

**P849**

**Resveratrol Protects Retinal Pigment Epithelial Cells From Acrolein-Induced Oxidative Damage and Cigarette Smoke-Induced Choroidal Neovascularization via Increase in Mitochondrial Bioenergetics**

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**Backgrounds:**

Resveratrol (RSV) alleviates the oxidative damage on human adult retinal pigment epithelial (ARPE) cell. Similar protection was observed in the UV irradiation damage model of human RPE cells. The purpose of this study was to study the role of mitochondrial bioenergetics in the cytoprotective effect of RSV. Its role in protection against the adverse effect of cigarette smoke (CS) in experimental choroidal neovascularization (CNV) was also examined.

**Materials and Methods:**

Cultured ARPE-19 cells were treated with acrolein alone or with additional of RSV. Temporal changes in cell viability, expression of the antioxidant protein, and mitochondrial bioenergetics were evaluated. In animal study, CNV lesions were created in Brown Norway rats by laser-induced photocoagulation. Effects of CS alone or with additional treatment of RSV on CNV lesion were quantified by fluorescein isothiocyanate-dextran labeling.

**Results:**

In ARPE-19 cells, RSV reduced acrolein-induced cell death. This was accompanied by reversal of acrolein-induced superoxide dismutase expression and the increase in mitochondrial bioenergetics, including basal respiratory rate, ATP turnover, and maximal mitochondrial capacity. In animal experiments, we found that CS-induced CNV following laser injury was appreciably prevented in rats subjected to peripheral infusion of RSV.

**Conclusion:**

Our results indicated that RSV, a major polyphenol found in red wine, exerts protection against acrolein-induced cytotoxicity in human ARPE-19 cells via increase in the mitochondrial bioenergetics. In addition, the antioxidant effect of RSV may contribute to the protection against the laser-induced CNV in animals exposed to CS. Therefore, RSV might be beneficial for treatment of acrolein-induced or CS-evoked RPE degeneration.

**P850**

**MicroRNA-125b Regulates Poteasome Pthway in Oral Squamous Cell Carcinoma(OSCC)**

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**Backgrounds:**

Aberrant expression of miRNAs has been implicated in the pathogenesis of a variety of human cancers. Recently, proteasome pathway has gathered much attention in cancer studies as the main degradation system for oxidatively damaged proteins and also for several proteins in the cell cycle regulation and transcription, which are important for cancer initiation and progression. However, the interaction between miRNAs and proteasome system has not been well characterized.

**Materials and Methods:**

Microarray experiment: oral cancer tissue using Affymetrix Human Genome U133A 2.0 Array (Santa Clara, CA) and Illumina HumanHT-12 v4 Expression BeadChip.

Pathway analyses with the Gene Set Enrichment Analysis (GSEA) algorithm: To identify pathways regulated in cancer tissue and OECM1 cell line (knockdown miR-125b). Using gene sets from KEGG pathway, KEGG database, Two-layer regulatory network modeling: To construct the miR-125b-regulated network, we generated proteasome related genes and miR-125b to the target relationship as customized interactions in KEGG database (GeneGo, St Joseph, MI, USA). The uploaded dataset was used to construct a network consisting of the shortest paths.

miRNA and mRNA expression analysis: The expression levels of miRNAs were determined by qPCR. For quantitating mRNA expression, the total RNA was reverse transcribed using oligo-dT RT-PCR using ABI Prism 7900 Fast Real-Time PCR system (Foster City, CA, USA).

Western blot analysis: The protein bands were visualized by enhanced chemiluminescence detection. GAPDH for verification of loading control.

**Results:**

Previously, our laboratory simultaneously profiled the expression levels of 270 miRNAs using loop RT-PCR and the mRNA expression levels by microarray in 49 oral cancer tissues. We found that 11 miRNAs are up-regulated and 38 miRNAs down-regulated in oral cancer tissues. GSEA analysis revealed that several up-regulated genes are located in proteasome-related pathways. GSEA analysis revealed a strong inverse correlation with miR-125b. But most of these proteasome-related genes are not miR-125b direct target. Recent studies show that miRNAs may modulate the levels of multiple targets in a pathway by targeting critical transcription factors. Therefore, we used the shortest-path algorithm and the GeneGo MetaCore database to perform network modeling. The resulting model suggests that miR-125b has the ability to regulate multiple proteasome-related genes in a two-layer regulatory network by targeting multiple transcription factors (TFs).

**Conclusion:**

To test this hypothesis, we established the miR-125b overexpression and knockdown system in OECM1 cell (OECM1). Initial study confirmed that overexpression of miR-125b down-regulated those proteasome-related genes and TFs in mRNA and protein levels; knockdown miR-125b up-regulated the proteasome-related genes and TFs on mRNA and protein levels. Our study confirmed that miR-125b regulated multiple proteasome-related genes mainly through c-Myc. Further studies to confirm the interaction not only between miR-125b and c-Myc also between c-Myc and candidate proteasome-related genes are currently underway.

**P851**

**Early Administration of Probiotics Attenuates Bacterial-mediated Intestinal Inflammation and Smad 7 Pro-Inflammatory Cell Signaling**

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**Backgrounds:**

Probiotics such as *L. acidophilus* play an important role to microflora homeostasis in gastric-intestinal duct. To determine the cellular mechanisms by which early administration of probiotics and/or prebiotics in the presence of enteric pathogens altered host response via Smad 7 and NF- $\kappa$ B /I- $\kappa$ B $\alpha$  expression in the human intestinal epithelium in vitro.

**Materials and Methods:**

Intestinal epithelial cells (Caco-2 or T84 cells) were exposed to *Salmonella typhimurium*. Culture supernatants (medium) were collected for IL-8 cytokine detection at 1, 2, 3 hours post-pathogen exposure. The cell lysates were used to detect Smad7, NF- $\kappa$ B and I- $\kappa$ B $\alpha$  by Western Blot analysis. Furthermore, Caco-2 cells (or T84 cells) were pre-administered with probiotic (*L. acidophilus*) and/or prebiotic (inulin supplemented with oligofructose). Subsequently, the cells were infected with *S. typhimurium* for one hour. Post pathogen exposure, the culture supernatants were used for cytokine determination and cell lysates were used for determination of gene or protein expression with real-time PCR and western blot analysis, respectively.

**Results:**

Pathogens activated the NF- $\kappa$ B pathway within 30 min to 1 hour in T84 cells, while Smad 7 induction occurred within 1 hour in T84, Caco-2 cells. Smad 7 induction was attenuated by pre-treatment with probiotics, while *Salmonella* infection alone enhanced Smad 7 intracellular production in Caco-2 cells. Probiotic pre-treatment prevented I- $\kappa$ B $\alpha$  degradation and the activation of the NF- $\kappa$ B pathway, while pre-treatment with prebiotics or *Salmonella* alone enhanced I- $\kappa$ B $\alpha$  degradation and activation of NF- $\kappa$ B pathway in Caco-2 cells. Additionally, there was approximately a 2-fold reduction in total IL-8 production in Caco-2 cells pre-treated with probiotics prior to *Salmonella* inoculation 24 hours post infection.

**Conclusion:**

The NF- $\kappa$ B pathways were activated early in the inflammatory response to enteric pathogens. However, Smad 7 was activated much later in the inflammatory response to enteric pathogens. Smad 7 and NF- $\kappa$ B induction confer to pro-inflammatory cytokine secretion (IL-8). Pro-inflammatory cytokines enhanced Smad 7 accumulation within the cell. Furthermore, probiotics attenuated Smad 7 to induce I- $\kappa$ B $\alpha$  expression while infection in human epithelial cells.

**P852**

**Resveratrol enhances chemosensitivity in mouse melanoma model through connexin 43 upregulation.**

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**Backgrounds :**

Gap junctions mediate cell communication by allowing the passage of molecules from one cell to another. Gap junctions are formed by connexons, called connexons, each made of six connexin (Cx) proteins. Cx43 is ubiquitous and reduced in a variety of tumor cells. Cx43 may influence the response of tumor cells to treatments by facilitating the passage of antitumor drugs or death signals between neighboring tumor cells. Although some studies indicate that resveratrol exhibits potential antitumor activities, the precise mechanisms of its beneficial effects are not fully understood. Therefore, it is warranted to elucidate the underlying mechanism of antitumor effects of combination therapy of resveratrol and cisplatin. The presence of functional gap junctions is highly relevant for the success of chemotherapy.

**Materials and Methods :**

The melanoma cancer cell lines were treated with resveratrol and cisplatin. Cell viability was determined by WST-1 assay and the protein expression was determined by Western blot analysis.

**Results :**

Following resveratrol treatment, dose-dependent upregulation of Cx43 expressions were observed. To study the pathway underlying these resveratrol-induced effects, we found that resveratrol induced a significant increase in mitogen-activated protein kinases (MAPK) signaling pathways.

**Conclusion :**

That resveratrol cotherapy leads to increase Cx43 gap junction communication and enhances the combination of cisplatin therapeutic effects.