2019台灣胸腔暨重症加護醫學會年會

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2019 Taiwan Society of Pulmonary and Critical Care Medicine

Mesenchymal stem cell protects against intermittent hypoxia-induced apoptosis and cytotoxicity via ameliorating autophagy impairment in obstructive sleep apnea

間質幹細胞可藉由改善阻塞性睡眠呼吸中止症的細胞自噬不足來保護細胞免於間歇性缺氧誘發的細胞凋零和細胞毒性

陳永哲 醫師　高雄長庚醫院　胸腔內科

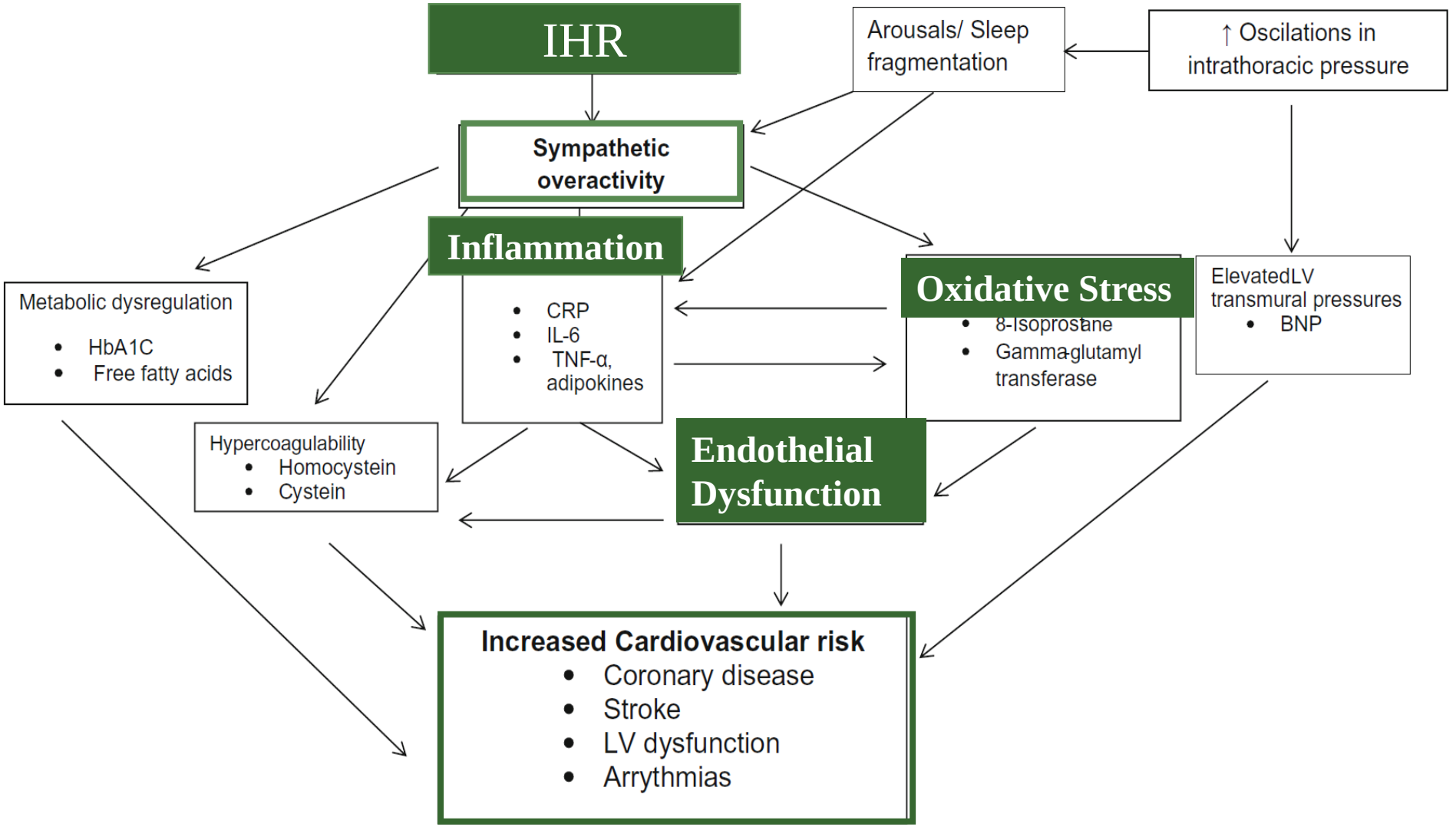
時間：2019/12/07 PM 15:50 ~ 16:00

地點：高雄展覽館3樓 301B

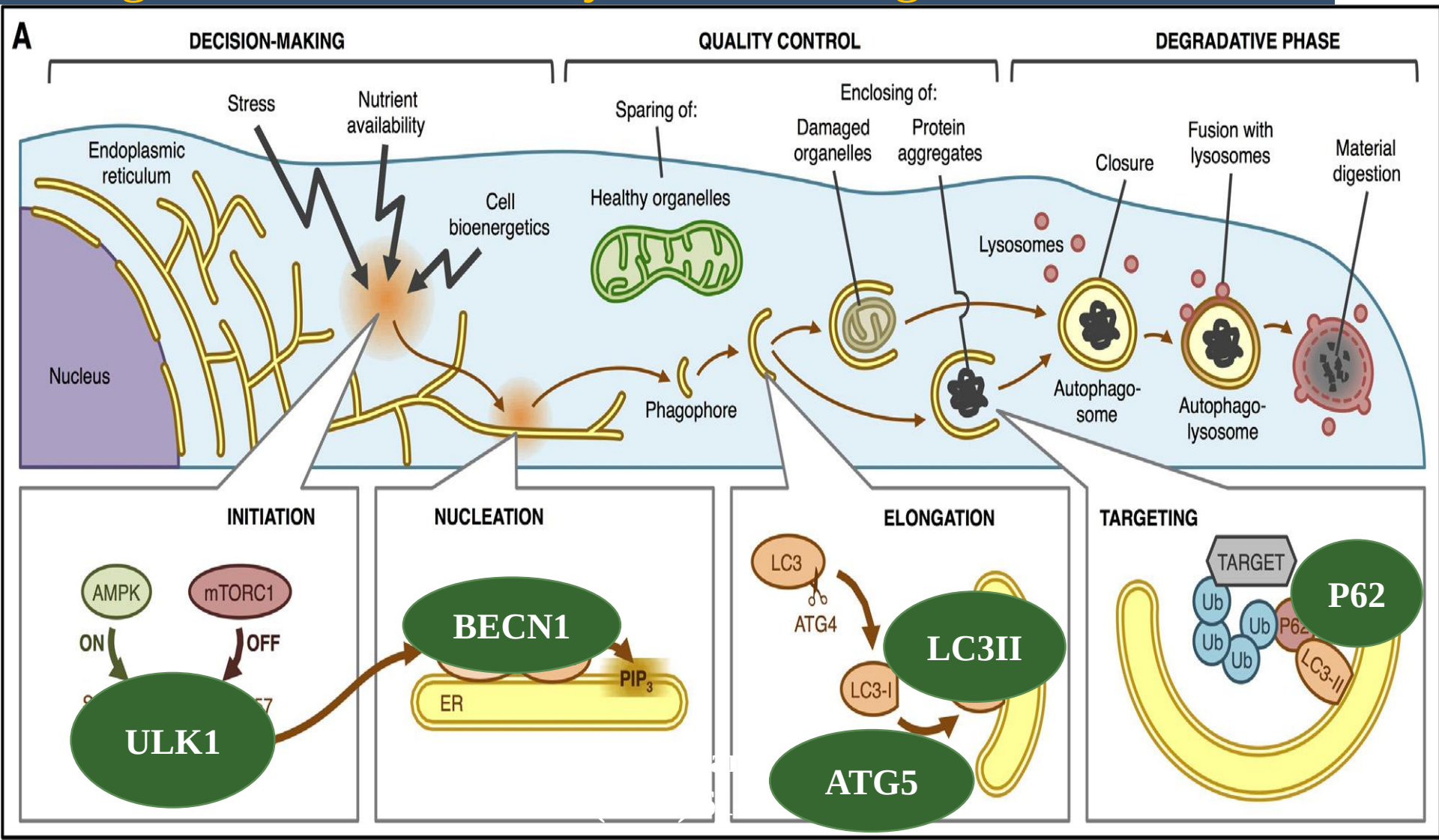
長庚 高雄

Intermittent Hypoxia with Re-Oxygenation (IHR)-induced chronic inflammation/oxidative stress is involved in the increase in CV risk in obstructive sleep apnea (OSA).

Sleep Breath (2015) 19:755–768

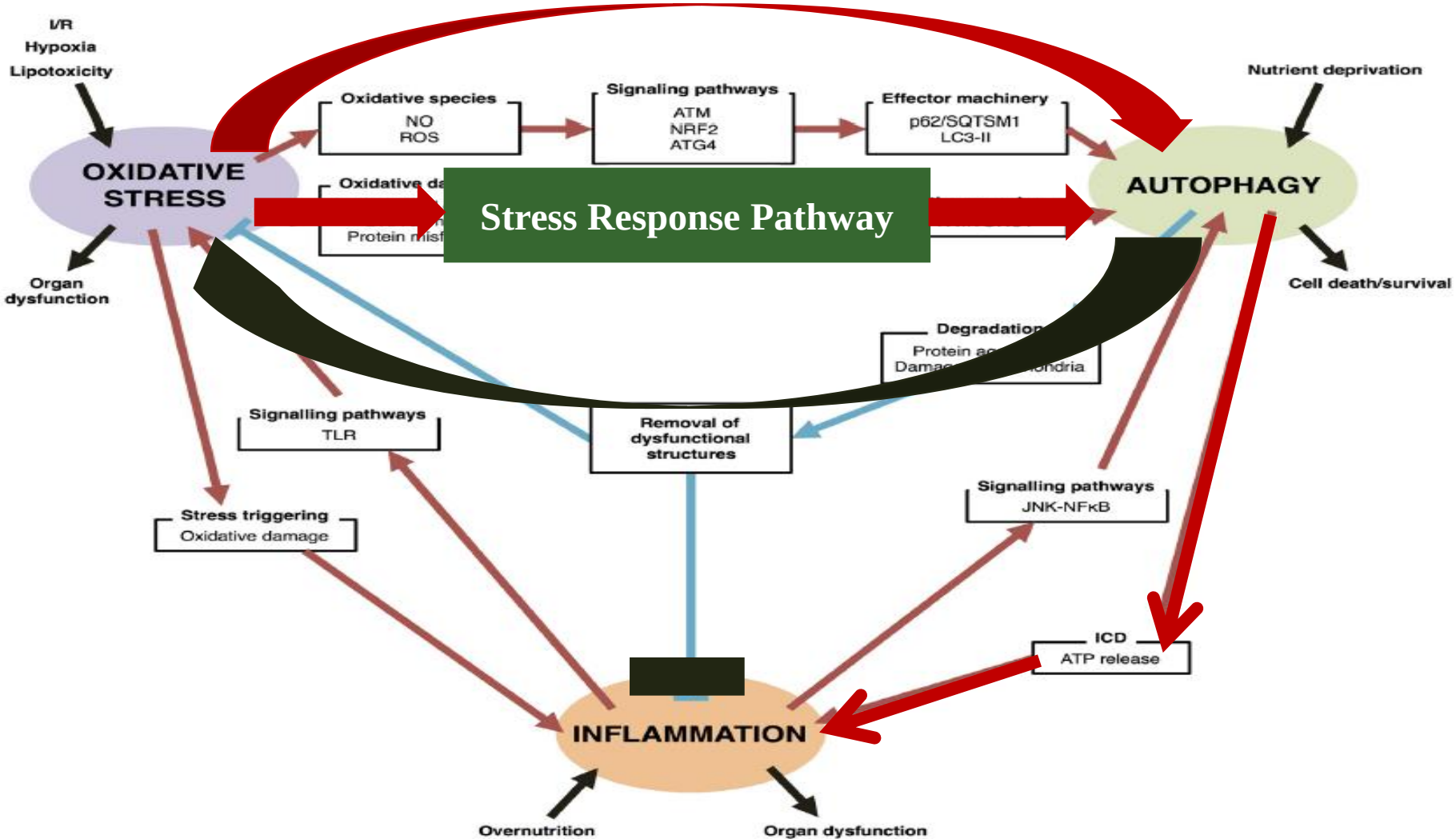


Autophagy is a self-degradative process to remove mis-folded/aggregated proteins and damaged organelles. Divided into five stages: Initiation, Nucleation, Elongation, Fusion with lysosomes, Degradation.



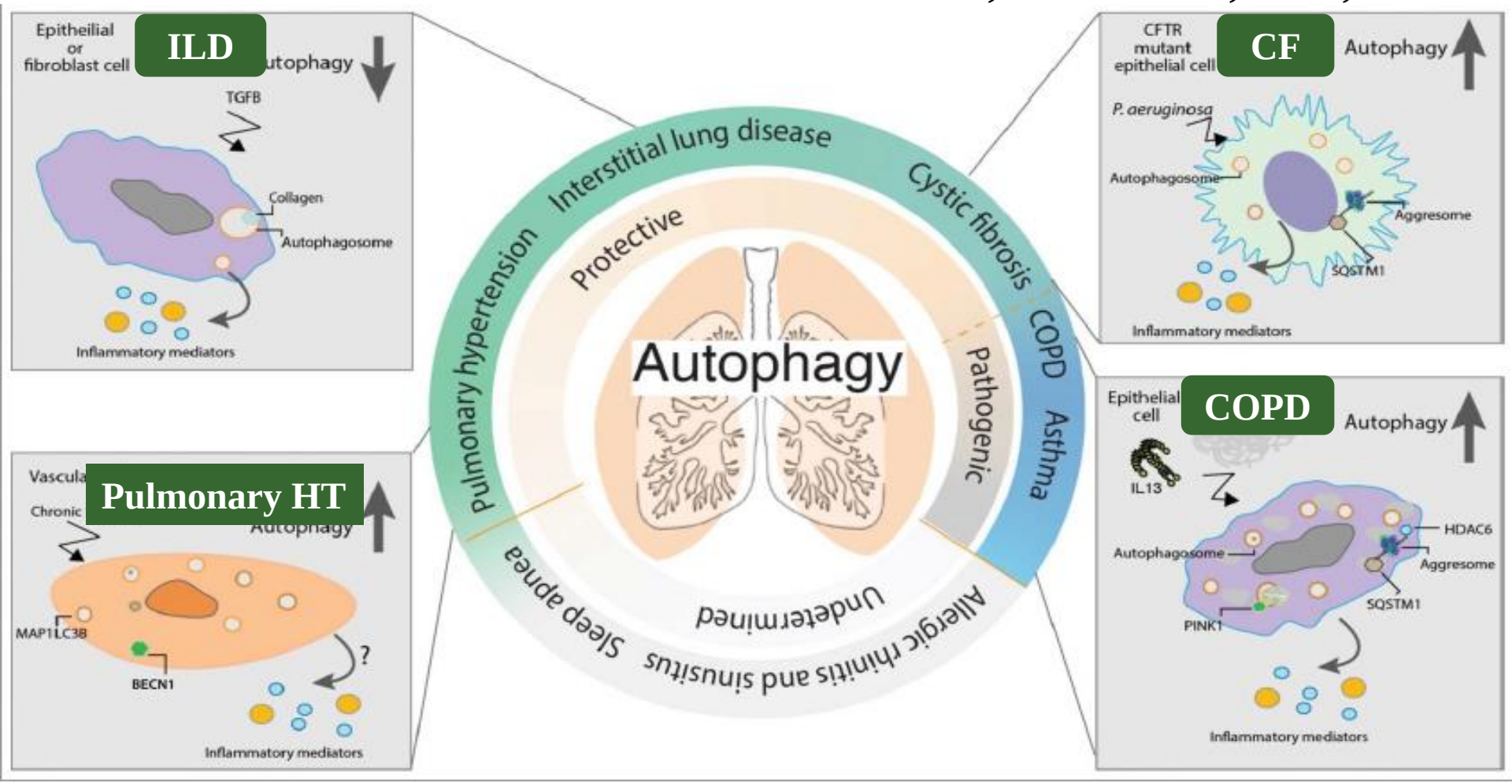
When oxidative stress is excessive, autophagy becomes dysregulated by activation of stress response pathways (sestrins, p53, ER stress), diminishing removal of dysfunctional structures and provokes chronic inflammation.

Free Radical Biology and Medicine 124 (2018) 61–78



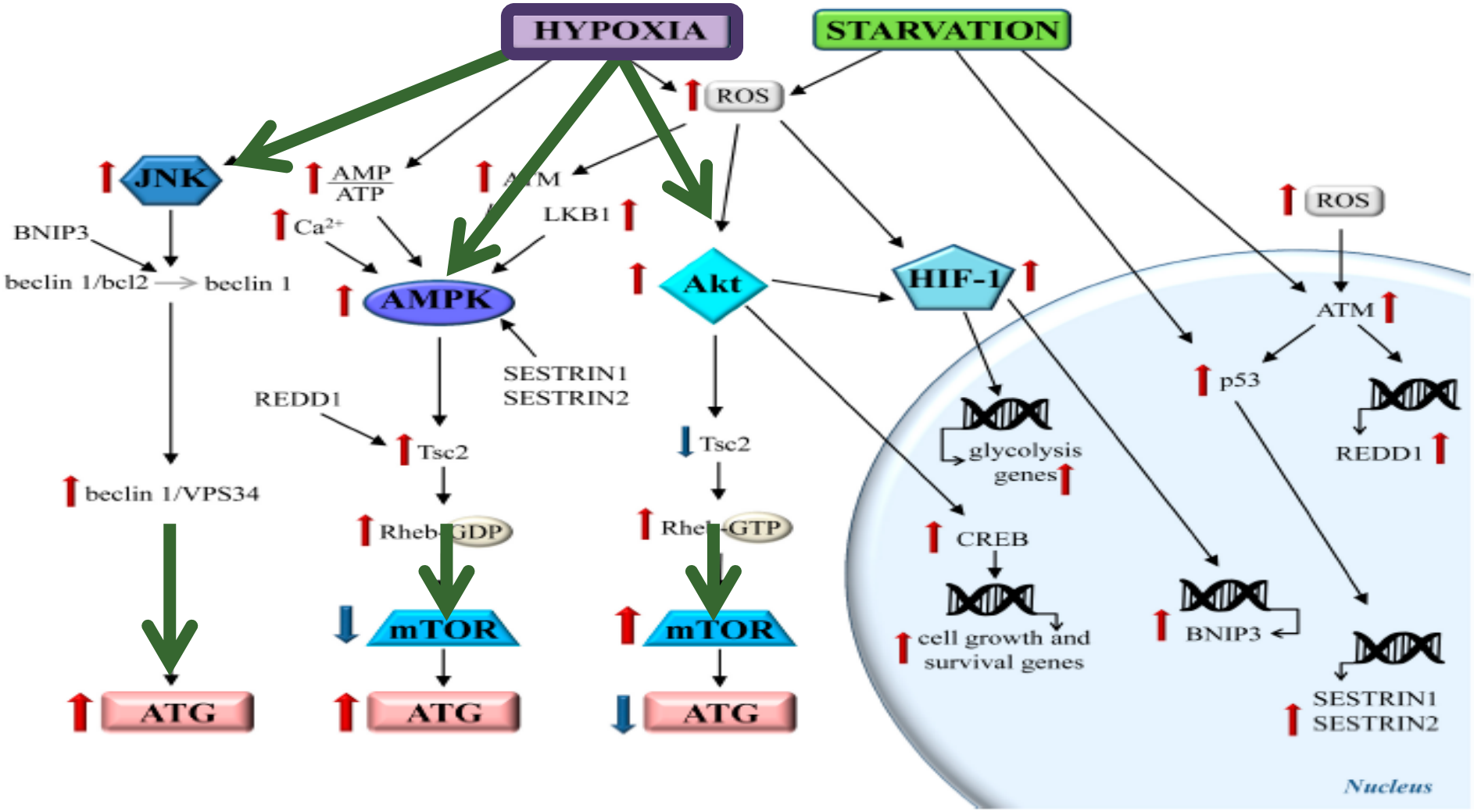
The up-regulation of autophagy may act as a pro-survival mechanism responsible for the clearance of damaged organelles. In the chronic lung diseases IPF, PH and CF, loss of autophagy or dysregulated autophagy drives lung inflammation and injury, suggesting autophagy plays a protective role.

AUTOPHAGY, 2018 VOL. 14, NO. 2, 221–232



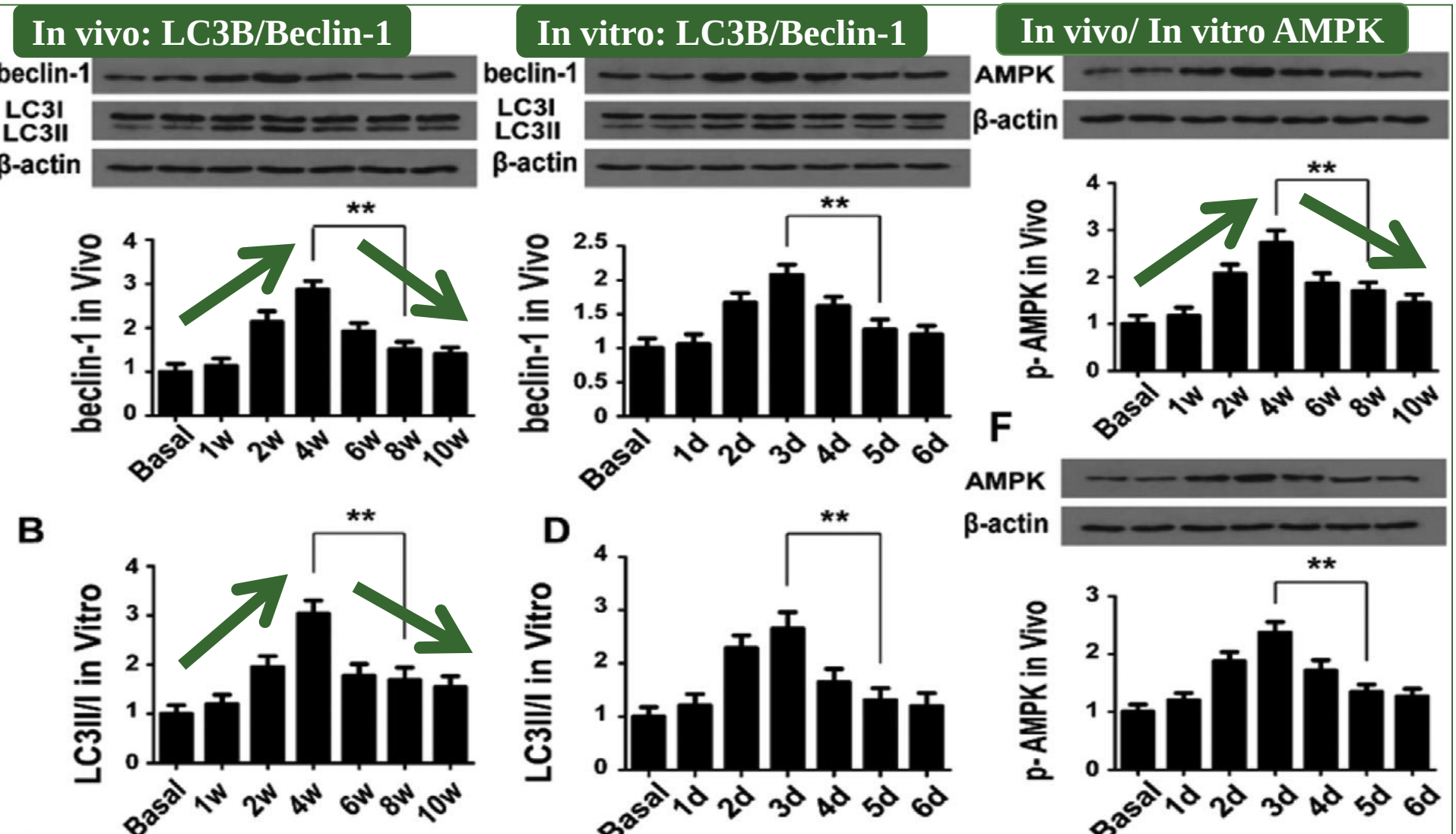
Hypoxia and starvation occurring during chronic brain hypoperfusion activate several cell pathways.
Induce autophagy through JNK/AMPK.
Inhibit autophagy through AKT.

Int. J. Mol. Sci. 2018, 19, 2756



Intermittent hypoxia with Re-oxygenation (IHR) induces cardiac hypertrophy by impairing autophagy through the adenosine 50-monophosphateactivated protein kinase (AMPK) pathway

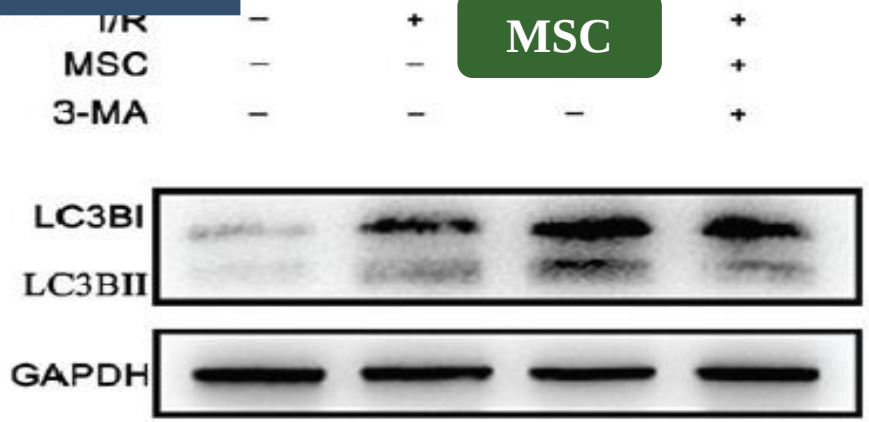
Archives of Biochemistry & Biophysics 606 (2016) 41-52



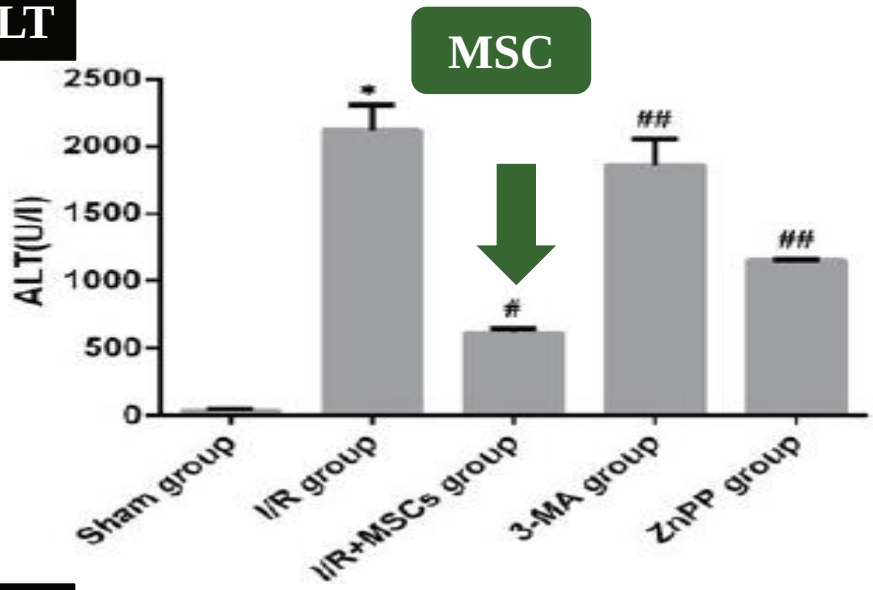
BM-mesenchymal stem cell (MSCs) protect against liver ischemia/reperfusion injury via HO-1 mediated autophagy.

MOLECULAR MEDICINE REPORTS 18: 2253-2262, 2018

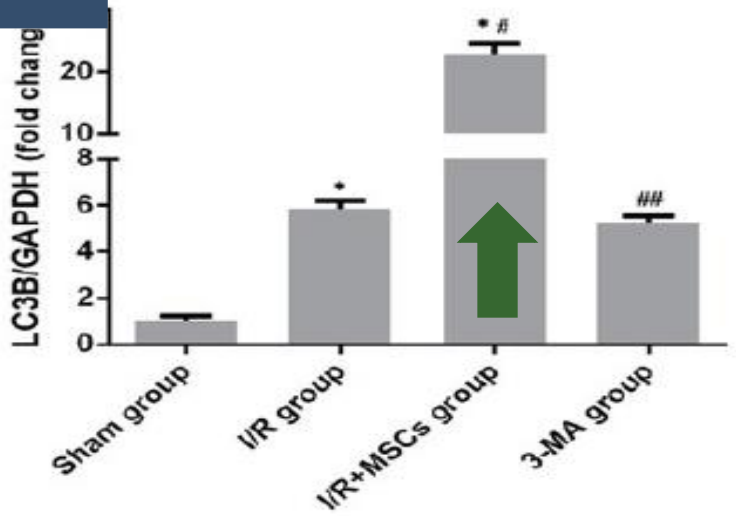
LC3B



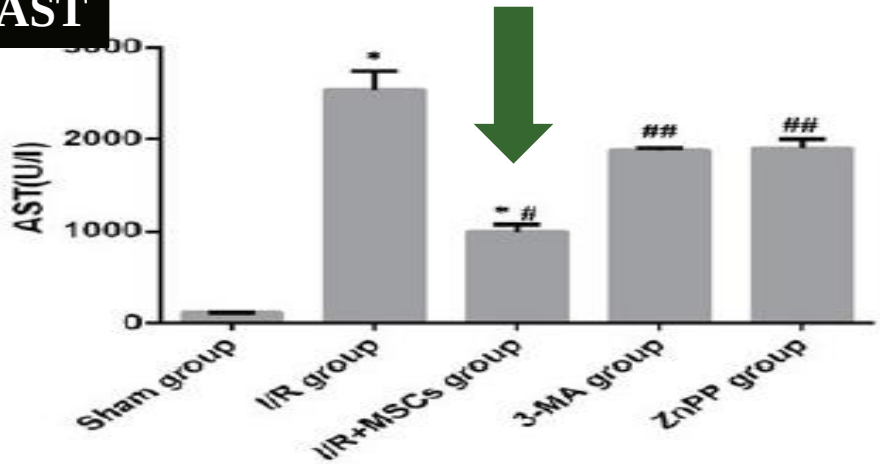
ALT



LC3B



AST



Hypothesis of the current study

(A)Gene and protein expression levels of autophagy related genes (ATG) in peripheral blood immune cells may be different between

(A) OSA patients and primary snoring (PS) subjects

(B) OSA patients with and without specific clinical phenotype, such as EDS, hypertension, heart disease, metabolic/neurocognitive/endothelial dysfunction, etc---.

(B)In vitro IHR stimuli may

(A)Induce changes in gene/protein expression levels of the ATGs in THP-1 macrophage cell lines.

(B)Augment ROS generation, cell apoptosis, cytotoxicity

(C) These changes may be reversed with umbilical cord MSC-CM treatment or agents.

Study Design: Clinical Samples & in vitro Experiments

- Peripheral Blood Mononuclear Cells (PBMC), samples from
  - 48 treatment-naïve OSA patients
  - 12 primary snoring (PS) subjects,
- Gene expression levels of the core ATGs, including LC3II, ATG5, p62, ATG9a, ULK1, BECN1
  - Real-time quantitative RT-PCR
- Protein expression levels of LC3II, ATG5, p62 of blood $CD14^{+}$monocyte,$CD16^{+}$neutrophil, $CD3^{+}CD4^{+}$ T helper (Th) cell
  - Flowcytometry

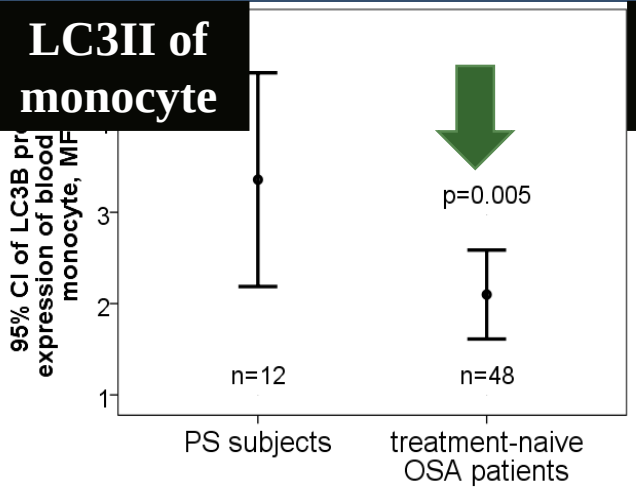
- Human monocytic THP-1 cell line
  In vitro IHR stimuli
  - 17-min hypoxic period
    (0 % O2 and 5 % CO2)
  - 13 min of re-oxygenation
    (21 % O2 and 5 % CO2)
  - 2 events/hour, 7 cycles/Day, 1-4 days
  - vs. normoxic (NOX) condition
- Cell viability: WST1
- Cell apoptosis: annexin V/PI stain by flowcytometry
- ROS: H2DCFDA by flowcytomeetry
- Cytotoxicity: LDH assay
- Umbilical cord mesenchymal stem cell (MSC)-conditional medium

Demographic, biochemistry, and sleep data of all the 60 study participants

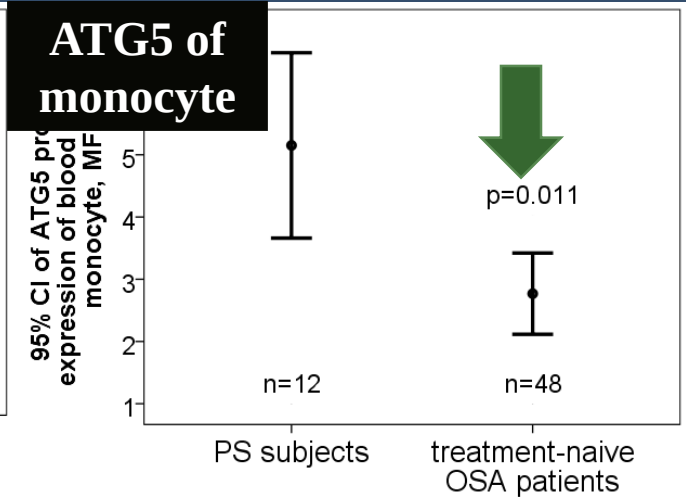
	PS subjects (n=12)	Severe OSA patients (n=48)	p value
Age, years	45.7±12.3	45.7±10.5	1.0
Male Sex, n (%)	14 (68.8)	3 (77.1)	0.505
BMI, kg/m^2	24.8±3.8	25.4±3.6	0.356
AHI, events/hour	4.2±3	47.1.±19.4	<0.001
ODI, events/hour	1.4±1.1	32.2±22.5	<0.001
Mean SaO2, %	94.8±1.5	94.1±2.8	0.621
Minimum SaO2, %	89.4±1.9	76.1±15.9	0.074
Snoring index, counts/hour	139±126	390±214	0.008
ESS	5.5±4.1	9.5±4.2	0.032
Smoking history, n (%)	3 (25)	15 (16.2)	0.393
Cholesterol, mg/dl	200.2±46.4	199.5±32.8	0.95
Triglycerides, mg/dl	123.6±68.7	145.2±99.2	0.497
Co-morbidities			
Hypertension, n (%)	1 (0.8)	16 (21.6)	0.305
Diabetes mellitus, n (%)	1 (0.8)	3 (6.2)	0.542
Heart disease, n (%)	1 (0.8)	3 (6.2)	0.164
Stroke, n (%)	0 (0)	0	1
CKD, n (%)	0 (0)	0 (0)	1

ATG5 / LC3II protein expression of blood CD14^{+}monocytes, and ATG5 protein expression of blood CD16^{+}neutrophils were decreased in treatment-naïve OSA patients versus PS subjects. OSA patients with insomnia had lower LC3II protein expressions of CD3^{+}CD4^{+}helper T cell, while OSA patients with memory impairment had higher P62 protein expressions of CD14+monocyte.

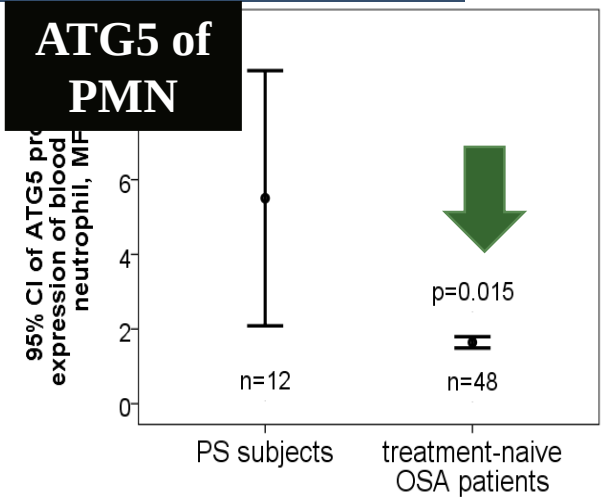
LC3II of monocyte



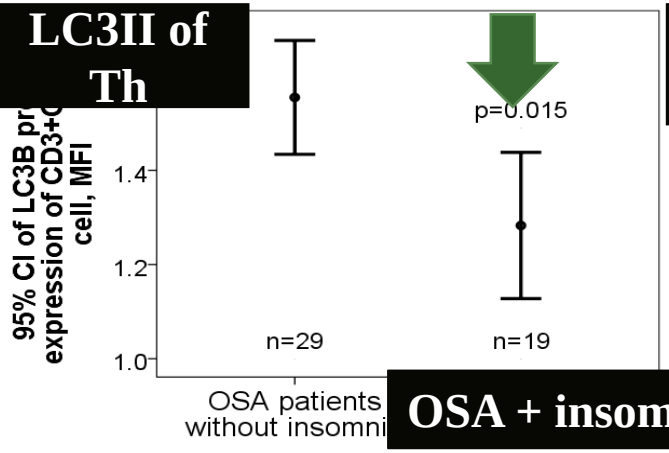
ATG5 of monocyte



ATG5 of PMN

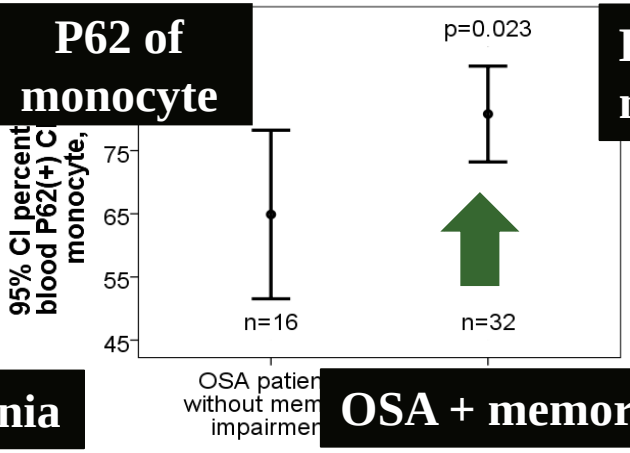


LC3II of Th



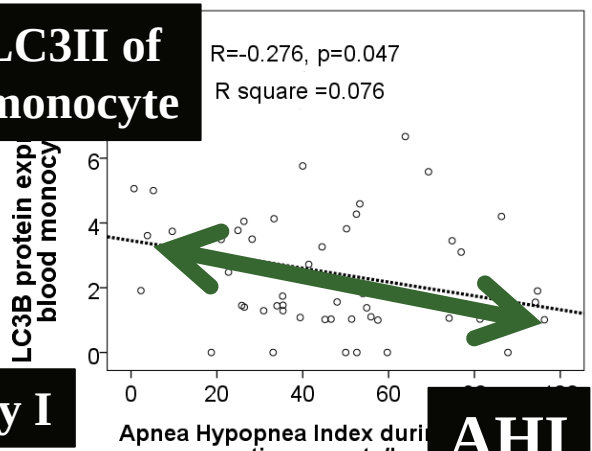
OSA + insomnia

P62 of monocyte



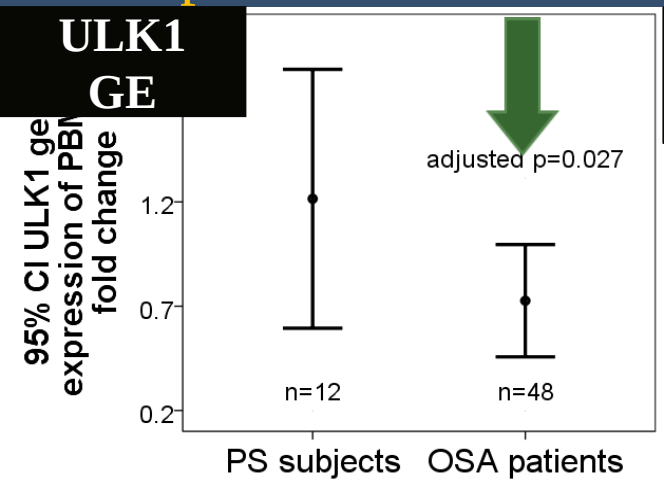
OSA + memory I

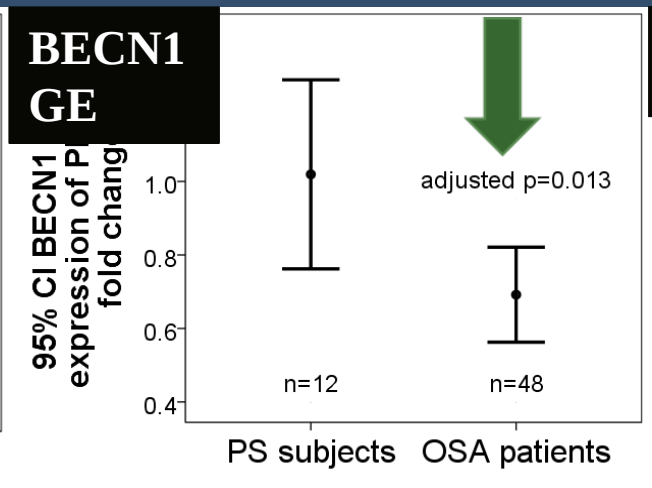
LC3II of monocyte

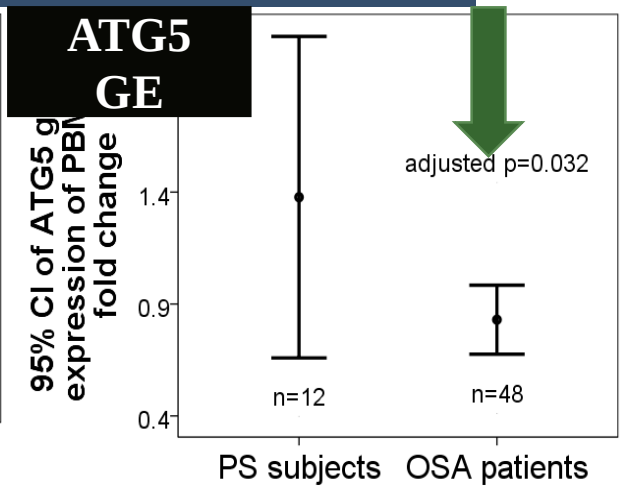


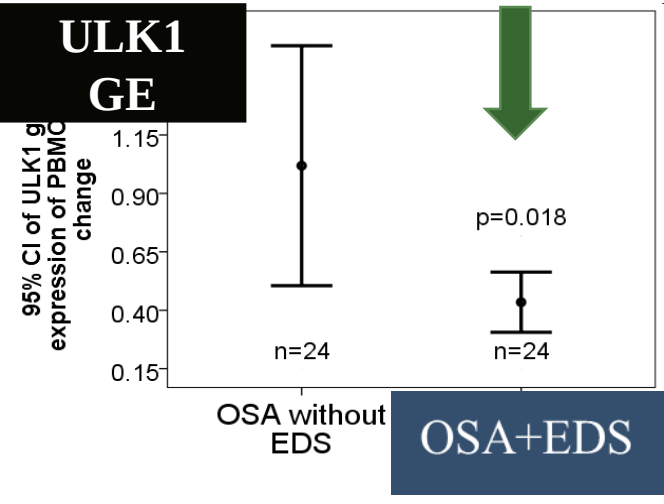
AHI

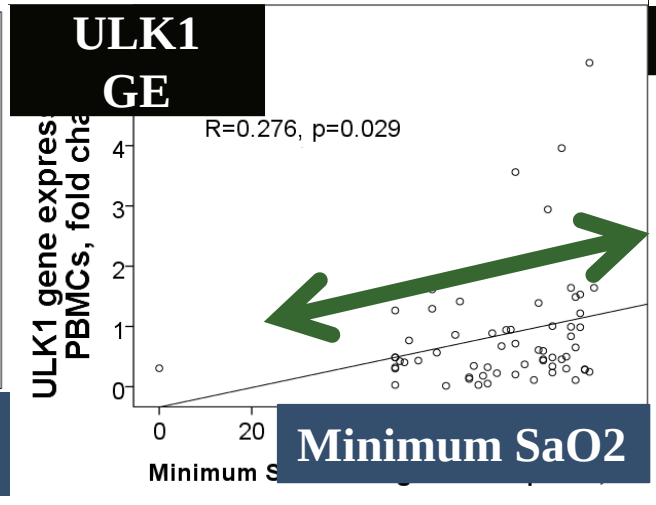
ATG5, ULK1, and BECN1 gene expressions of PBMCs were decreased in treatment-naïve OSA patients versus PS subjects. ULK1 gene expressions were further decreased in those with excessive daytime sleepiness (EDS: ESS>10), and negatively correlated with minimum SaO2 / positively with absolute neutrophil count.

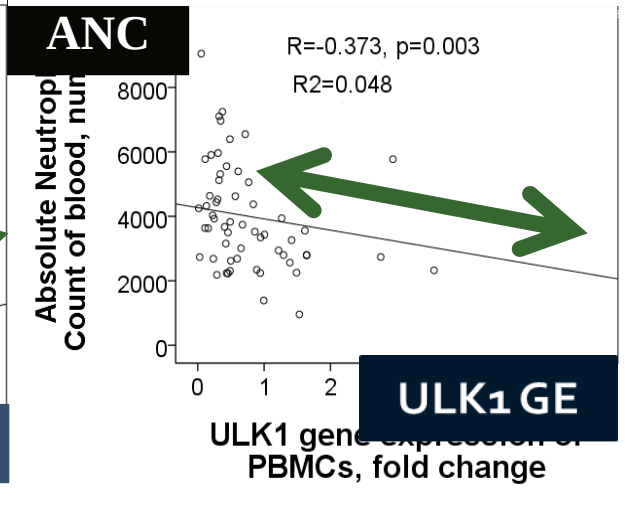




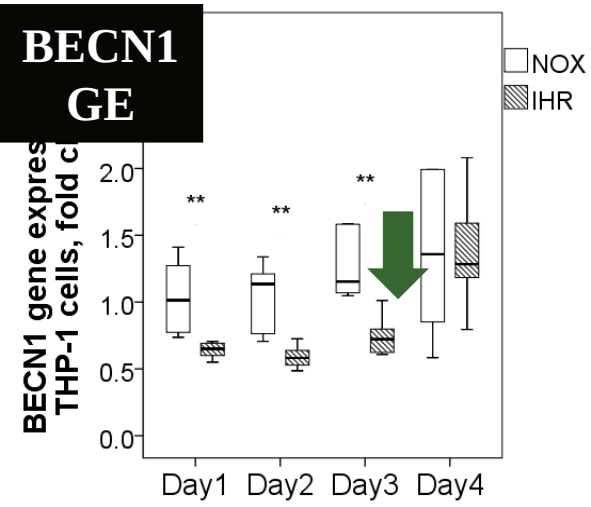


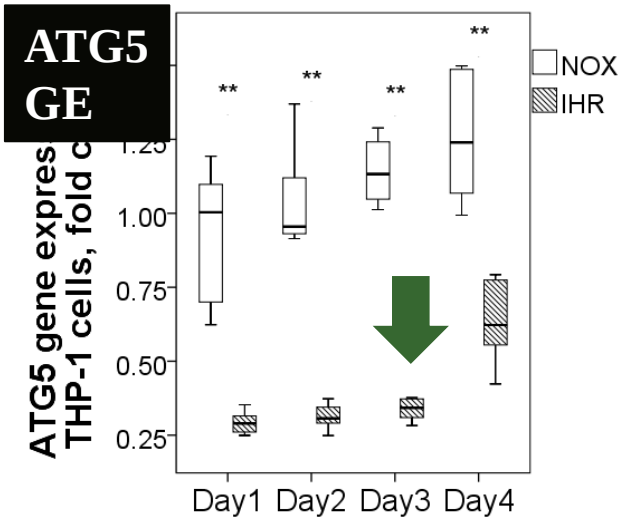


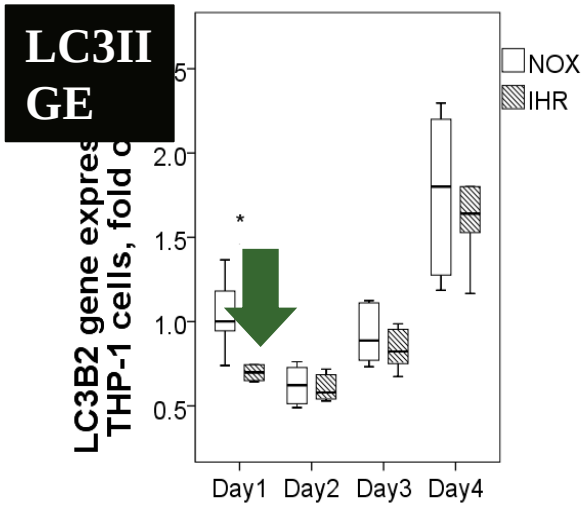


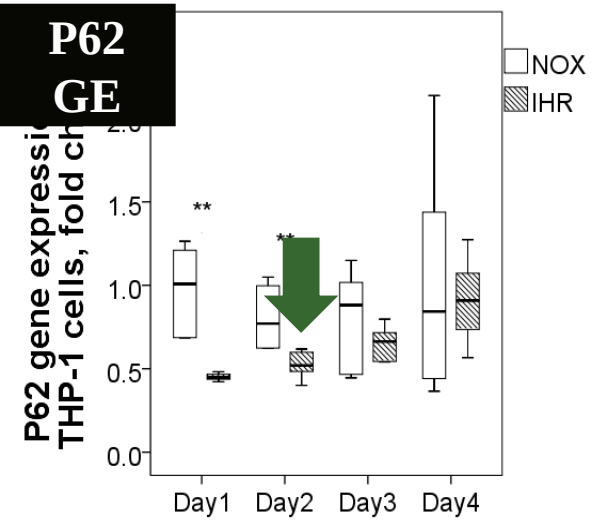


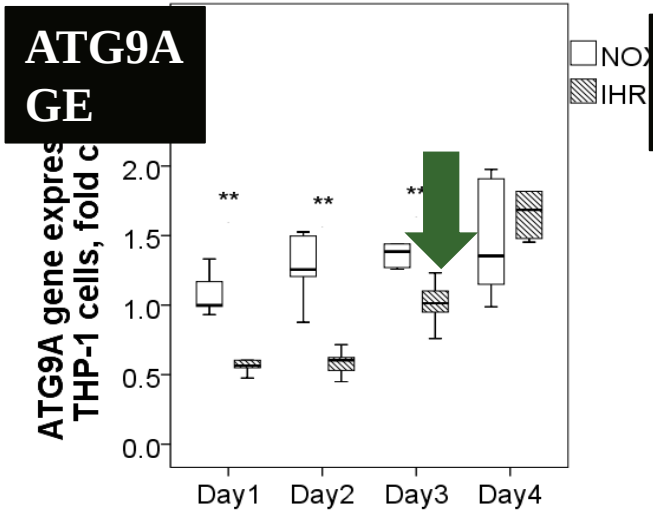
In vitro IHR exposure in THP-1 cells for 1-4 days resulted in down-regulations of ATG5, ULK1, BECN1, ATG9A, p62, and LC3II genes.

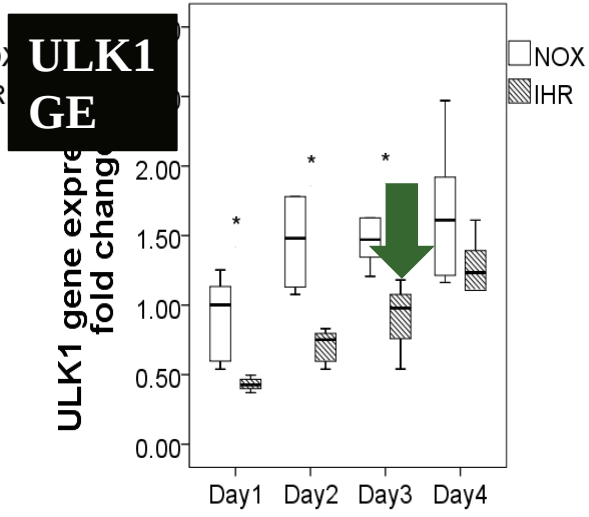






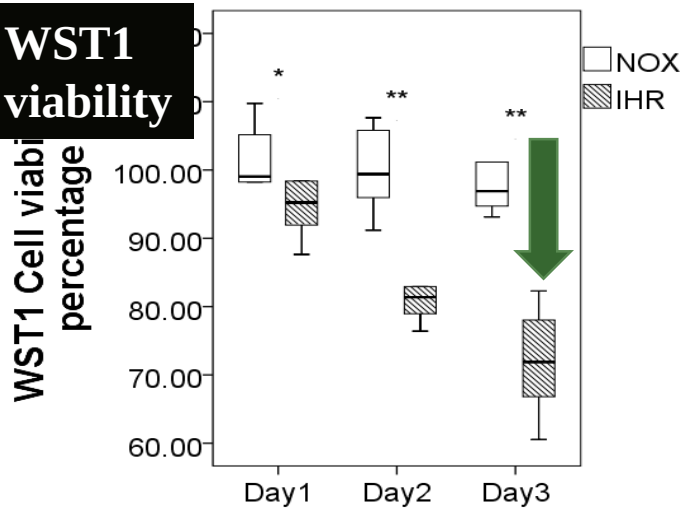




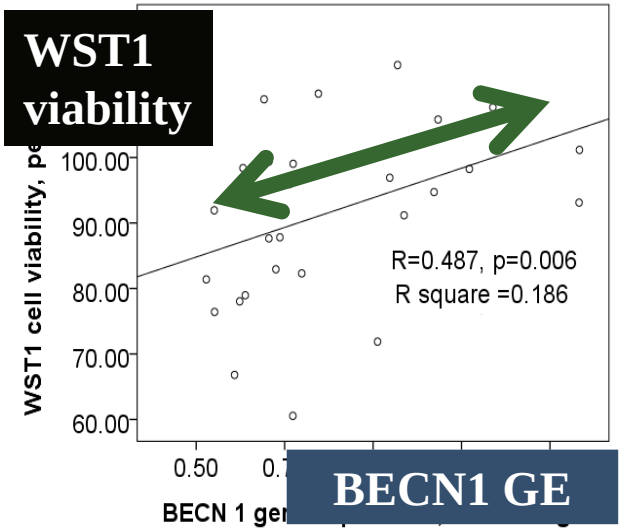


In vitro IHR exposure resulted in, decreased cell viability, increased ROS generation and increased apoptosis/cytotoxicity, while mesenchymal stem cell (MSC)-conditional medium (CM) treatment reversed these abnormalities partly.

WST1 viability

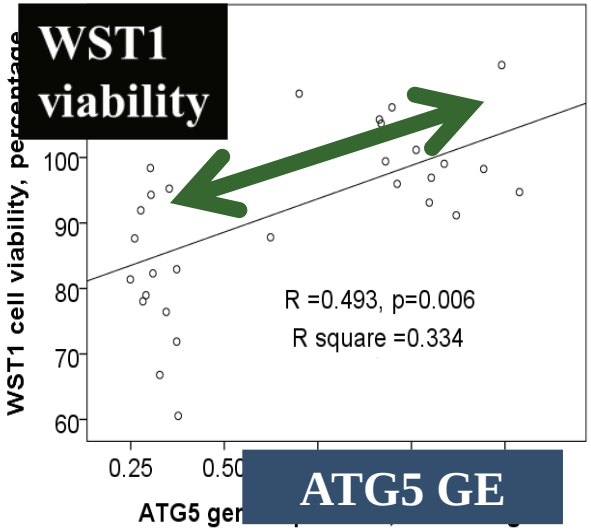


WST1 viability



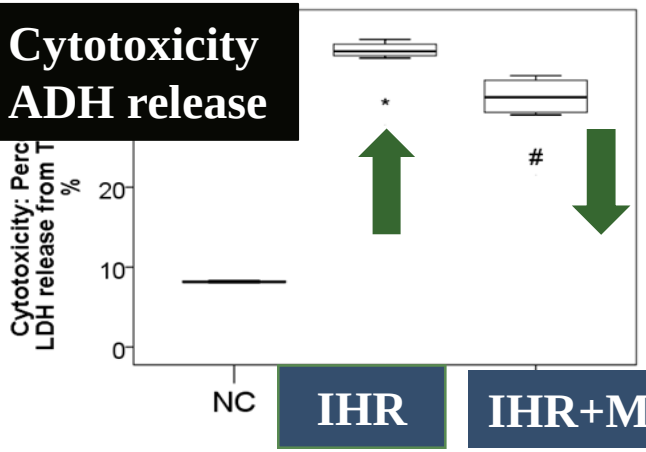
BECN1 GE

WST1 viability



ATG5 GE

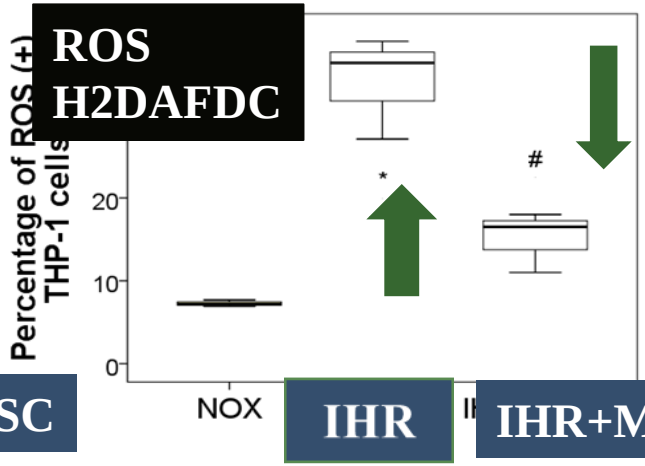
Cytotoxicity ADH release



IHR

IHR+MSC

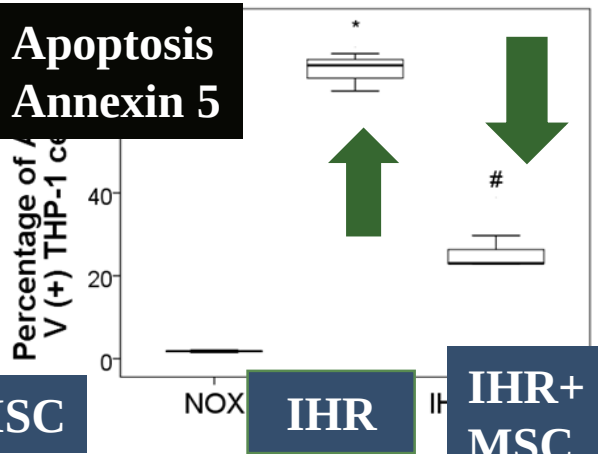
ROS H2DAFDC



IHR

IHR+MSC

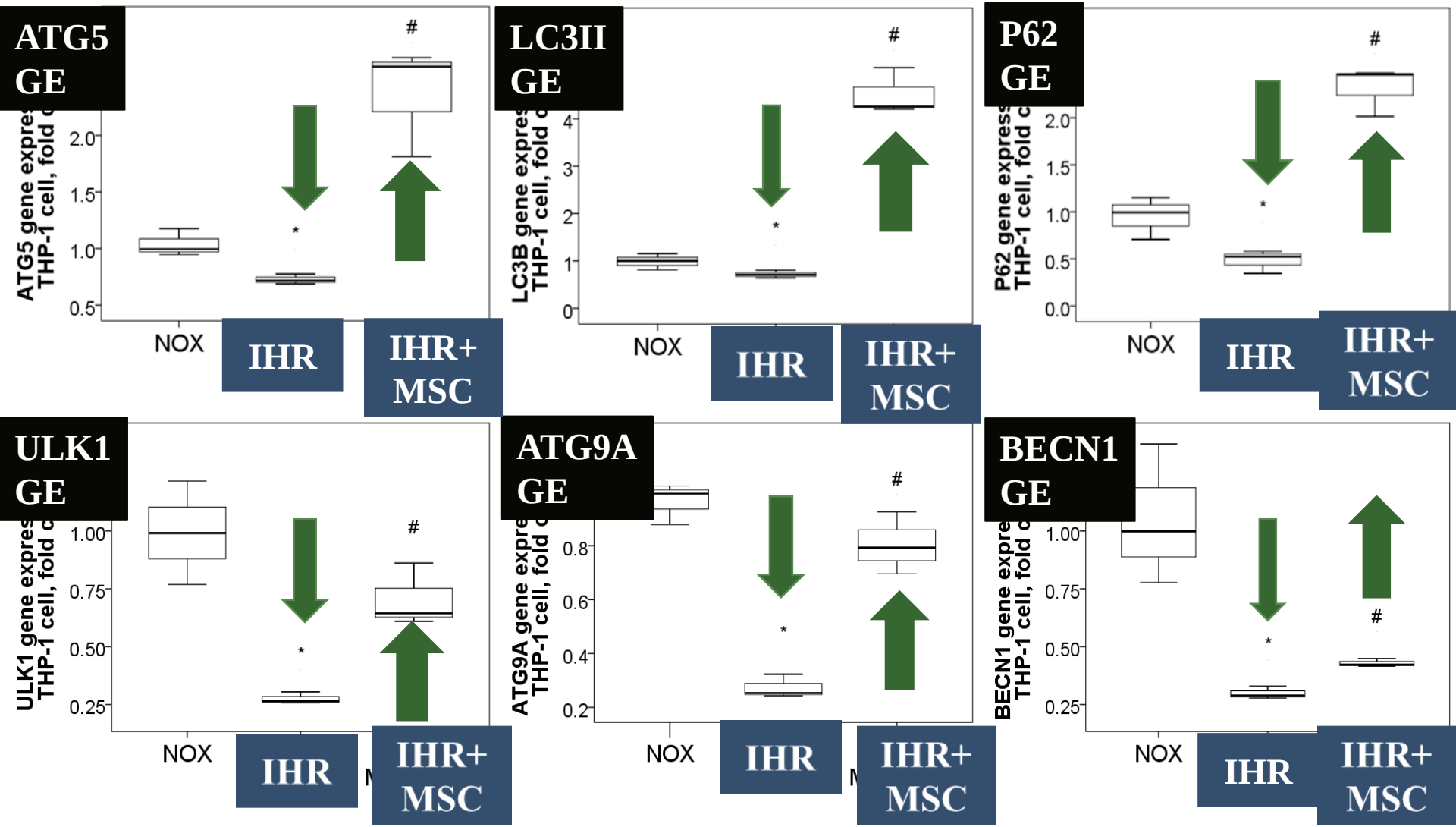
Apoptosis Annexin 5



IHR

IHR+ MSC

In vitro IHR exposure resulted in down-regulations of the LC3B, ATG5, p62, ULK1, ATG9a, and BECN1 genes, which were reversed with mesenchymal stem cell (MSC)-conditional medium (CM) treatment.



Summary and Conclusions

- ATG5, ULK1, and BECN1 gene expressions of peripheral blood mononuclear cells were decreased in OSA patients versus PS subjects.
- ATG5 and LC3BII protein expression of blood monocytes, and ATG5 protein expression of blood neutrophils were decreased in OSA patients versus PS subjects.
- OSA patients with insomnia had lower LC3B protein expressions of helper T cell, while OSA patients with memory impairment had higher P62 protein expressions of monocyte.
- In vitro IHR exposure resulted in down-regulations of ATG5, ULK1, BECN1, ATG9A, and LC3BII genes along with increased reactive oxygen species generation and increased apoptosis/cytotoxicity, while mesenchymal stem cell (MSC)-conditional medium treatment reversed these abnormalities.
- Impaired autophagy in OSA patients
  - correlated with disease severity and clinical phenotypes
- Autophagy Insufficiency with in vitro IHR exposure in THP-1 cell
  - Increased ROS production, and Increased apoptosis and cytotoxicity
  - All of which could be reversed with MSC-CM treatment

Acknowledgement

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  - Dr. Chia-Wei Liou
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- Medical Research Department, KCGMH
  - Dr. Po-Yuan Hsu
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  - Yong-Yong Lin, Master
  - Yi-Xin Zheng, Master
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  - Mr. Wei-Zhe Liu
  - Ms. Yi-Jing Li