in green, Collagen 1 A1 in red, and nuclei are counterstained with DAPI (blue). Scale bar: 50 µm

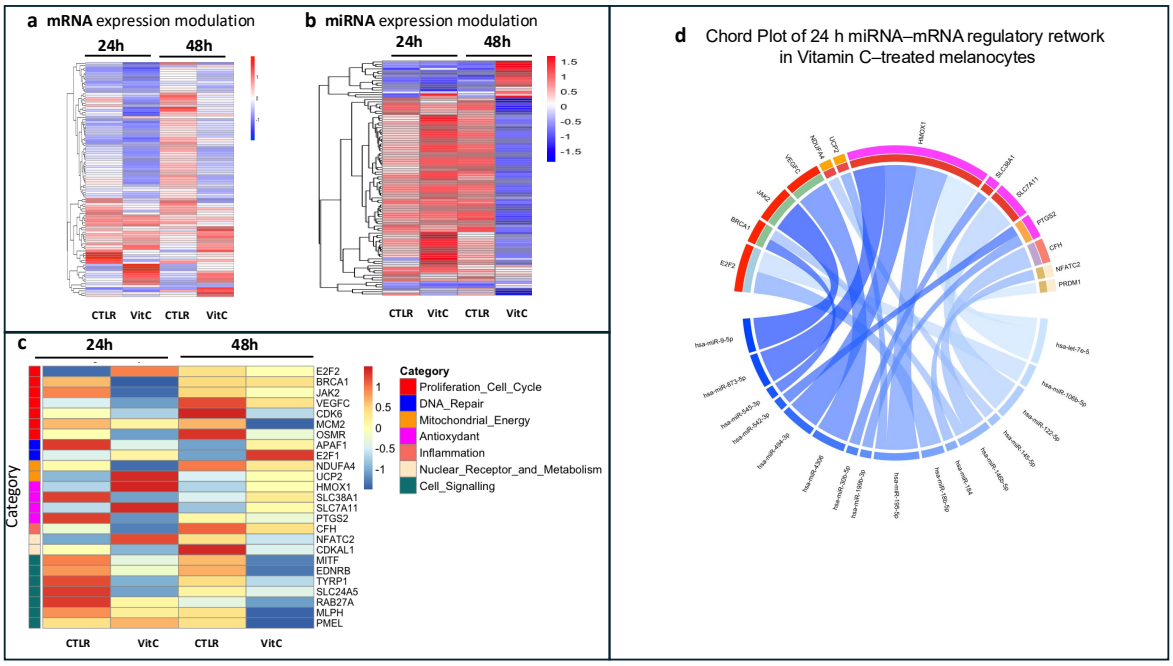

Figure 5: Vitamin C–Induced Antioxidant Activation and Melanogenesis Suppression in Melanocytes. Heatmaps of the 100 most variable transcripts across all Vitamin C treatment conditions (0 vs. 100 µg/mL at 24 h and 48 h), showing mRNAs (**a**) and miRNAs (**b**). Rows represent individual transcripts; columns represent samples. Data are row-wise Z-score normalized (blue = low, red = high) and clustered by Euclidean distance. (**c**) Heatmap of selected Vitamin C–responsive genes, grouped by functional category. Expression is row-scaled (Z-score) across the same treatment conditions; red and blue indicate above- or below-average expression, respectively. (**d**) Chord plot depicting the network of miRNAs modulated at 24 h following 100 µg/mL Vitamin C treatment and their experimentally validated mRNA targets. Outer arcs on the upper semicircle are color-coded by functional category, and inner arc labels indicate each gene's pathway affiliation. Ribbons connect each miRNA to its confirmed mRNA targets.

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