Resveratrol is a Nuclear Receptor 4A1 (NR4A1) Ligand the Antagonizes NR4A1-Regulated Prooncogenic Pathways in Lung Cancers

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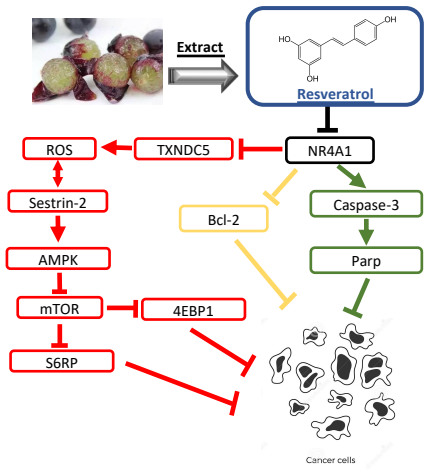
Abstract

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytochemical found in fruits, nuts, and vegetables and there is evidence that this compound offers protection from several human diseases including cancer. In cancer cell lines, resveratrol inhibits cell growth, survival, migration/invasion and genes/pathways associated with these anticancer activities. Many of the same anticancer activities reported for resveratrol have previously been observed in this laboratory using bis-indole derived nuclear receptor 4A1 (NR4A1) ligands that antagonize of NR4A1-regulated pro-oncogenic pathways. Treatment of A549, H460, H1299 lung cancer cells with 50-125 μM resveratrol for 24, 48, 72 hours inhibited cell growth and IC_{50} values for growth inhibition decreased with time. In addition, resveratrol inhibited the mTOR signaling pathway and other responses in lung cancer cells as previously observed for NR4A1 antagonists in the same cell lines. Therefore, we investigated the interactions of resveratrol with the ligand binding domain of NR4A1 in an assay that measures fluorescent quenching of a tryptophan residue in the NR4A1 ligand binding pocket. Resveratrol bound NR4A1 and the K_D value was 1.4 μM. H460 and H1299 lung cancer cells were transfected with the yeast Gal4-NR4A1 fusion construct and UAS-luciferase which contains tandem Gal4 response elements, and treatment with 125+150 μM resveratrol decreased transactivation. Thus, resveratrol directly bound NR4A1, inhibited NR4A1-dependent transactivation, inhibited cell growth and mTOR signaling, and the role of NR4A1 in mediating the responses induced by resveratrol is currently being investigated.

Introduction



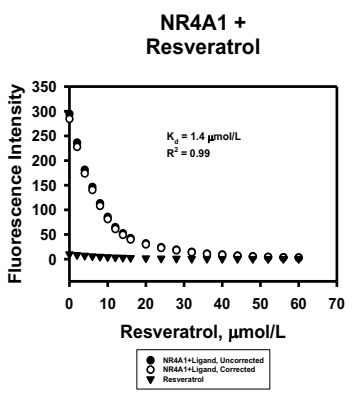


Figure 1. Directly binding of resveratrol NR4A1(LBD)

Methods

- **Cell line:** A549, H460 and H1299 non-small lung cancer cell lines were purchased from American Type Culture Collection.
- **Cell proliferation Assay:** A549, H460, H1299 cells are seeded ($2x10^4$ per well) in 96-well plates. A549 cells were maintained with DEME and 10% FBS. H460 and H1299 cells were maintained in RPMI 1640 and 10% FBS. Cells were allowed to attach for 24 hours. Then, the cells were treated at given concentrations with appropriate media and 2.5% stripe FBS for 24, 48, and 72 hours. XTT cell viability kit that was purchased from Cell Signaling was applied to stain live cells after treatment. Plates were read with the absorbance at wavelength 450 nm.
- **Transactivation assay:** Luciferase activity was determined in cells transfection performed with GAL4-NR4A1 (human)/GAL4-luc constructs and the values were normalized against corresponding β-gal activity.
- **Western blotting:** Cells were lysed and whole-cell lysates were resolved in 10% SDS-PAGE gels and proteins were transferred using PVDF membrane by wet blotting followed by primary and secondary antibody incubation and detection using ECL reagent.
- **Cell migration:** The cells were scratched using a pipette tip when the cell reached around 95% confluence, and were further incubated with fresh medium for 24 hrs.

Results

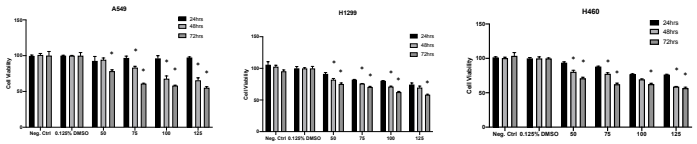


Figure 2. Resveratrol inhibits cell growth and survival. Results are expressed as mean ± SD for triplicates per treatment and significant (P<0.05) difference from control treatments are indicated (*).

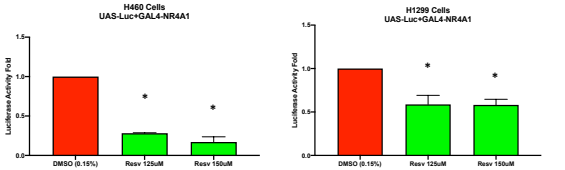


Figure 3. Effects of NR4A1 Ligands on transactivation. H460 and H1299 cells Gal4-NR4A1 and UAS-luc reporter gene luciferase activity was determined after treatment with Resveratrol. Results are expressed as mean ± SD for at least 3 replicate determinations for each treatment group and significant (p<0.05) inhibition is indicated (*).

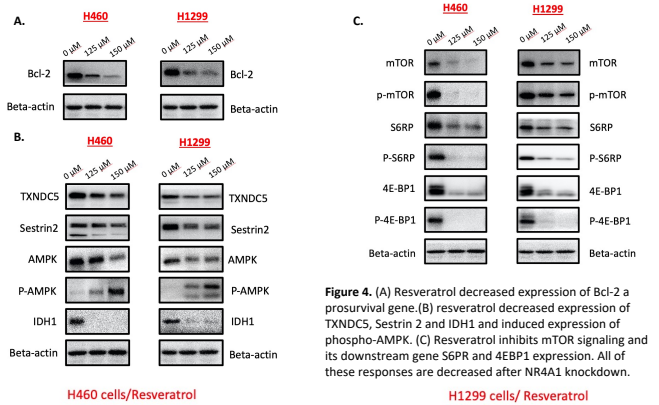


Figure 4. (A) Resveratrol decreased expression of Bcl-2 a prosurvival gene.(B) resveratrol decreased expression of TXNDC5, Sestrin 2 and IDH1 and induced expression of phospho-AMPK. (C) Resveratrol inhibits mTOR signaling and its downstream gene S6PR and 4EBP1 expression. All of these responses are decreased after NR4A1 knockdown.

Figure 5. Resveratrol treatment decreases the migration ability of H460 and H1299 lung cancer cells in vitro. H460 and H1299 cells were treatment with 125 μM and 150 μM resveratrol for 24 hours and the mobility rates were observed via wound healing assays.

Conclusion

- Resveratrol inhibits both H460 and H1299 cell proliferation and survival at concentration 125 and 150 μM.
- NR4A1 expression is inhibited by resveratrol in H460 and H1299 cells.
- Resveratrol induces apoptosis in H460 and H1299 cells by inhibiting expression of Bcl-2.
- Resveratrol induces phospho-AMPK but inhibited TXNDC5, Sestrin2 and IDH1 expression.
- Resveratrol inhibits mTOR signaling and it also indicated by the decrease of S6RP and 4EBP1 expression.
- Resveratrol decreases the migration ability of H460 and H1299.
- All of these responses have previously been observed after knockdown of a NR4A1 antagonists.

Acknowledgement

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