



# Resveratrol's Effects on the Androgen Receptor Pathway as a Target for Decreasing Growth and Invasion of Melanoma and Methods of Application

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## Introduction

Although melanoma affects both sexes, current literature shows it is more prevalent and more deadly in men. The androgen receptor has been proven to play a role in melanoma growth and metastasis, and may play a part in the difference in incidence and mortality rates between sexes. Resveratrol, a naturally occurring compound with poor bioavailability, has been shown to decrease melanoma cell growth in both in vitro and in vivo studies and has known effects on the androgen receptor signaling pathway. We propose that resveratrol is a potential therapeutic that can decrease melanoma growth and invasion by targeting the androgen receptor pathway, and we review current and future perspectives for resveratrol application methods.

## Methods

**Cell culturing:** A375-MA2 metastatic melanoma cells were maintained according to American Type Culture Collection (ATCC) protocol.

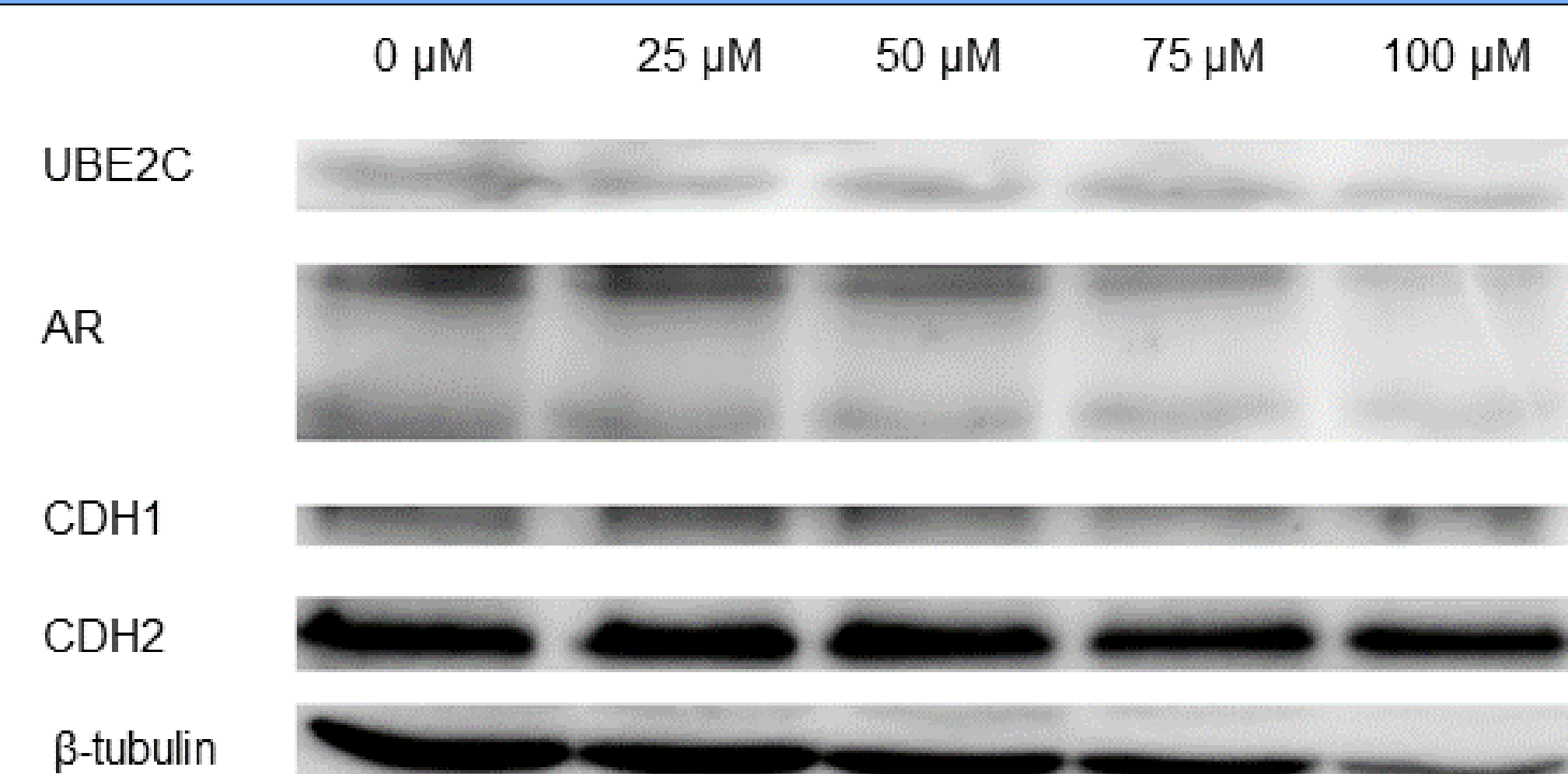
**Western Blotting:** 50 µg of each protein sample were run through SDS-PAGE and transferred to nitrocellulose for blotting. Western blots were performed for Ubiquitin-conjugating enzyme E2 C (UBE2C), androgen receptor (AR), E-Cadherin (CDH1), N-Cadherin (CDH2), and β-Tubulin. (Figure 1)

**Scratch Wound Assay:** Five separate 10 cm plates of melanoma cells were sub-cultured. After they reached total confluency they were washed with PBS two times, scratched with a pipette tip, and immediately bathed in medium containing varying concentrations of RSV. Pictures were taken at 0, 6, 12, 18, and 24 hours. (Figure 2)

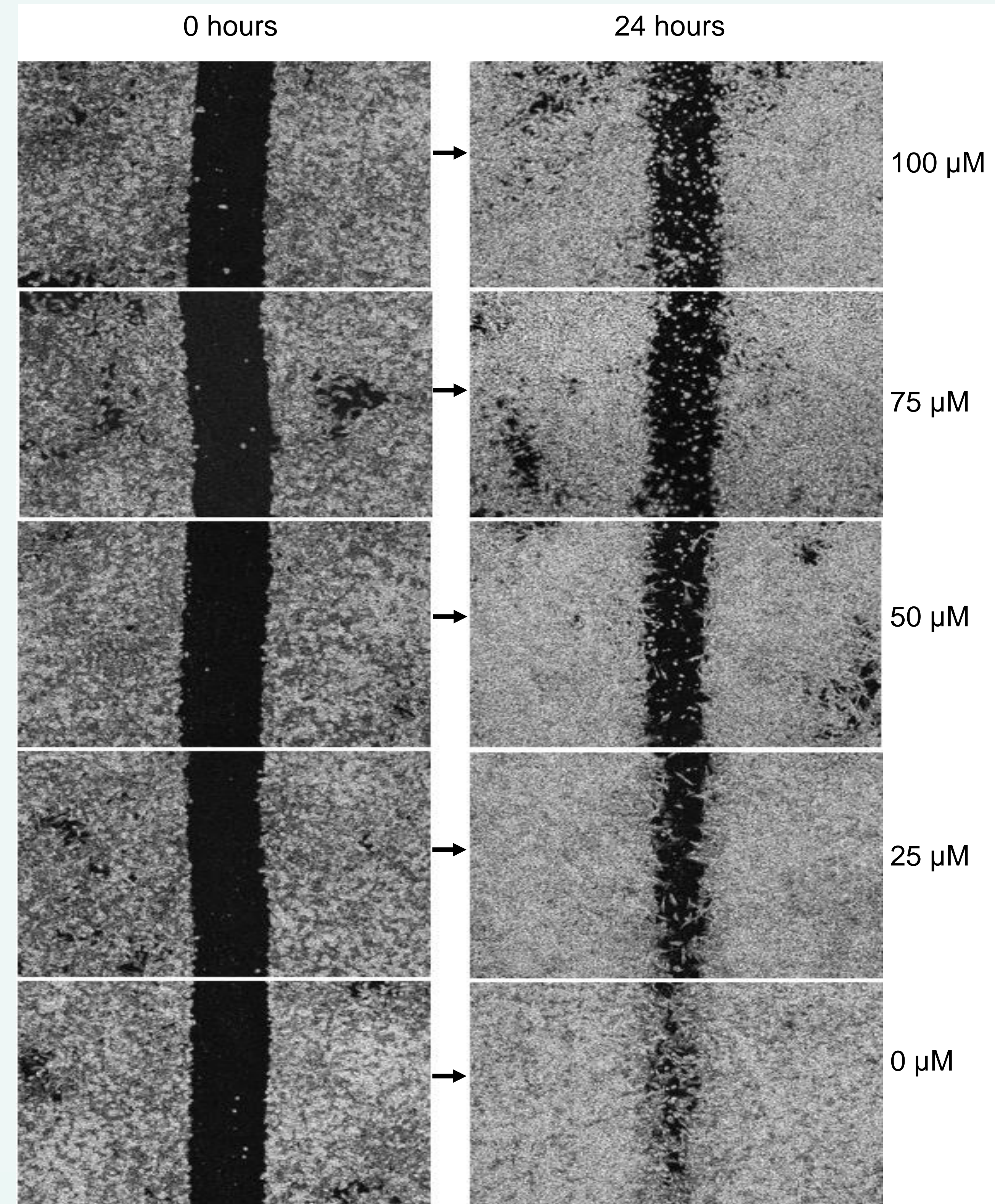
**RNA Extraction and qPCR:** RNA isolation from the cultured cells was performed using the QIAGEN RNeasy kit and quantified with a Nanodrop 2000. cDNA was synthesized and gene expression was measured using StepOnePlus Real Time PCR System. Taqman probes CDH1, CDH2, UBE2C, and CCAR2 were utilized as AR activity indicators. All samples were done in quadruplicate. (Figure 3)

## Western Blot Results

**Figure 1. Protein Levels.** Protein samples from MA-2 cells treated with RSV were analyzed via Western Blot for UBE2C, AR, CDH1, and CDH2. Western blots were treated with β-Tubulin to control for loading.

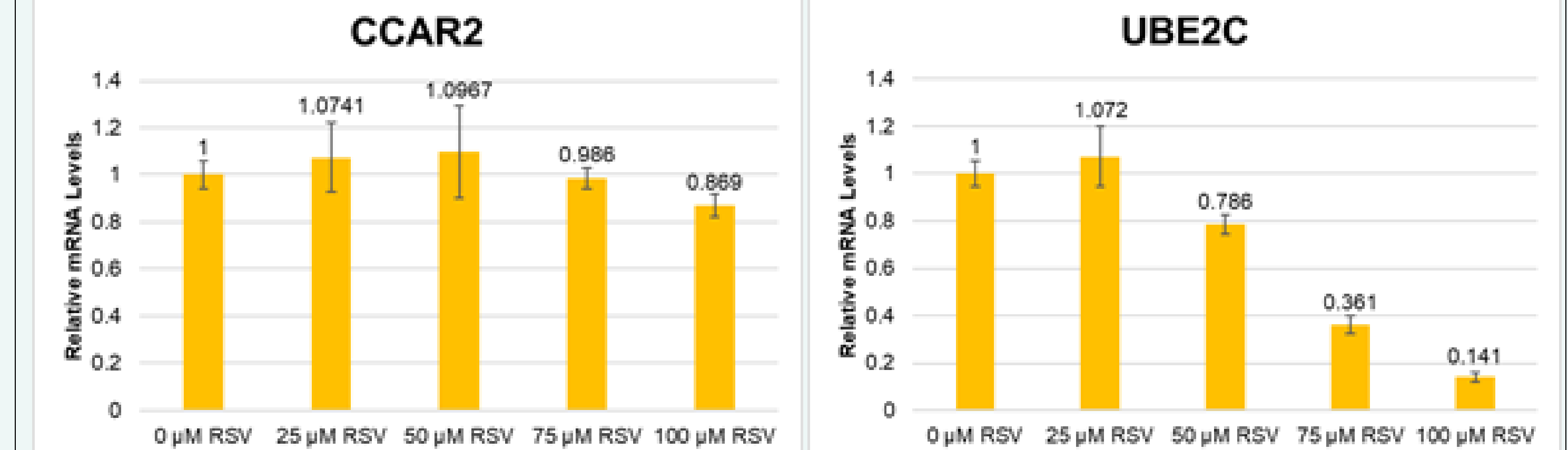


## Scratch Wound Assay Results



**Figure 2. Migration of Melanoma Cells in Scratch Wound Assay.** A dose-dependent decrease in melanoma migration was visualized with increasing concentrations of RSV. The untreated plate almost reached total confluency 24 hours after the scratch.

## qPCR Results

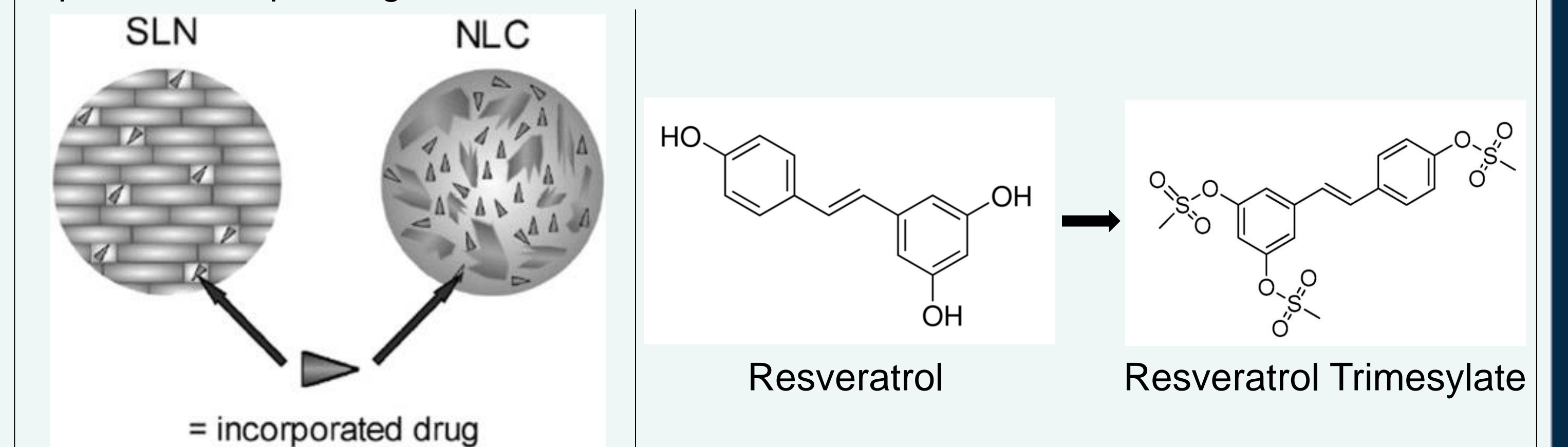


**Figure 3. Relative mRNA Expression.** No difference in cadherin or CCAR2 expression was visualized. There was a dose-dependent decrease in UBE2C expression with increasing RSV treatment.

## Bench to Bedside

**Problem:** Resveratrol is lipophilic and has poor bioavailability due to high rates of first pass metabolism by the liver, intestines, and intestinal bacteria.

**Potential Solutions:** Topical application with solid lipid nanoparticles, nanostructured lipid carriers, prodrugs.



## Conclusion

Resveratrol is a promising therapeutic capable of altering melanoma growth and metastasis at least partially through its interactions with the androgen receptor signaling pathway. Future studies should examine the effects of resveratrol on melanoma that has not metastasized yet to further characterize its ability to prevent metastasis. Additional in vivo studies examining its effects on melanoma through innovative methods of delivery will be useful in bringing resveratrol from bench to bedside.