

N-acetyl glucosamine and niacinamide in the regulation of melanin production in vitro

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INTRODUCTION

In vitro studies have shown that the inhibitory effects of niacinamide and N-acetyl glucosamine (NAG) on melanin production can be enhanced with the addition of undecyl-10-enoyl-phenylalanine (MSH WHITE). Here we show the combinatorial benefits of niacinamide, which inhibits melanosome transfer between the melanocyte and the keratinocyte, NAG, an inhibitor of tyrosinase glycosylation, and MSH WHITE, an α -MSH antagonist, on the *in vitro* production of melanin. We also investigate the differential gene expression of cultures treated.

OBJECTIVE

To explore the *in vitro* benefits of niacinamide, NAG, and MSH WHITE in reducing melanin production and to investigate potential mechanisms of action using genomic comparisons.

METHODS

Skin-Equivalent Cultures

- Melanocyte- and keratinocyte-containing cultures, reconstituted from human epidermis, were purchased from SkinEthic (Nice, France).
- Niacinamide and niacinamide + MSH WHITE were applied topically to the cultures for 2 days (n=3).
- Water was applied topically to the control cultures daily for 2 days (n=4).
- Cultures were harvested after the second day of treatment.
- RNA was isolated from the harvested cultures and preserved using RNAlater (Ambion, Austin, Texas).

Genomic Analysis

- RNA was isolated and GeneChip targets were generated using the Enzo BioArray™ HighYield™ labeling kit and standard procedures.
- Affymetrix HG-U133 Plus 2.0 GeneChips were hybridized, washed, stained, and scanned using standard parameters.
- Summary statistics were calculated using standard methods
- Comparisons were also made to previous studies to identify genes unique to the treatment of MSH WHITE + niacinamide compared to niacinamide + NAG (previous study) and niacinamide alone (control leg)

Total Melanin Analysis

- Mouse melanoma F1 cells were cultured to a confluency of 20 - 30 % and treated with media containing test compounds.
- Two days post treatment, a small amount of media was removed to determine cell viability using a ToxiLight viability assay kit.
- A solution containing 0.75% sodium hydroxide was then added to the remaining culture to lyse the cells and neutralize pH.
- Melanin was detected colorimetrically at 405nm using a spectrophotometer.

RESULTS

Statistical analysis of pigmentation-relevant genes revealed two interesting expression profiles:

Genes specifically up-regulated by the niacinamide + MSH WHITE combination (Figure 1):

Dual specificity phosphatase 1 (DUSP1): A negative regulator of the MAP kinase pathway. Inhibiting the MAP kinase pathway would lessen the cellular inflammatory response.

Arylsulfatase B (ARSB): responsible for lysosomal transport. Small changes in lysosomal transport could significantly increase the rate of lysosomal melanin degradation.

TGF- β 1, GADD45, and Kip1-2: All three genes encode cell-cycle regulators that help cells deal with stress. Up-regulation of these genes could aid the cell in preventing or responding to external stress (e.g. UV damage or free radicals).

Genes specifically down-regulated by the niacinamide + MSH WHITE (Figure 1):

Phospholipase A2, group IVA (PLA2G4A): responsible for the release of arachidonic acid from the cell membrane. Limiting the release of arachidonic acid, which is converted to eicosanoids (including prostaglandins) would dampen the cell's inflammatory response and reduce post-inflammatory pigmentation.

Chemokine (C-C-motif) receptor 1 (CCR1): a key cytokine and chemokine receptor, which is responsible for initiating cytokine-mediated inflammatory responses.

Leucine rich repeat (in FLII) interacting protein 2 (LRRFIP2): an initiator of the Wnt signaling pathway, which is responsible for the transcription of pigmentation-relevant genes (TYR, TRP-1, TRP-2).

Total Melanin Synthesis: (Figure 2)

Mouse melanoma F1 cells treated with niacinamide + NAG produced only 45% \pm 1.3% of the melanin produced by cells receiving no active treatment.

Mouse melanoma F1 cells treated with niacinamide, NAG, and MSH WHITE produced only 15% \pm 1.4% of the melanin produced by cells receiving no active treatment, thereby significantly decreasing melanin production.

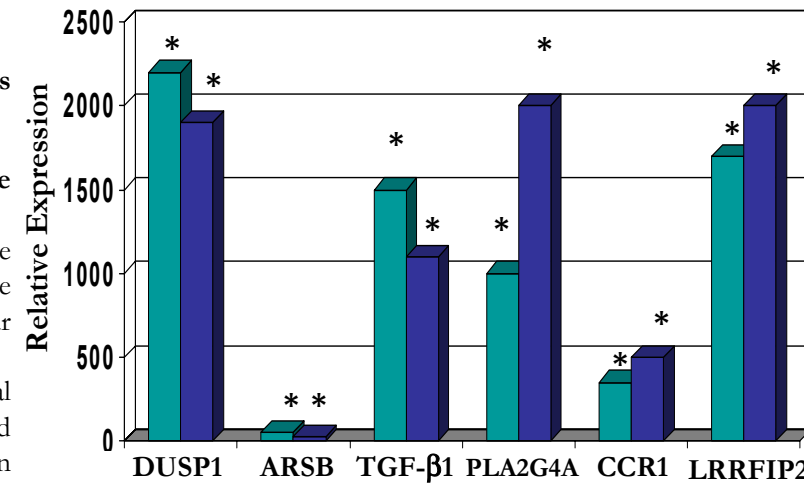


Figure 1. DUSP1, ARSB, and TGF- β 1 were up-regulated according to the profile: niacinamide + MSH WHITE (green) > niacinamide (blue) while PLA2G4A, CCR1, and LRRFIP2 were down-regulated according to the profile: niacinamide + MSH WHITE (green) < niacinamide (blue). * For all genes p<0.05.

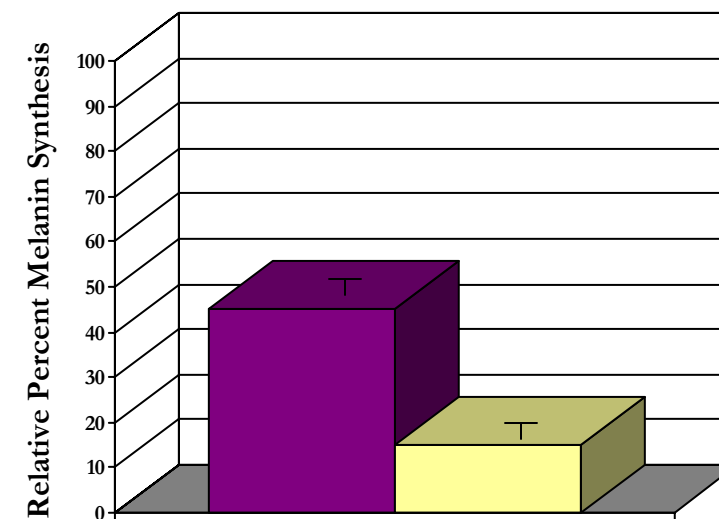


Figure 2. Relative percent melanin synthesis from mouse melanoma F1 cells treated with niacinamide and NAG (■) vs. niacinamide and NAG and MSH WHITE (□).

CONCLUSIONS

The combination of niacinamide, NAG, and MSH WHITE provides an additional benefit for the *in vitro* inhibition of melanin synthesis compared to treatment with niacinamide + NAG alone.

Genomic insights into the mechanism of action suggests that MSH WHITE regulates melanin production by up-regulating genes responsible for the turnover of melanin, by inhibiting inflammatory signaling, and by increasing the cell's capacity to deal with stressors. MSH WHITE also down-regulates genes responsible for the transcription of pigment genes as well as mediating inflammation from both prostaglandin-mediated inflammatory signaling as well as cytokine/chemokine regulated inflammatory signaling.

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