

# Niacinamide Inhibits Melanogenesis Related Gene Expression in Melanocytes When Co-cultured with Keratinocytes

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## INTRODUCTION

Niacinamide (vitamin B3) containing products have been clinically demonstrated<sup>1)</sup> to reduce the appearance of facial hyperpigmentation. We previously reported niacinamide inhibits melanosome transfer from melanocytes to surrounding keratinocytes *in-vitro*, although it does not directly inhibit melanogenesis in human melanocytes, nor in B16 melanoma cells<sup>2)</sup>.

## OBJECTIVE

To determine the effect of niacinamide on the expression of melanogenesis related genes and proteins in melanocytes when co-cultured with keratinocytes.

## METHODS

Human keratinocyte cultures were seeded with human melanocyte cells, and treated with niacinamide. Melanocyte:keratinocyte (M:K) seeding ratios were 1:8. Some cultures were stimulated with  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) after a 2 day period in which all sources of  $\alpha$ -MSH were removed from the system. Unless otherwise noted, the data presented here are from  $\alpha$ -MSH stimulated cultures<sup>3,4)</sup>.

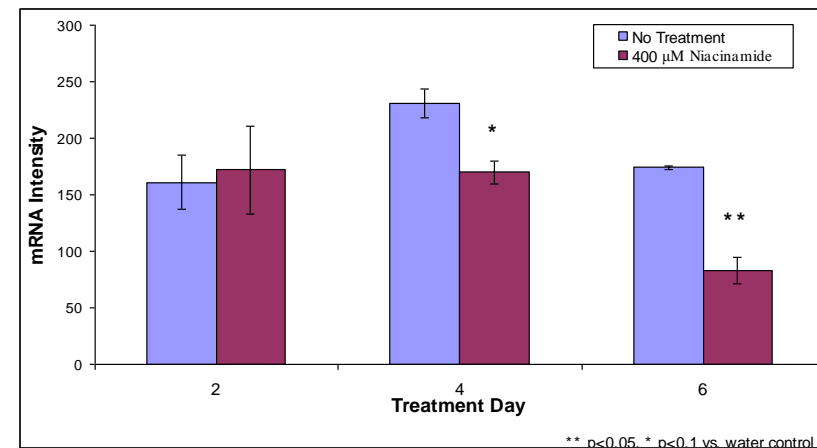
For RNA research, cultures were harvested for 0, 2, 4 & 6 days post treatment. Melanogenesis related RNA was quantitated using multiplex techniques.

For protein research, cultures were harvested for 6 days at the ratio of M:K = 1:25. MITF (microphthalmia-associated transcription factor) protein was quantitated using beads conjugated with anti-MITF antibodies.

## RESULTS

### Fig 1. Niacinamide Decreases MITF messenger RNA (mRNA) Expression Levels in Co-culture Model

Chart below shows mRNA for MITF is decreased after niacinamide treatment compared to no treatment controls (N=3 each). The effect becomes apparent between Day 2 and Day 4 and becomes more pronounced by Day 6.



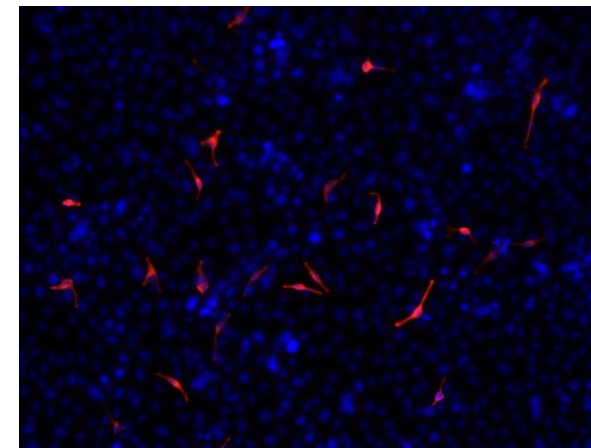
### Table 1. Pigmentation Gene Panel Data

mRNA levels from co-cultures treated with IBMX (3-isobutyl-1-methylxanthine) and niacinamide normalized to no treatment control (N=3 each). 10 nM  $\alpha$ -MSH is present in all legs of this study. IBMX up-regulation peaks at day 2 while niacinamide down-regulation of pigmentation genes occurs at day 4 and later. MITF data highlighted in bar graph above. (\* p<0.05, + p<0.1 vs. control)

	TYR	TYRP1	TYRP2	MITF	PMEL17	
Control	100	100	100	100	100	Day 2
300uM IBMX	*131	*124	*274	*145	*162	
400uM Niacinamide	83	93	109	108	94	
Control	100	100	100	100	100	Day 4
300uM IBMX	104	96	*174	103	115	
400uM Niacinamide	*74	*80	110	+79	92	
Control	100	100	100	100	100	Day 6
300uM IBMX	*39	103	*162	*67	77	
400uM Niacinamide	*29	*59	*43	*50	*47	

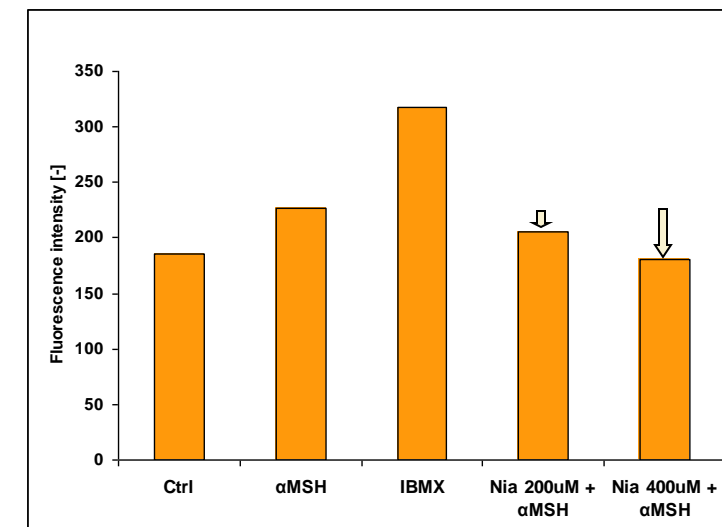
### Fig 2. Melanocytes in Fluorescent Photograph of Co-culture

Melanocytes (red) was stained with NK1btep-Alexafluor 592-{red} in co-culture to confirm that melanocytes were still looking dendritic and that the numbers were constant throughout the experiment. The counted melanocyte number at day 6 was the same as day 0 (data not shown).



### Fig 3. Niacinamide Decreases MITF Protein

Niacinamide decreases MITF protein level down towards non-stimulated levels (no  $\alpha$ -MSH) in co-culture. Melanocyte-keratinocyte co-cultures were treated with  $\alpha$ -MSH to increase melanogenesis. Co-cultures were treated with niacinamide for 6 days. MITF protein is decreased after 6 days of treatment.



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## CONCLUSIONS

The melanocyte-keratinocyte co-culture system is suitable for probing signaling interactions that are impossible to monitor in mono-culture systems<sup>2,3)</sup>. Modern mRNA quantitation methodology allows measurement of key pigmentation gene expression regulation with good signal-to-noise. Bead-based protein detection methodologies allow protein level detection. Both protein and mRNA data support the conclusion that niacinamide decreases the pigmentation machinery. We have previously presented that mono-cultures of B16 melanoma cells do not show reduction of melanin synthesis with niacinamide treatment. Taken together, the data implicate the existence of a communication loop between keratinocytes and melanocytes upon which niacinamide exerts its pigmentation reducing action.

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