Insulin-Like Growth Factor-II Receptor Mediates Angiotensin II-Induced Rab9-Dependent Macroautophagy and Apoptosis in H9c2 Cardiomyoblast Cells

類胰島素生長因子第二型受體調控血管收縮素II在H9c2心肌纖維母細胞所誘導的Rab9依賴型自噬作用與細胞凋亡

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Background

- Macroautophagy (hereafter referred to as autophagy) has emerged as an important process in the pathogenesis of cardiovascular diseases.
- Angiotensin II (AngII) plays an important role in pathogenesis of heart disease. Our previous studies demonstrated that up-regulation of IGF-IIR (Insulin-like growth factor -II receptor) gene and the subsequent activation of IGF-IIR signaling contributed to AngII-induced myocardial cell hypertrophy, and apoptosis.

Figure 1. (A.) Illustration of the IGF-IIR dependent signaling pathway in cardiac remodeling (Endocrinology, 2009). (B).Comparison of canonical and non-canonical autophagy (Nature. 2009).

Fig 1 (A).

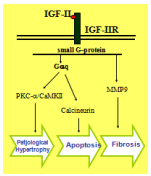
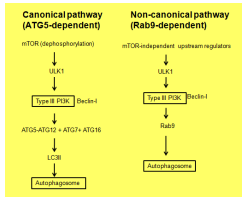


Fig 1. (B.)



Objectives

To investigate whether autophagy is involved in AngII-induced IGF2R signaling and further clarify the induced autophagy is associated with IGF2R-induced apoptosis in H9c2 cells..

Methods

Study design

- Cell line: H9c2 cardiomyoblast cells were obtained from the American Type Culture Collection (ATCC) .
- H9c2 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 IGF2 which interacts selectively with the IGF-IIR.

Autophagy assessments

- si-RNAs (small interference RNAs) were transfected into H9c2 cells to specifically knockdown the target genes; Gene silencing efficiency was 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 formation inhibition.
- Autophagy level was quantified by flow cytometr with LysoTracker Red dye.

Results

- Ang II-induced Rab9-dependtdent autophagy is mediated by IGF-IIR and this non-canonical autophagy contributes to AngII-induced apoptosis in H9c2 cardiomyoblast cells.
- Selective activation of IGF-IIR by Leu27IGF-II confirm that the induction of Rab9-depedent autophagy is mediated by IGF-IIR-Gαq signaling in H9c2 cells, which may in turn contribute to mitochondria-mediated apoptosis.

Figure 2. Autophagy was induced by IGF2R activation. (A).Typical autophagic vacuoles were observed in Leu27-IGFII treated H9c2 cells by transmission electron microscopy. AU: autophagosome; AV: Autolysosome . (B).Increase of autophagy level by IGF2R activation. Measurement by Lyso Tracker Red staining of flow cytometry.

Fig2(A).

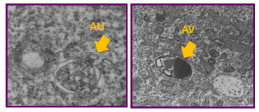


Fig2 (B.)

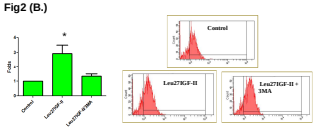


Figure 3. Canonical autophagy was suppressed by IGF2R activation. LC3 turnover assay indicated that significant reduction of canonical autophagy after Leu27IGF-II 24hr incubation compared with no-stimuli cells .

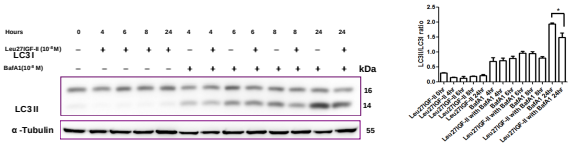


Figure 4. Rab9-dependet autophagy is mediated through IGF2R-G αq signal. (A).Up-regulation of *rab9* by IGF2R activating. (B).Rab9-depednet autophagy was induced by IGF2R signaling. (C).Inhibition of Rab9-depedent autophagy by Type III PI3K inhibition or G αq knockdown.

Fig 4 (A)

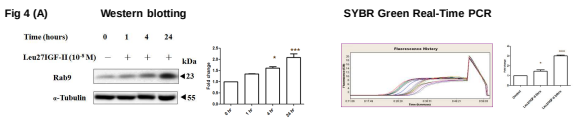


Fig 4 (B)

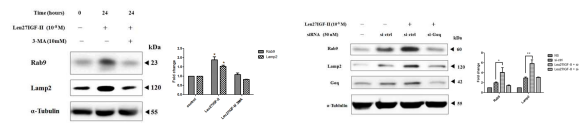


Fig 4 (C) Determine autophagy by Lysotracker Red stain. The data shown are representative of three independent experiments with similar findings.

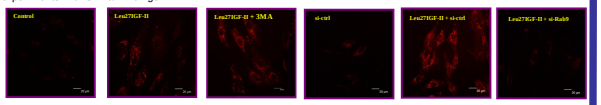


Figure 5. Rab9-depedent autophagy contributes to IGF2R-induced H9c2 cell death. Cell viability were assessed by Trypan Blue Exclusion Test.

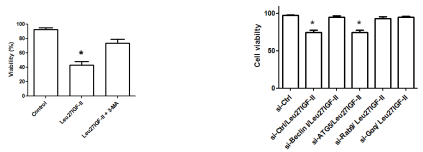


Figure 6. Rab9-dependent autophagy contributes to IGF2R-induced apoptosis in H9c2 cells..

Fig 6 (A). Leu27IGF-II induced apoptosis were attenuated markedly by autophagy inhibition.

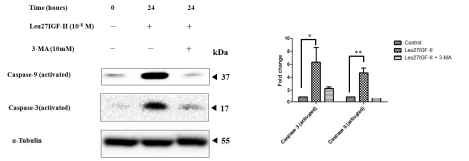


Fig 6B. Leu27IGF-II induced activated-caspase3 were reduced by Rab9-depedent autophagy inhibition.

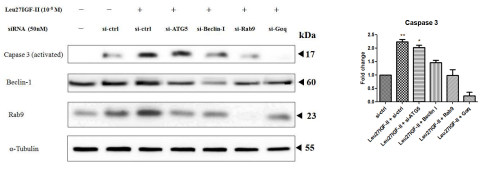


Fig 6(C). Apoptosis level were detected by TUNEL assay. Selective knockdown of Rab9-dependet autophagy required ATGs (Autophagy related genes) attenuated IGF2R-induced apoptosis markedly.

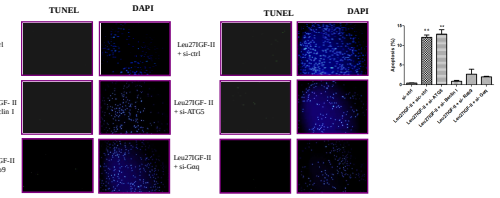


Fig 6 D. Annexin V/PI staining assay of cell apoptosis induced by Leu27IGF-II. The total apoptotic cells (early and late-stage apoptosis) are represented by the right side of the panel (Annexin V staining alone or together with PI) in which the total cell death number. Results indicated that Rab9-depedent autophagy contributes to IGF2R-induced apoptosis.

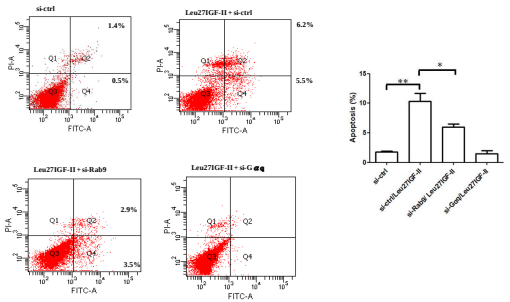


Figure 7. Rab9-depedent autophagy induced by AngII is mediated by IGF2R.

Fig 7 (A). Increase of *igf2r* gene expression by AngII in a time depenent manner. Transcription levels were analyzed by RT-PCR.

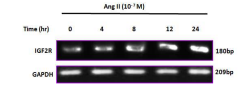


Fig 7 (B). AngII mediated up-regulation of *igf2* gene via AT-1 receptor .Transcription levels were analyzed by RT-PCR. PD 123319: AT-1 receptor antagonist; Losartan: AT-2 receptor antagonist.

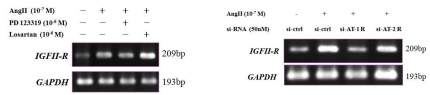


Fig 7 (C). IGF2R mediated AngII-induced Rab9-depedent autophagy and cell apoptosis.

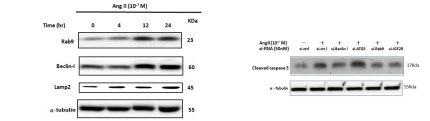
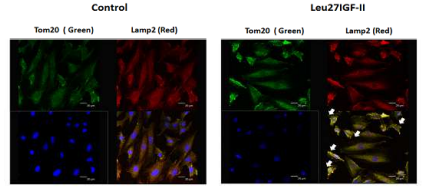


Figure 8. Rab9-depedent autophagy is a mitophagy which may contribute to IGF2R mediated mitochondria-dependent apoptosis. Arrows indicated the image colorization of Lamp2 and Tom20.



Conclusions

Our findings provide the novel evidence that the non-canonical autophagy exists in myocardial cells and implicates that IGF2R may be an important nexus point between autophagy, endosomal trafficking and apoptosis. Further studies are warranted to reveal the detail molecular mechanisms governing IGF2R-mediated Rab9-dependent autophagy. The knowledge may help in the development of future therapeutic autophagy agonists/antagonists to cure cardiomyopathy.

References

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