Insulin-Like Growth Factor-II Receptor Mediates Angiotensin II-Induced Rab9-Dependent Macroautophagy and Apoptosis in Cardiomyocytes

第二型類胰島素生長因子受體調控血管收縮素II在心肌細胞所誘導的Rab9依賴型自噬作用與細胞凋亡

Ding-Yu Lin (林町餘) [1], Wei-Wen Kuo (郭薇雯) [2], Fu-Jen Tsai (蔡輔仁) [3], Chang-Hai Tsai (蔡長海) [4], Chih-Yang Huang (黃志揚) [1,5]

1Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan; 2 Department of Biological Science and Technology, China Medical University, Taichung, Taiwan 3 Department of Pediatrics, Medical Research and Medical Genetics, China Medical University Hospital, Taichung, Taiwan 4 Department of Healthcare Administration Asia University, Taichung, Taiwan 5Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan.

BACKGROUND

●Macroautophagy (hereafter referred to as autophagy) has emerged as an important process in the pathogenesis of cardiovascular diseases.

●Angiotensin II (Ang II) plays an important role in pathogenesis of heart disease. Our previous studies demonstrated that up-regulation of *igf2r* (Insulin-like growth factor -II receptor) gene and the subsequent activation of IGF2R-Gαq signaling contributed to AngII-induced myocardial cell hypertrophy, and apoptosis.

Figure 1. (A.) Illustrations of Ang II-mediated IGF2/IGF2R signals contributing to cardiac remodeling (Chu *et al*., Endocrinology, 2009; Am J Physiol *Endocrinol Metab*, 2006). (B).Comparison of canonical and non-canonical autophagy (Nishida Y et al., Nature. 2009).

Fig 1 (A)

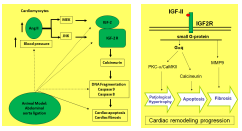
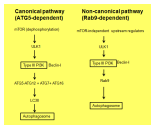


Fig 1 (B)



OBJECTIVES

To investigate whether autophagy is involved in AngII-induced IGF2R signaling and further clarify whether the induced autophagy is associated with IGF2R-mediated apoptosis in myocardial cells.

METHODS

Study design

●Cells: H9c2 rat cardiomyoblast cells were obtained from the American Type Culture Collection (ATCC) and primary neonatal rat ventricular myocytes (NRVMs) were harvested from1 to 3 days old Sprague Dawley rats.

H9c2 cells **NRVMs**

●Cells were incubated with Ang II(10^{-7} M) or Leu27IGF-II (10^{-8} M) for the subsequent analysis. Leu27IGF-II is an analog of IGF-II which interacts selectively with the IGF-IIR.

Autophagy assessments

● si-RNAs (small interference RNAs) were transfected into myocardial cells to specifically knockdown the target genes; Gene silencing efficiency were over 60% in all experiment groups.
● 3-Methyladenine(3-MA;10mM) was used for autophagy inhibitor and Bafilomycin A1(BafA1,100nM) was used for autolysosome inhibitor.
● Autophagy levels were quantified by flow cytometr with LysoTracker Red dye.

RESULTS

●Ang II-induced Rab9-depentdent autophagy is mediated by IGF-IIR and this non-canonical autophagy contributes to Ang II-induced apoptosis in cardiomyocytes.

●Selective activation of IGF-IIR by Leu27IGF-II confirmed that the induction of Rab9-depedent autophagy is mediated by IGF-IIR-Gαq signaling in cardiomyocytes, which may in turn contribute to mitochondria-mediated apoptosis.

Figure 2. Induction of Autophagy by IGF2R activation. (A).Typical autophagic vacuoles were observed in Leu27-IGFII treated myocardial cells by transmission electron microscopy (TEM). AU: autophagosome; AV: Autolysosome. (B). Increase of autophagy levels by IGF2R activation.

Fig2(A) Fig2 (B)

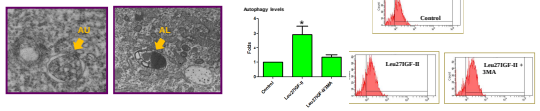


Figure 3. Canonical autophagy was suppressed by IGF2R activation. LC3 turnover assay indicated that canonical autophagy was inhibited after Leu27IGF-II 24hrs incubation compared with no-stimuli H9c2 cells .

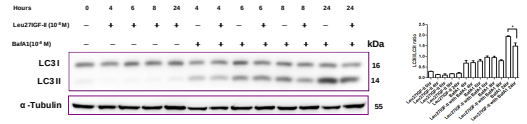


Figure 4. Rab9-dependet autophagy is mediated through IGF2R-Gαq signaling in cardiomyocytes. (A).Up-regulation of *rab9* gene by IGF2R activating. (B).Rab9-dependnet autophagy was induced by IGF2R-Gαq signaling. (C). Determine autophagy levels by Lysotracker Red stain. siRNA-scramble(si-ctrl) were used as the control si-RNA. *P<0.05; **P<0.01.

Fig 4 (A) Western blotting SYBR Green Real-Time PCR

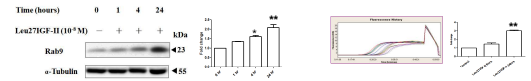


Fig 4 (B)

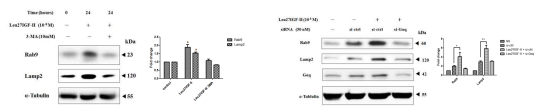


Fig 4 (C)

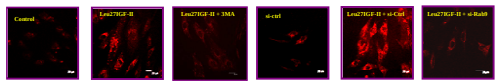


Figure 5. Rab9-depedent autophagy contributes to IGF2R-induced myocardial cells death. (A). H9c2 cells. (B). NVRMs. Cell viability were assessed by Trypan Blue Exclusion Test.

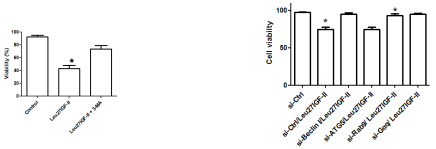


Figure 6. Rab9-dependent autophagy contributes to IGF2R-induced apoptosis in cardiomyocytes.

Fig 6 (A). Pro-apoptotic proteins induced by IGF2R activation were attenuated markedly by autophagy inhibition or knockdown of Rab9-dependet autophagy required ATGs (Autophagy related genes) in H9c2 cells and NRVMs.

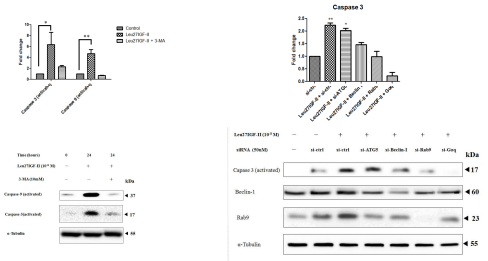


Fig 6 (B). Apoptosis levels were detected by TUNEL assay. Suppressed IGF2R-induced apoptosis by inhibition of Rab9-depedent autophagy. (Cells: NVRMs; *P<0.05; **P<0.01)

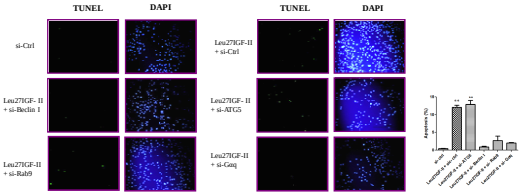


Fig 6 (C). Annexin V/PI staining assay of cell apoptosis induced by Leu27IGF-II. The total apoptotic cells (early and late-stage apoptosis) are represented by the right side of the panel (Annexin V staining alone or together with PI) in which the total cell death number. Results indicated that Rab9-depedent autophagy contributes to IGF2R-Gαq mediated apoptosis. (Cells: H9c2; *P<0.05; **P<0.01)

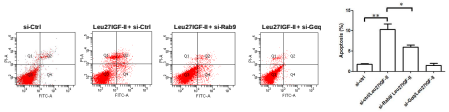


Figure 7. Rab9-depedent autophagy induced by Ang II is mediated by IGF2R in myocardial cells. (A). Increase of *igf2r* gene expression by Ang II in a time dependent manner. (B-C). Ang II mediated up-regulation of *igf2* gene *via* AT-1 receptor. Transcription levels were analyzed by RT-PCR. ; Losartan: AT-1 receptor antagonist; PD 123319: AT-2 receptor antagonist.

Fig7 (A)

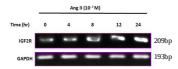


Fig7 (B)

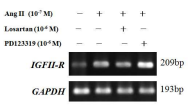


Fig7 (C)

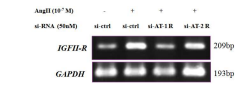


Figure 8. Rab9-depedent autophagy is associated with Ang II-induced myocardial cell apoptosis. (A).Time-course induction of Rab9-depedent autophagy and apoptosis in Ang II-treated H9c2 cells. (B).Suppressed Ang II-induced apoptosis by inhibition of Rab9-depedent autophagy in NRVMs. **P<0.01.

Fig7 (A)

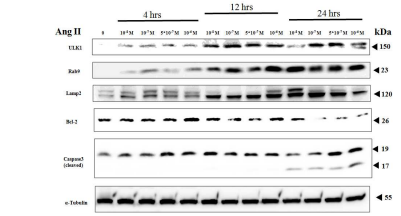


Fig7 (B)

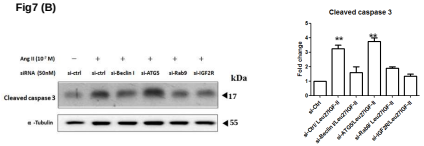


Figure 8. Rab9-depedent autophagy may contribute to IGF2R mediated mitochondria-dependent apoptosis through mitophagy. (A). Reduction of IGF2R-inudced depolarized mitochondria by autophagy inhibition. (B). Increase of mitophagy was observed in IGF2R-activated H9c2 cells.

Fig8 (A). JC-1 Mitochondrial membrane potential assay

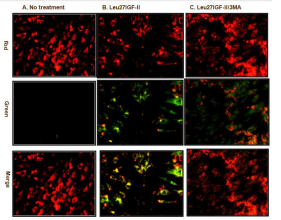
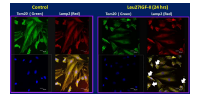


Fig8 (B). Immunofluorescence staining with mitochondria and lysosomes. Increase of mitochondria-containing lysosomes in IGF2R-activated H9c2 cells.



Arrows indicated the image colorization with Tom20 and Lamp2.

CONCLUSIONS

Schematic IGF2R death signal in cardiomyocytes