Resveratrol protects Leydig cells from nicotine-induced oxidative damage through enhanced autophagy

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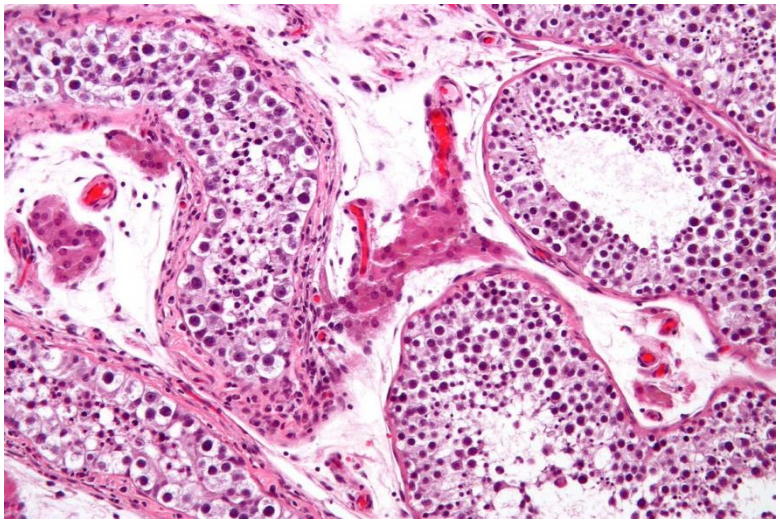

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Introduction

Leydig cells, also known as interstitial cells of Leydig, are found adjacent to the seminiferous tubules in the testicle.

Histological section through testicular parenchyma:

1 Lumen of seminiferous tubules

2 spermatids

3 spermatocytes

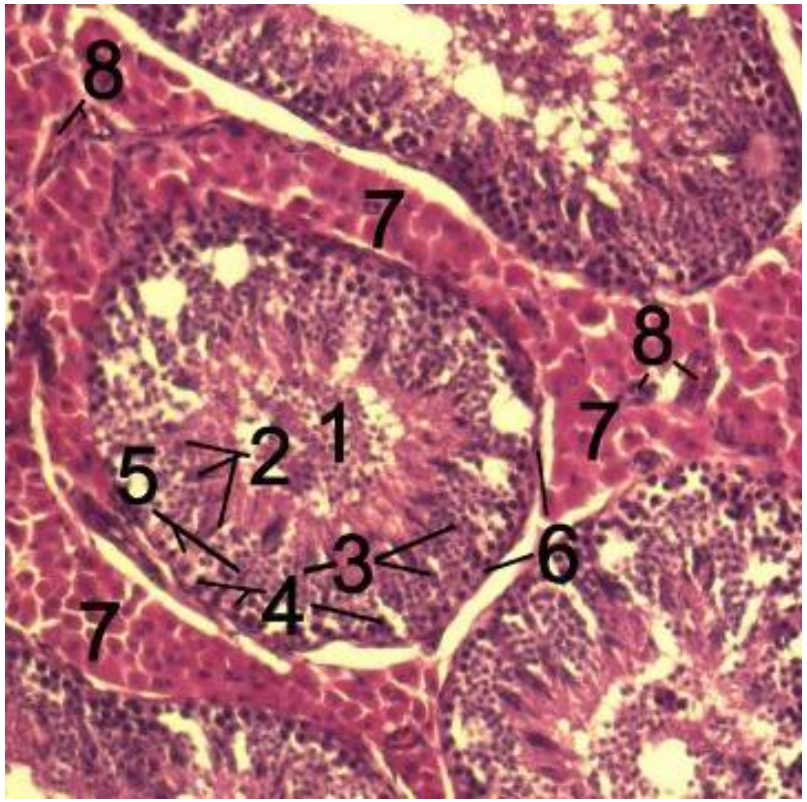
4 spermatogonia

5 Sertoli cell

6 Myofibroblasts

7 Leydig cells

8 capillaries



Introduction

- In recent years, the growing number of male infertility has aroused broad attention.
- Approximately 3% of all human couples are recognized as infertile, and about 50% of these infertility cases are related to male fertility factors.
- Male infertility can be caused by various factors:

❖ Testicular torsion or trauma

❖ Seminal tract infections

❖ Cryptorchidism

❖ Gonadal dysgenesis

❖ Reproductive channel obstruction

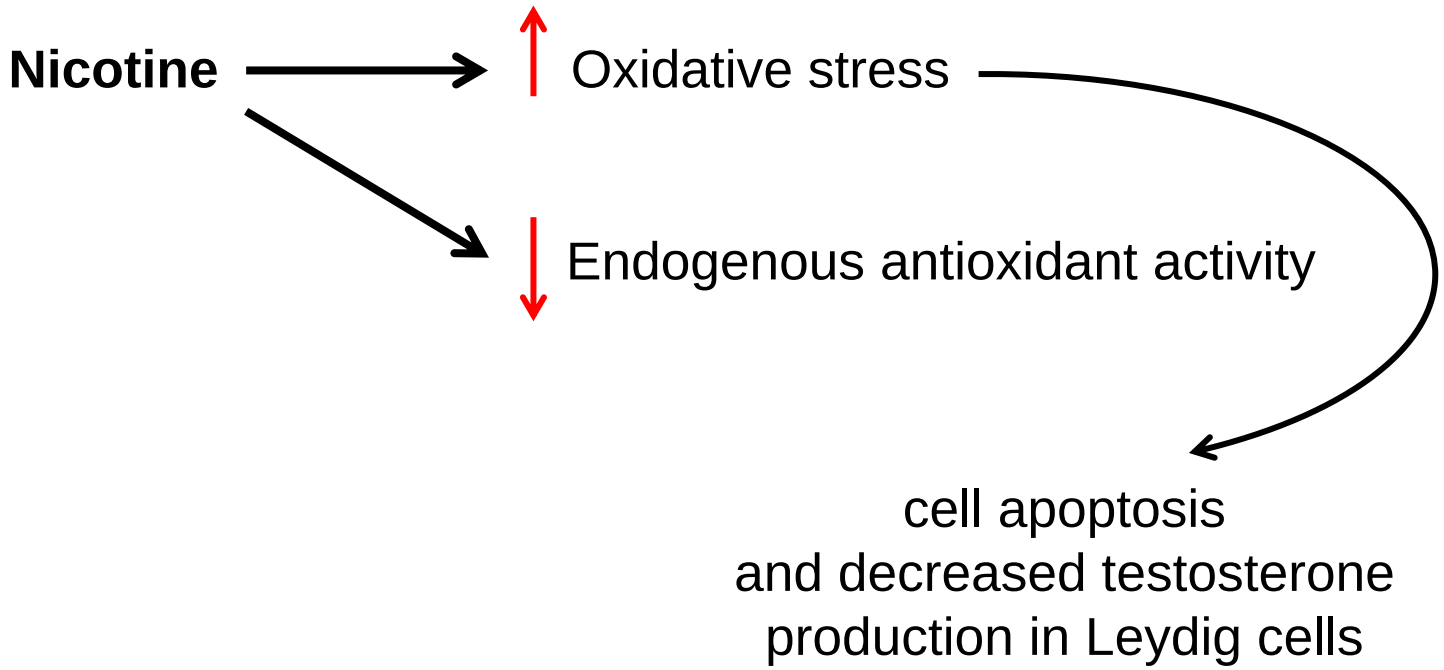
❖ Anti-sperm antibodies

❖ Genetic abnormalities and chromosomal aberrations

Introduction

- More than a billion cigarette smokers all over the world (man adults occupy a large proportion).
- Cigarette smoking is a well-known critical risk factor for various diseases including lung diseases, cardiovascular diseases, and cancers.
- Chronic exposure to water-pipe smoke (WPS) induces damaging effects to the reproductive system in male mice.
- Among the numerous alkaloids in tobacco, nicotine is the most active one and represents about 95% of the total alkaloid fraction.

Introduction



- oxidative stress is a primary factor in the pathogenesis of male infertility.

Antioxidant supplementation may be explored as a potential strategy to overcome reproductive disorders associated with male infertility, especially in cigarette smokers

Introduction

- Resveratrol (trans-3,5,4’-trihydroxystilbene), a type of natural phenol, and a phytoalexin.
- Sources of resveratrol in food include the skin of grapes, blueberries, raspberries, mulberries, peanuts and many other botanicals.
- It’s pharmacological properties include: anti-inflammatory, anti-oxidative, anti-thrombotic and anti-proliferative properties.
- Resveratrol reflected strong anti-oxidative capacities, particularly excellent scavenging activities.
- It has a capability to induce autophagy.

Introduction

What is Autophagy?

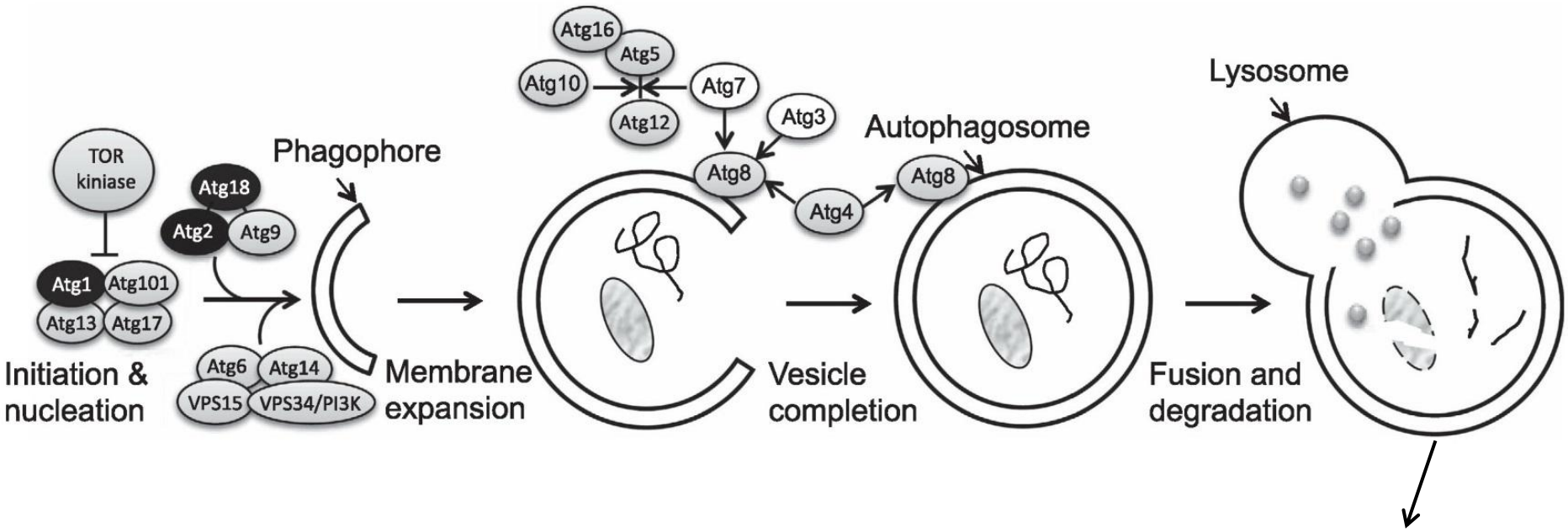
“Self-eating”

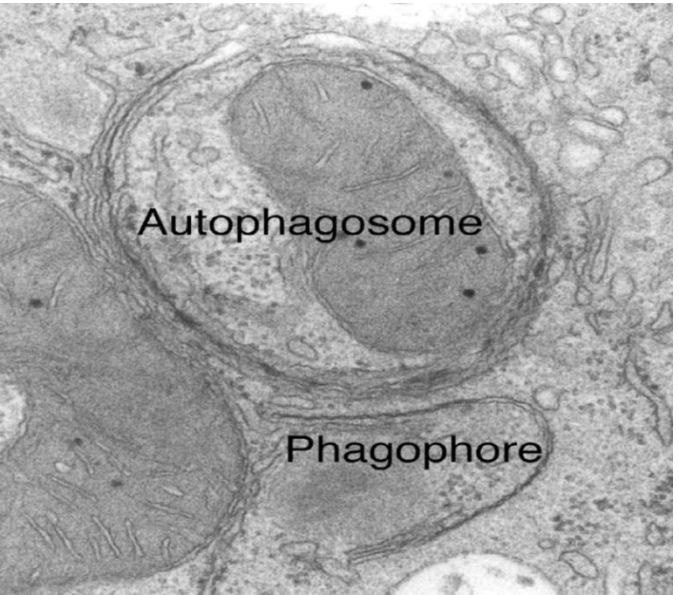
From the Greek words, *auto* "self" and *phagein* "to eat"

Catabolic process through which the cell recycles its own constituents.

Pathway that lead to the elimination of cytoplasmic components by delivering them into lysosomes.

Introduction: Mechanism of Autophagy

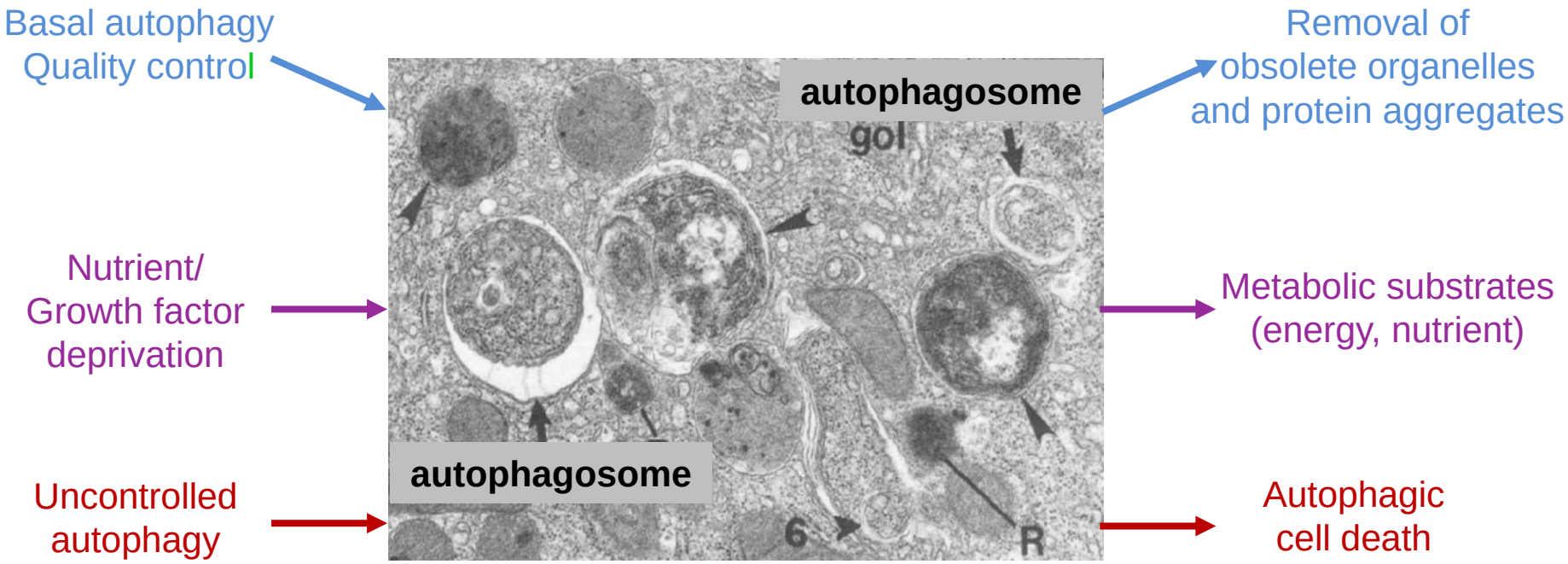




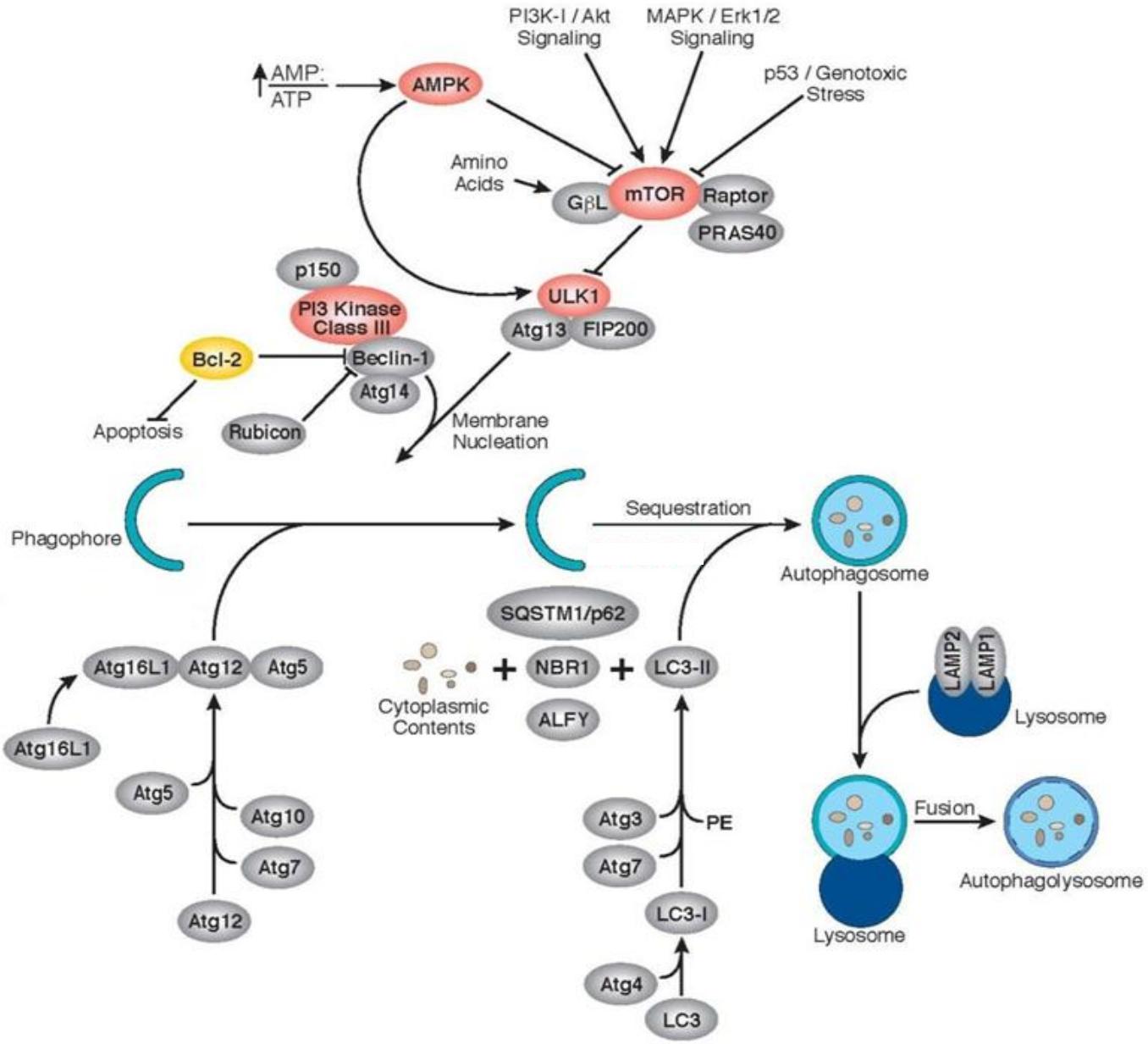
Denton et al., Immunol Cell Biol., 2015

Introduction: Multiple Functions of Autophagy

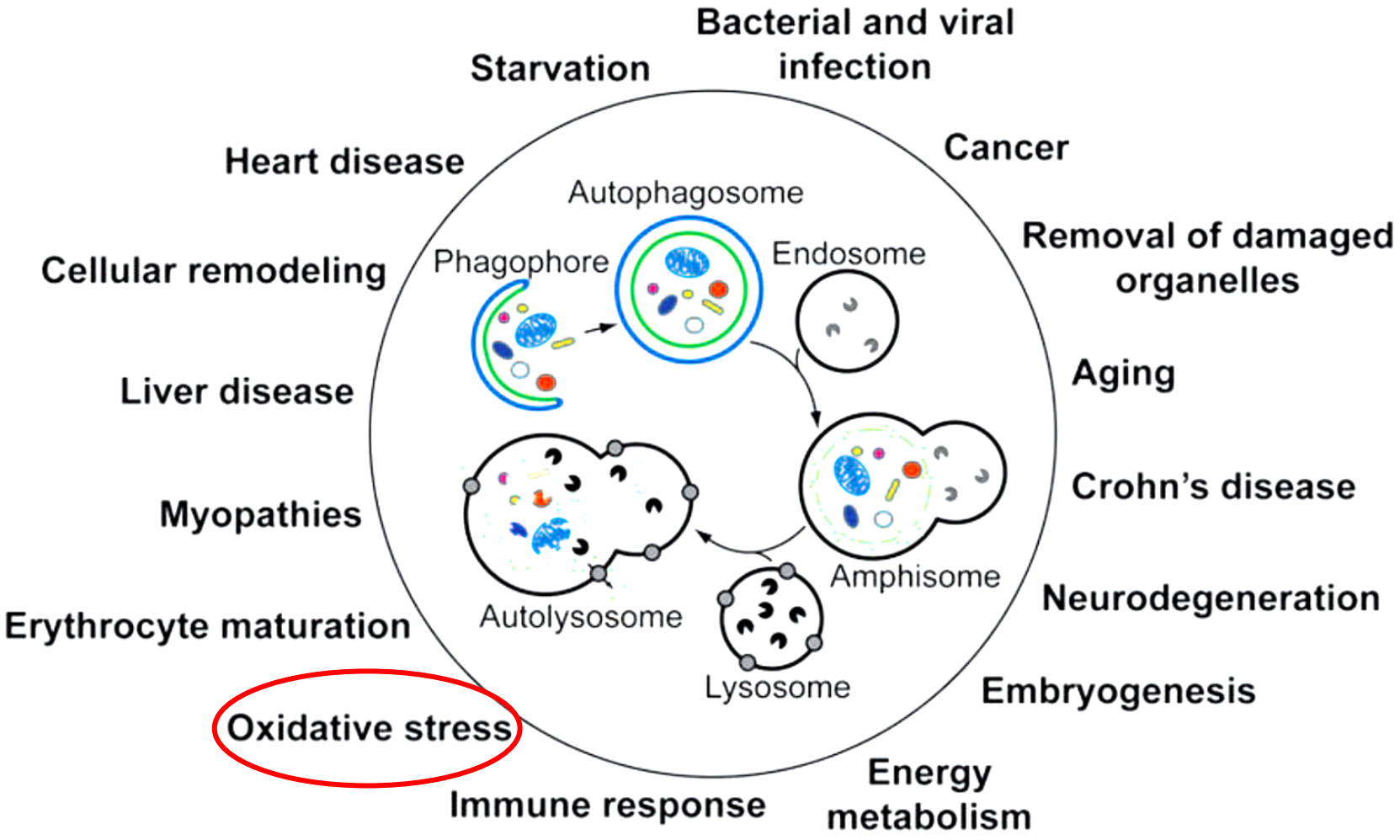
- Occurs in all eukaryotic cells
- Bulk degradative process that ends in lysosomes
- Degradation of intracellular components



Introduction: Autophagy Signalling Pathway

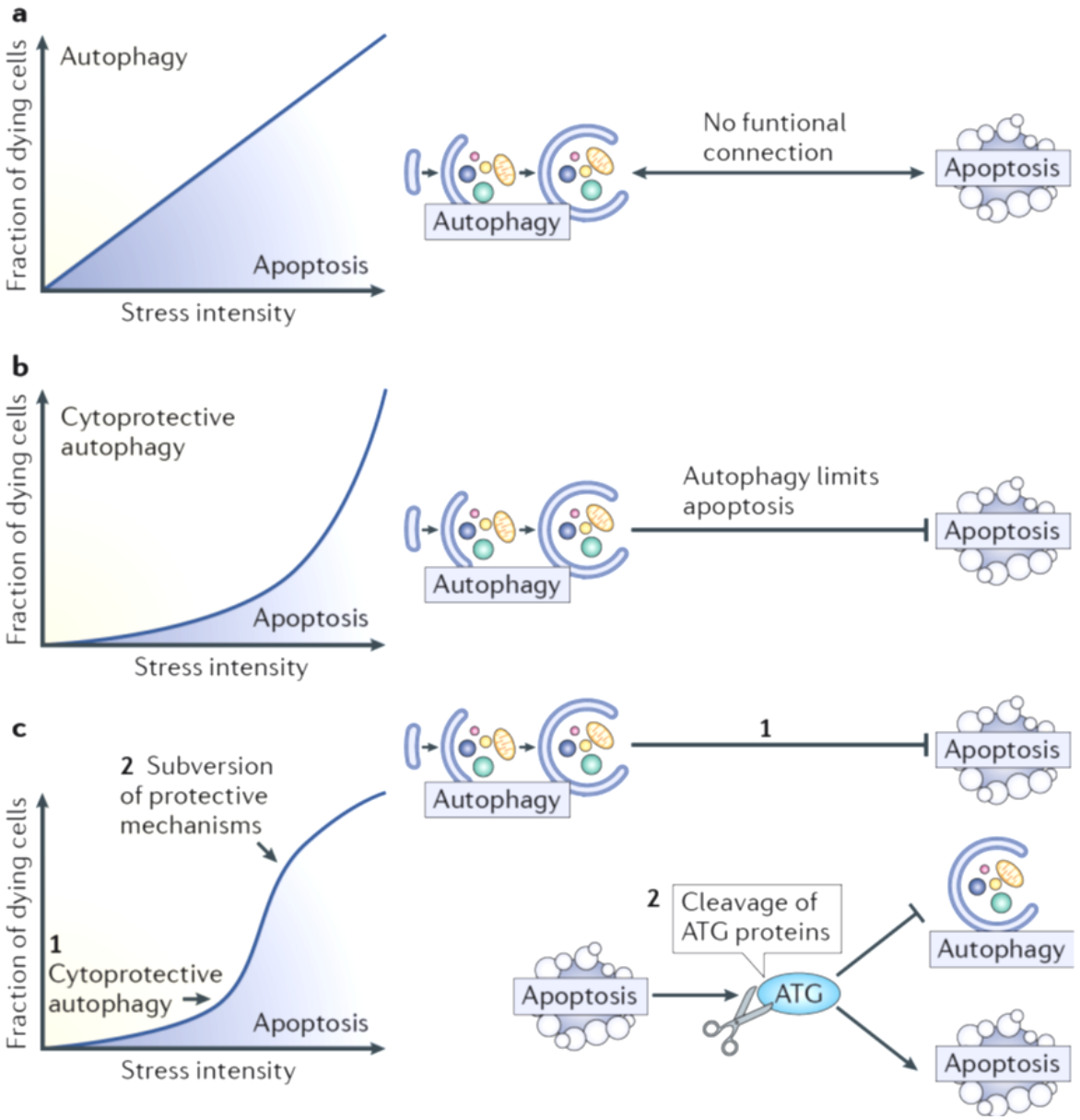


Introduction: Autophagy and Diseases



Klionsky DJ, Dev Cell. 2010

Introduction: Autophagy and Cell Death



Mariño et al., Nat Rev Mol Cell Biol 2014; 15: 81-94.

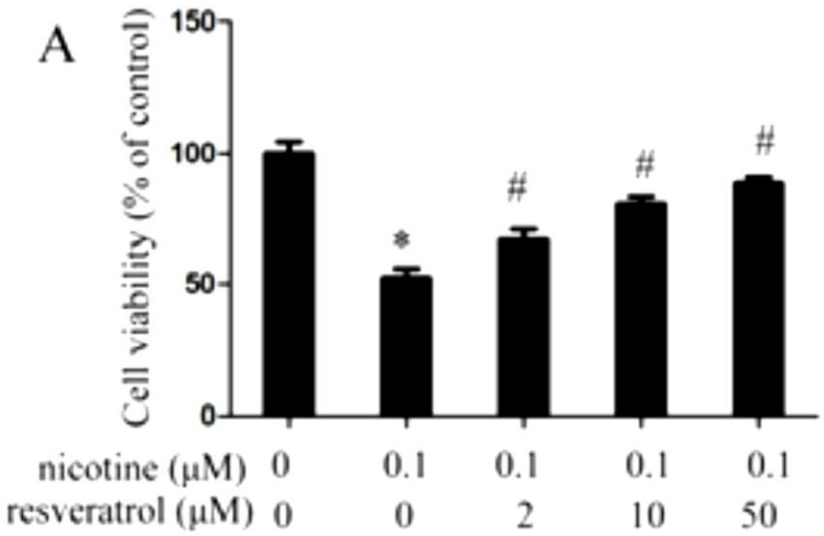
Aim of Study

- Investigate the effect of resveratrol on nicotine-induced oxidative damage in Leydig cells.
- Explore the underlying mechanism.

Methods

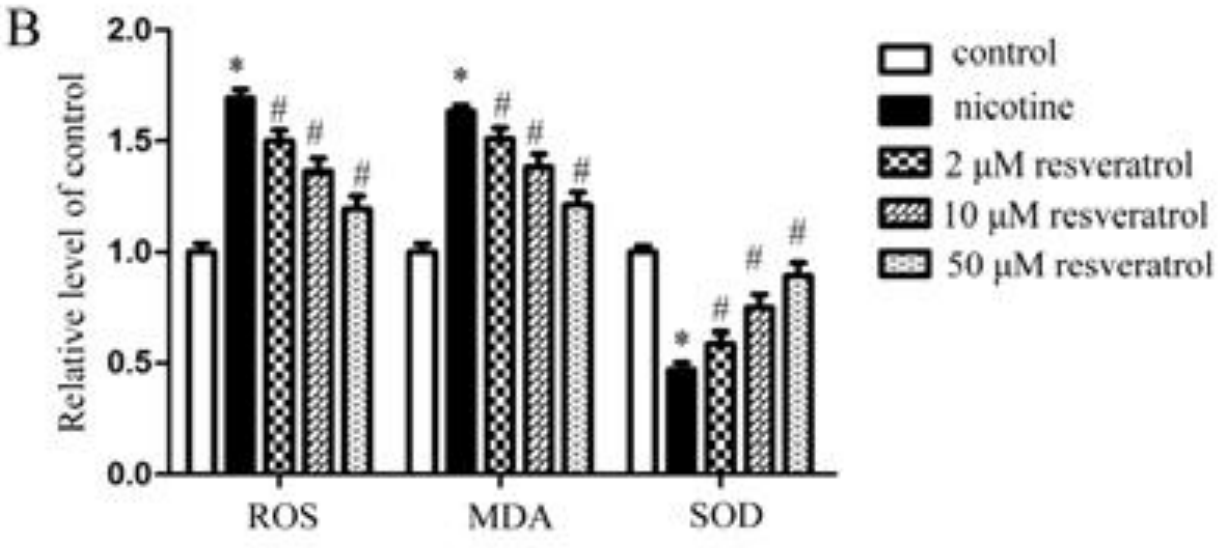
- **Cell culture:** The Leydig cell line TM3 (ATCC, USA) was derived from mouse Leydig cells, and selected as the cell model in this study.
- **MTT cytotoxicity assay**
- **ROS analysis:** General Oxidative Stress Indicator (H2DCFDA) was used to determine intracellular ROS accumulation in TM3 cells.
- **Measurement of MDA content and SOD activity in cell cultures:** by the Lipid Peroxidation MDA Assay Kit and Total Superoxide Dismutase Assay Kit.
- **TUNEL assay**
- **Autophagy detection (MDC and LysoTracker)**
- **Western blot assay**

Results: Resveratrol attenuated oxidative damage induced by nicotine



The cytoprotective effect of resveratrol were exerted in a concentration-dependent manner

Resveratrol suppressed nicotine-induced intracellular oxidant generation in Leydig cells



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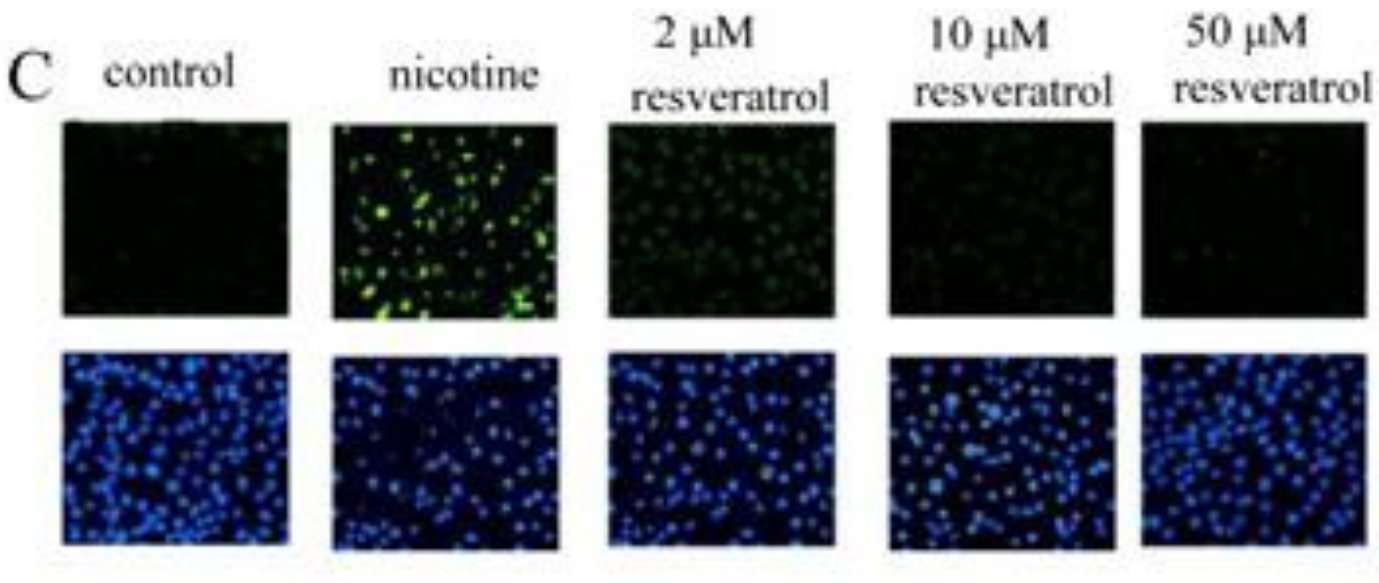
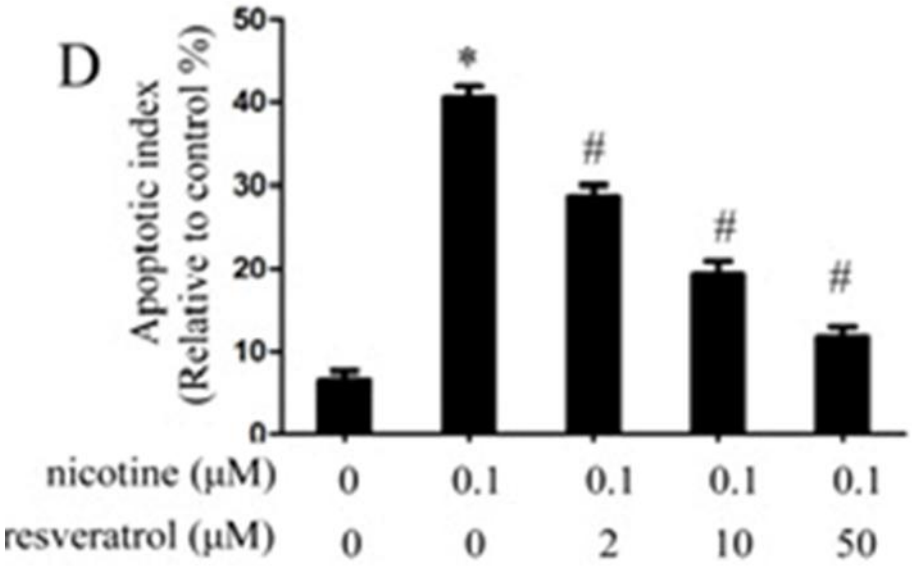


TUNEL assay

The anti-apoptotic effect of resveratrol were exerted in a concentration-dependent manner



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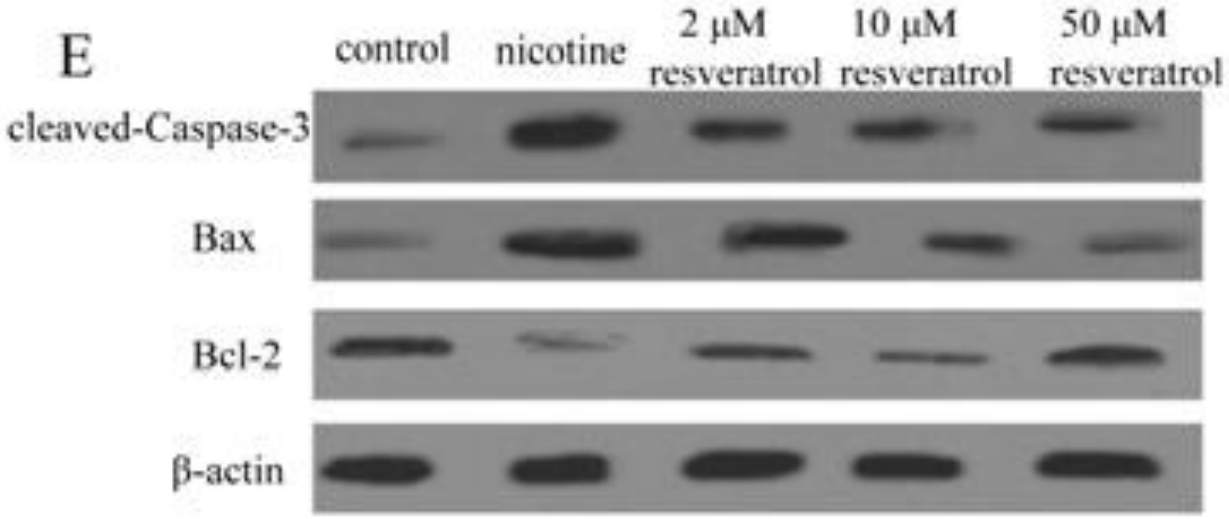
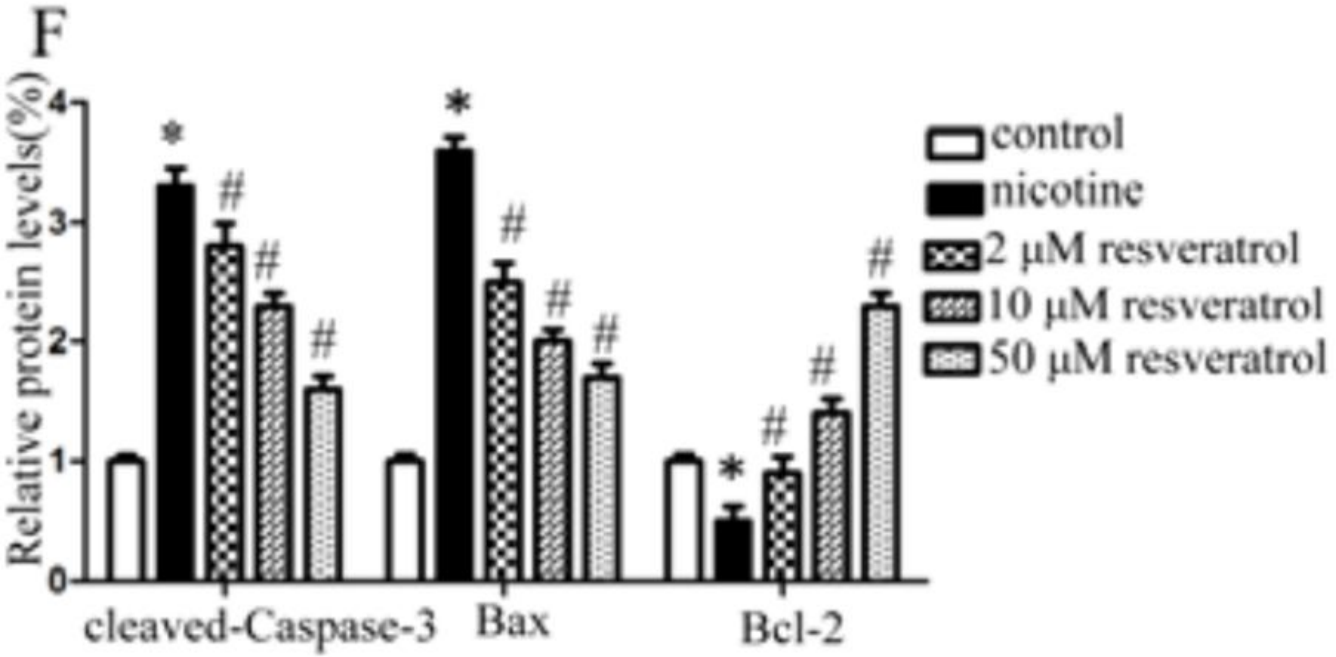
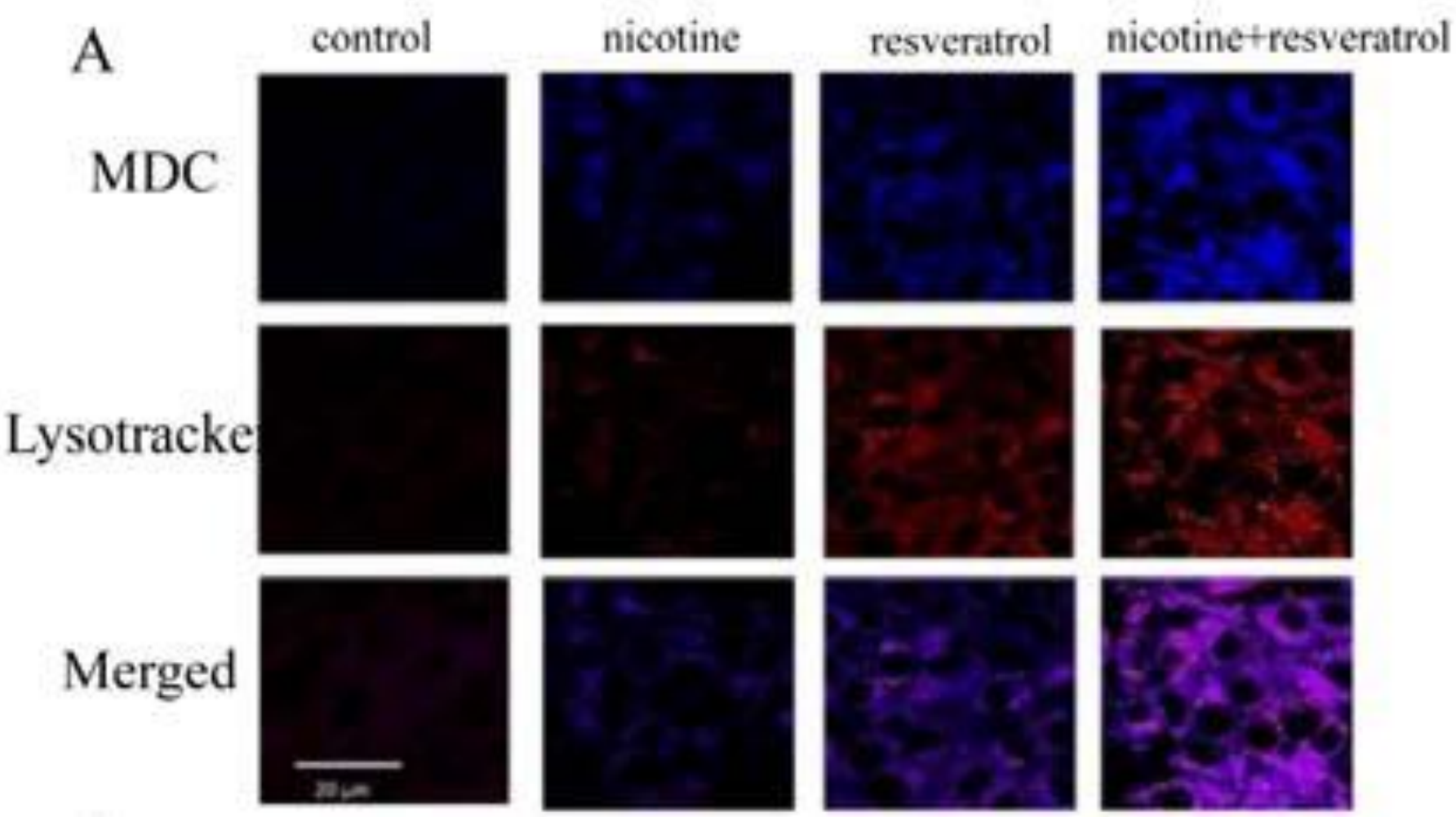




Results: Protective effect of resveratrol accompanied by autophagy upregulation



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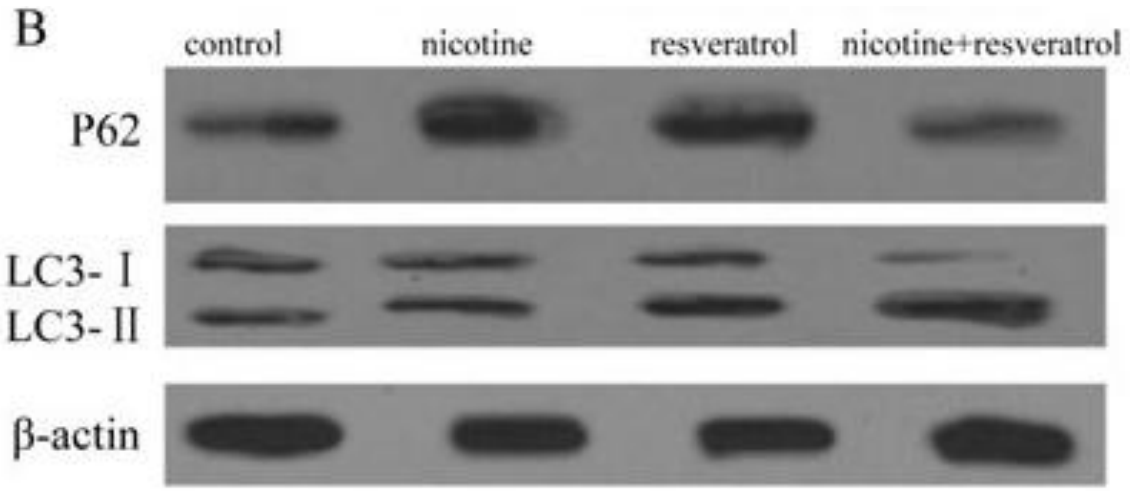

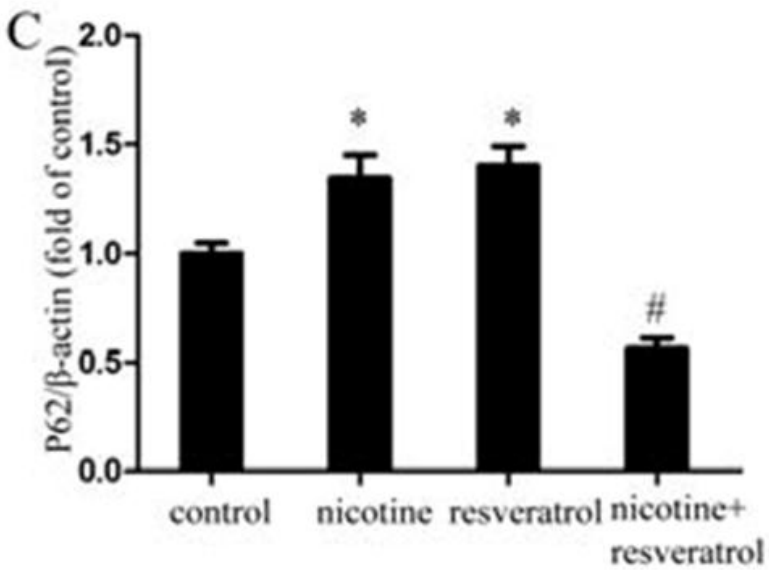

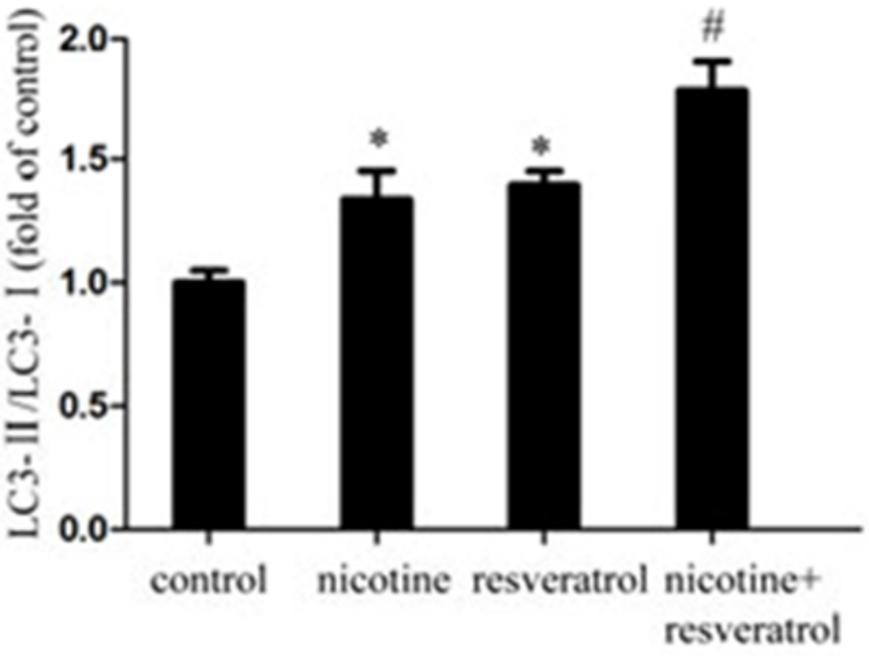

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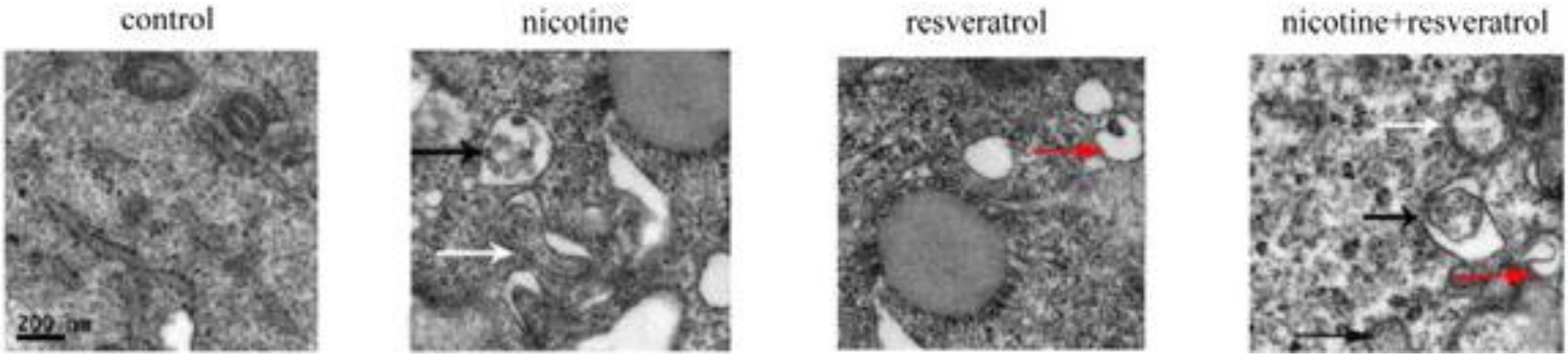
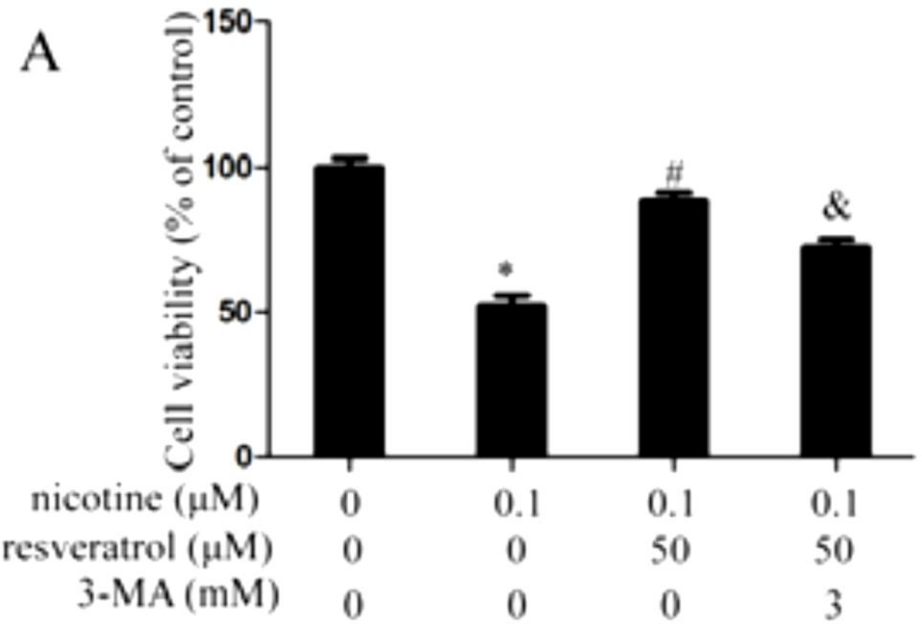


- Resveratrol not only increases autophagosomes formation but also manifests their subsequent fusion with lysosomes so as to degrade the internalized cargo.

Results: Autophagy inhibitors reduced protective effect of resveratrol



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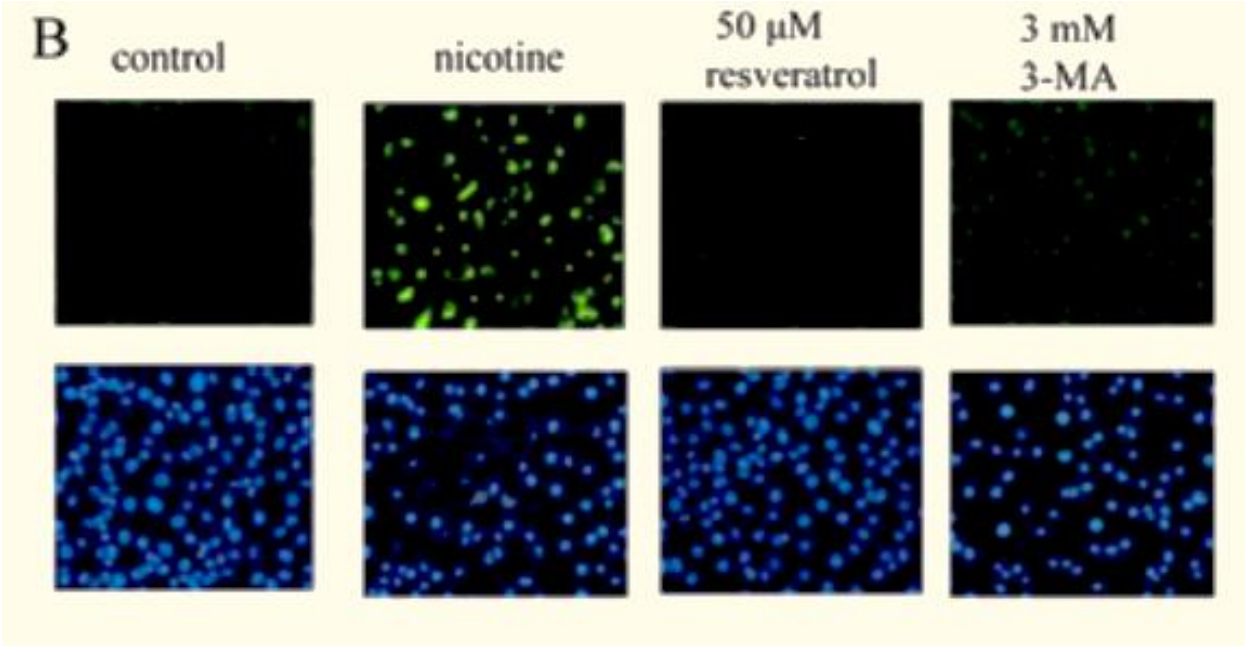

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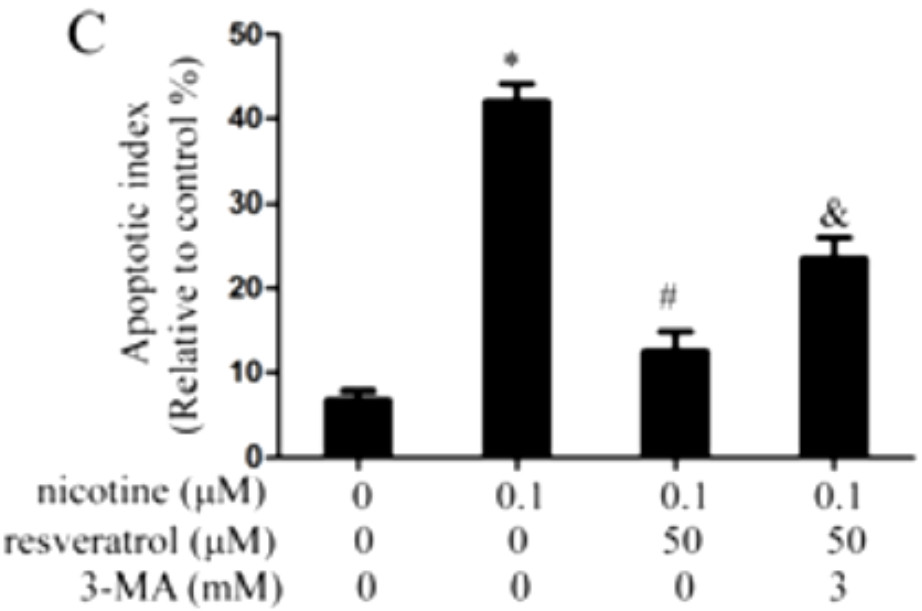
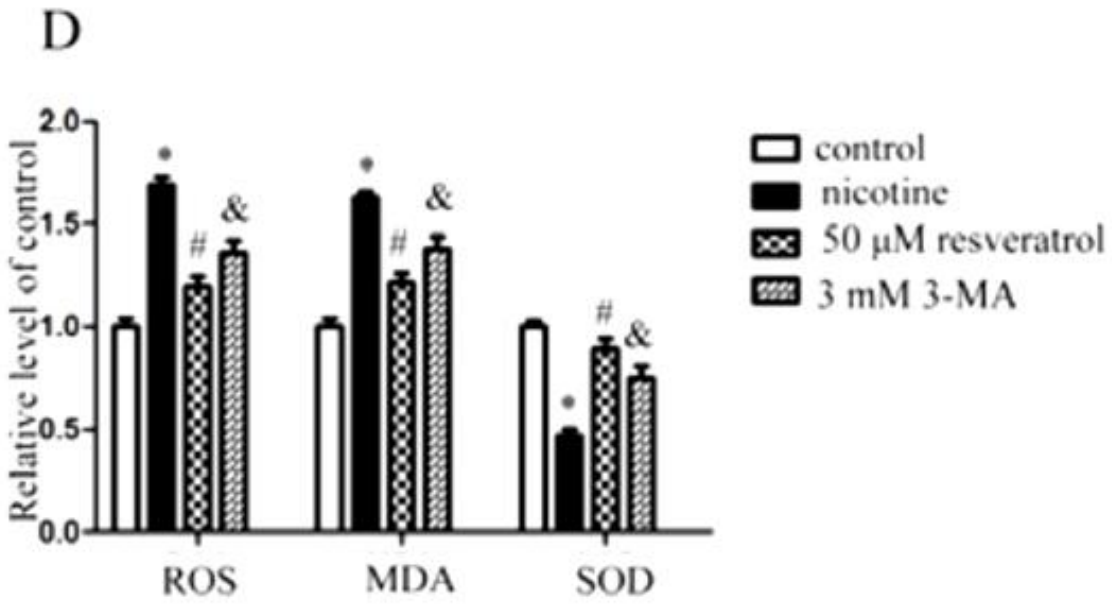
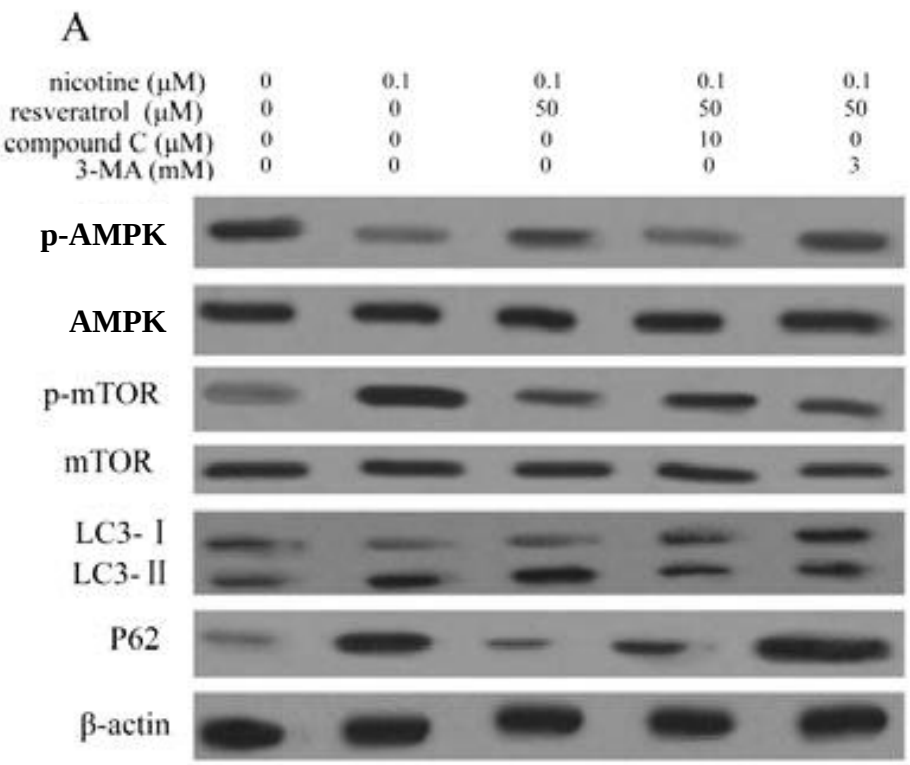
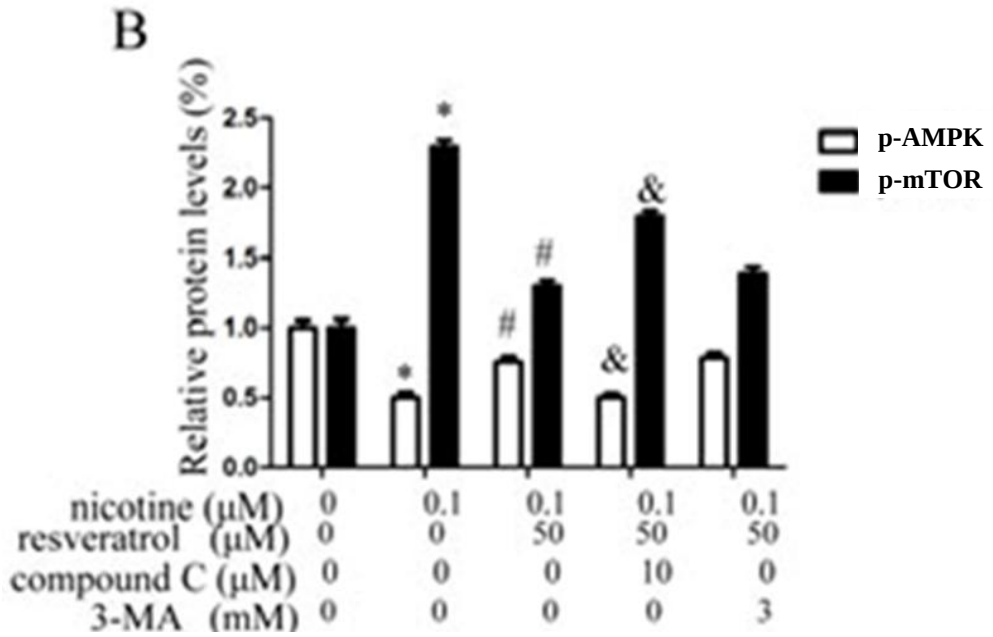




Results: Resveratrol instigates autophagy through AMPK/mTOR pathways





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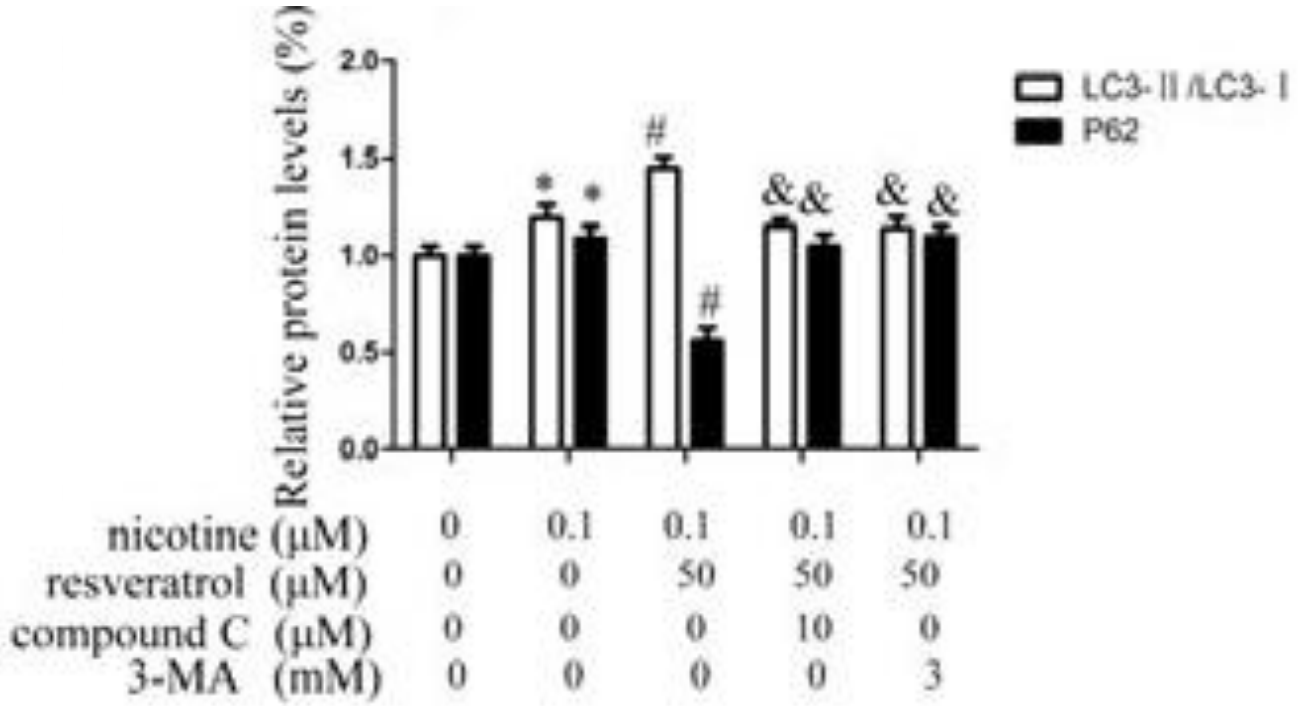

Resveratrol may exert it's autophagic mechanism through AMPK/mTOR pathways

Conclusions

- ✓ Resveratrol attenuates the detrimental effect of nicotine on the viability of Leydig cells and oxidative stress.
- ✓ The protective effects of resveratrol on the Leydig cells are mediated in part by autophagy.
- ✓ Resveratrol-induced autophagy is activated through the AMPK/mTOR pathway.

- ❖ **Taken together, the results of this study provide an evidence that resveratrol is a promising strategy to improve male infertility induced by oxidative stress.**
- ❖ **Resveratrol might be a choice to counteract Leydig cell injuries caused by nicotine exposure.**


Thank you for
attention