Natural Products in Upper Aerodigestive Cancer: Activation of PPARγ

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Introduction

Peroxisome proliferator-activated receptor (PPAR)

- A nuclear receptor which upregulates differentiation-associated genes
- Implicated in other important cell processes such as apoptosis, cell proliferation, and angiogenesis
- An interesting target for anti-cancer effects

Pioglitazone, a PPAR agonist

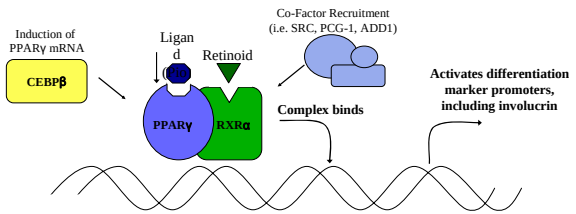
- Upregulates PPAR in cell culture and animal models
- Reduced leukoplakia lesions in a recently completed clinical trial at the U of M
- Costly for chemopreventive use

Natural Products

- Inexpensive, low toxicity
- Several implicated in chemoprevention and/or tested in ongoing chemopreventive studies at the U of M Masonic Cancer Center

Purpose of this study: To identify natural products which activate PPAR

Fig. 1: Activation of PPARγ and differentiation pathway.

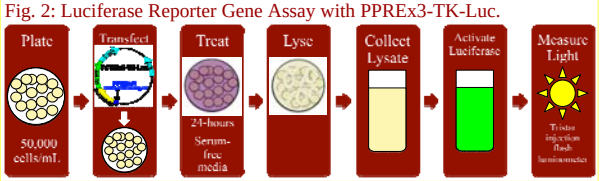


Materials & Methods

Cell Lines

- **HOK-16B**: HPV immortalized human oral keratinocytes from N.H. Park, UCLA
- **Beas 2B**: SV40 immortalized human bronchial epithelial cells from Reuben Lotan, MD Andersen Medical Center, Texas
- **CA 9-22**: Oral squamous carcinoma cells
- **NA**: Oral squamous carcinoma cells

Fig. 2: Luciferase Reporter Gene Assay with PPREx3-TK-Luc.



Luciferase Reporter Gene Assays

PPARγ ligand-mediated DNA binding activity and involucrin upregulation by transcription factor binding to its promoter were analyzed by Tropix Dual Light luciferase reporter gene assay.

- **PPARγ Reporter Gene** is a thymidine kinase luciferase containing reporter plasmid with a PPARγ response element (PPRE), PPREx3-TK-Luc, a kind gift from Dr. Ronald Evans (The Salk Institute, San Diego, CA).
- **Involucrin Reporter Gene** contains a 3.7 kb segment of the involucrin gene in a luciferase reporter plasmid, a kind gift from Dr. Daniel Bikle (UCSF).

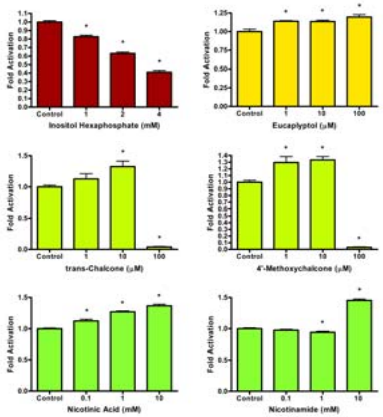
Co-transfection with a β-galactosidase reporter plasmid accounted for tranfection efficiency as an internal standard. 9 replicates were measured per data point.

MTT Assay

Cellular proliferation was evaluated via MTT assay. Cells were plated at $5X10^3$ cells/well in 96 well plates and treated the following day in RPMI serum free media. To assay, thiazolyl blue tetrazolium bromide (MTT) was added to the wells and incubated at 37°C for 4hrs. Mitochondrial dehydrogenases of live cells convert MTT to a water insoluble purple formazan, which was then solubilized with 100μL of dimethyl sulfoxide/isopropyl alcohol in a 1:1 mixture. 6 replicates were measured @ 560 nm per data point.

Results

Fig. 3: Effects of natural products on PPARγ activity are variable. PPARγ activity was measured for various natural products. Activities in CA 9-22 cells are shown (activities in multiple cell lines shown for resveratrol). Products which increased PPARγ activities by 100% or more were considered worth pursuing further. All natural products were obtained from Sigma-Aldrich. * = P value of < 0.05



Compound Name	Natural Source	PPARγ Activation
Inositol Hexaphosphoric Acid Sodium Salt	Corn and Soy Beans	-20%
Eucalyptol	Eucalyptus	+20%
trans-Chalcone	Derived from Natural Chalcones Found in Plants	+30%
4'-Methoxychalcone		+30%
Nicotinic Acid	Alfalfa, Corn, Legumes, Meats, Poultry, Fish	+40%
Nicotinamide		+50%
Resveratrol	Red Grapes	+100 to 180%

Fig. 4: Resveratrol activates PPARγ DNA binding activity in CA 9-22 cells. Luciferase reporter gene assays were performed with the PPREx3-TK-Luc plasmid. Pioglitazone (Pio) was used as a positive control. Cells treated with resveratrol (Res) showed increased luciferase expression in several experiments. Resveratrol-induced luciferase expression was reduced by a PPARγ ligand-binding inhibitor, T0070907 (Inh). * = P value of < 0.0001

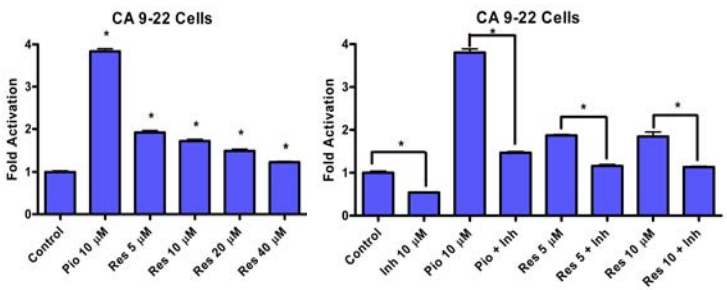


Fig. 5: Resveratrol increases PPARγ activity in multiple cell lines. Luciferase reporter gene assays were performed with the PPREx3-TK-Luc plasmid. Pioglitazone (Pio) was used as a positive control. Cells treated with resveratrol (Res) showed increased luciferase expression. * = P value of < 0.01

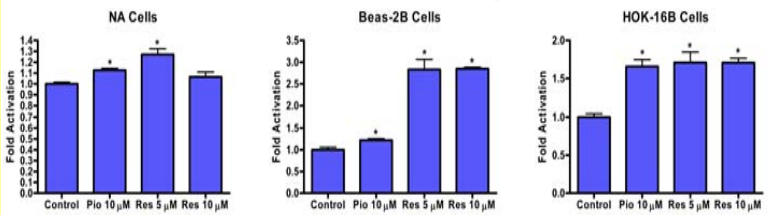


Fig. 5. Resveratrol increases involucrin expression. Luciferase assays were performed with the involucrin plasmid. Pioglitazone (Pio) was used as a positive control. Resveratrol (Res) increased luciferase expression in three cell lines. * = P value of < 0.005

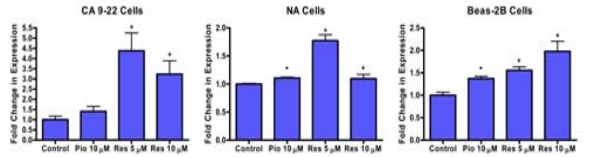
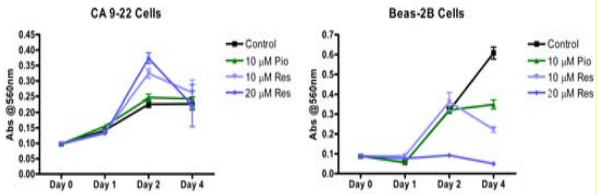


Fig. 6. Resveratrol has variable effects on cell proliferation. Cells were treated with pioglitazone (Pio) or resveratrol (Res). Plates were assayed using MTT at 0, 24, 48, and 96 hours from treatment.



Conclusions

Resveratrol is a promising chemopreventive agent in upper aerodigestive cancer.

- Increases PPARγ DNA binding activity in cancer cells
- Increases PPARγ activity in normal aerodigestive cells
- Increases involucrin expression
- May be less toxic than synthetic PPARγ ligands

Results indicate that resveratrol upregulates differentiation via ligand-mediated PPARγ DNA binding activity.

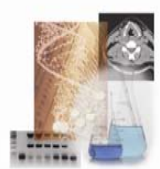
- Further investigation will target other differentiation markers and PPARγ pathways.
- This data may be applied to a clinical trial of precancerous lesions.

As an effective and inexpensive chemopreventive agent, resveratrol may be an important agent in the fight against cancer worldwide.

Acknowledgements

This work was supported by the Lions Multiple District 5M Hearing Foundation of Minnesota.

Special thanks to Dr. Lee Wattenberg's lab for support and donation of products.



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