低氧/厌氧产品案例——低氧与心梗研究

文章题目：Necroptosis mediated by impaired autophagy flux contributes to adverse ventricular remodeling after myocardial infarction

自噬流受损介导的坏死可导致心肌梗死后心室重构不良

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工作站使用情况：Ruskinn Invivo 500

使用气体 浓度：低氧（1% O_2）

摘要：心肌梗死后细胞死亡导致的功能性心肌细胞的丧失对于随后的心室重构、心功能障碍和心力衰竭最为关键。大量研究表明，自噬失调可能导致心肌细胞死亡。然而，自噬失调介导的细胞死亡的潜在机制仍不清楚。本文研究表明，在体内和体外对心肌缺血性损伤的反应中，自噬活性迅速增加，但随后是受损的自噬降解过程，这由到心肌梗死后 12 周的持续较高水平的 beclin1 所证明，同时，LC3 和 p62 的积累增加。串联 mRFP- GFP-LC3 腺病毒和溶酶体抑制剂氯喹的结果都支持缺血损伤诱导的缺陷性自噬。重要的是，我们发现受损的自噬流，不仅由自噬抑制剂绿奎诱导，也由 beclin1 敲除遗传学诱导，上调 RIP3 的表达，并加重 OGD 诱导的心肌细胞凋亡和心功能障碍。同时，心脏特异性 beclin1 过表达上调自噬部分改善了心梗后的心功能障碍。此外，通过强制心脏特异性过表达 RIP3 引起的坏死性激活加重了坏死性心肌细胞死亡、MI 后心脏重塑和心脏功能障碍，但所有这些都可以通过 RIP3 敲除抑制坏死而得到改善。总之，这些结果表明自噬功能障碍介导的神经细胞凋亡在机械上导致心肌梗死后心肌细胞丢失、心室重构不良和进行性心力衰竭。抑制坏死可能是预防梗死后心脏重构和心力衰竭的潜在靶点。

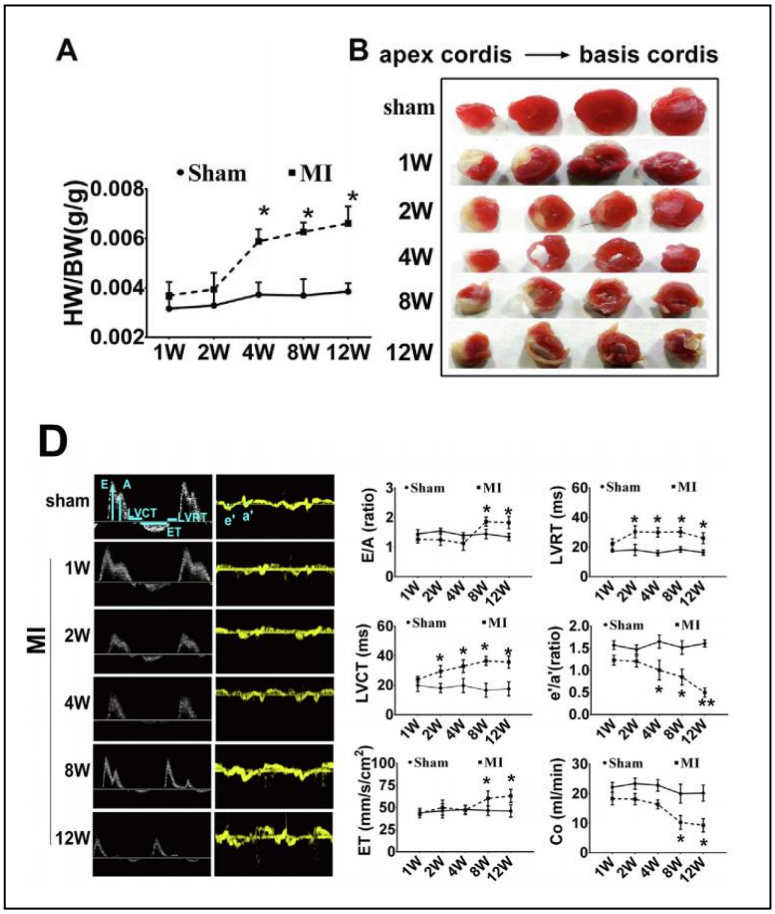

Fig. 1. Cardiac dysfunction induced by myocardial infarction (MI).Myocardial infarction was induced by permanent ligation of the left anterior descending (LAD) coronary artery of mice. (A) The ratio of heart weight to bodyBweight (HW/BW) was analyzed at indicated time point after ligation surgery in each experimental group (Student t test, n = 6, *P < 0.05 vs. sham). (B) Representative images of heart sections with TTC staining of the infarcted area.(C) Representative M-mode echocardiograms and the analyzed results of cardiac function obtained from mice in each experimental group (ANOVA for repeated measurements, n = 6, *P < 0.05 or **P < 0.01 vs. sham). (D) Representative transmitral flow and tissue Doppler echocardiograms and the analyzed results of cardiac function obtained from mice in each experimental group (ANOVA for repeated measurements, n = 6, *P < 0.05 or **P < 0.01vs. sham).

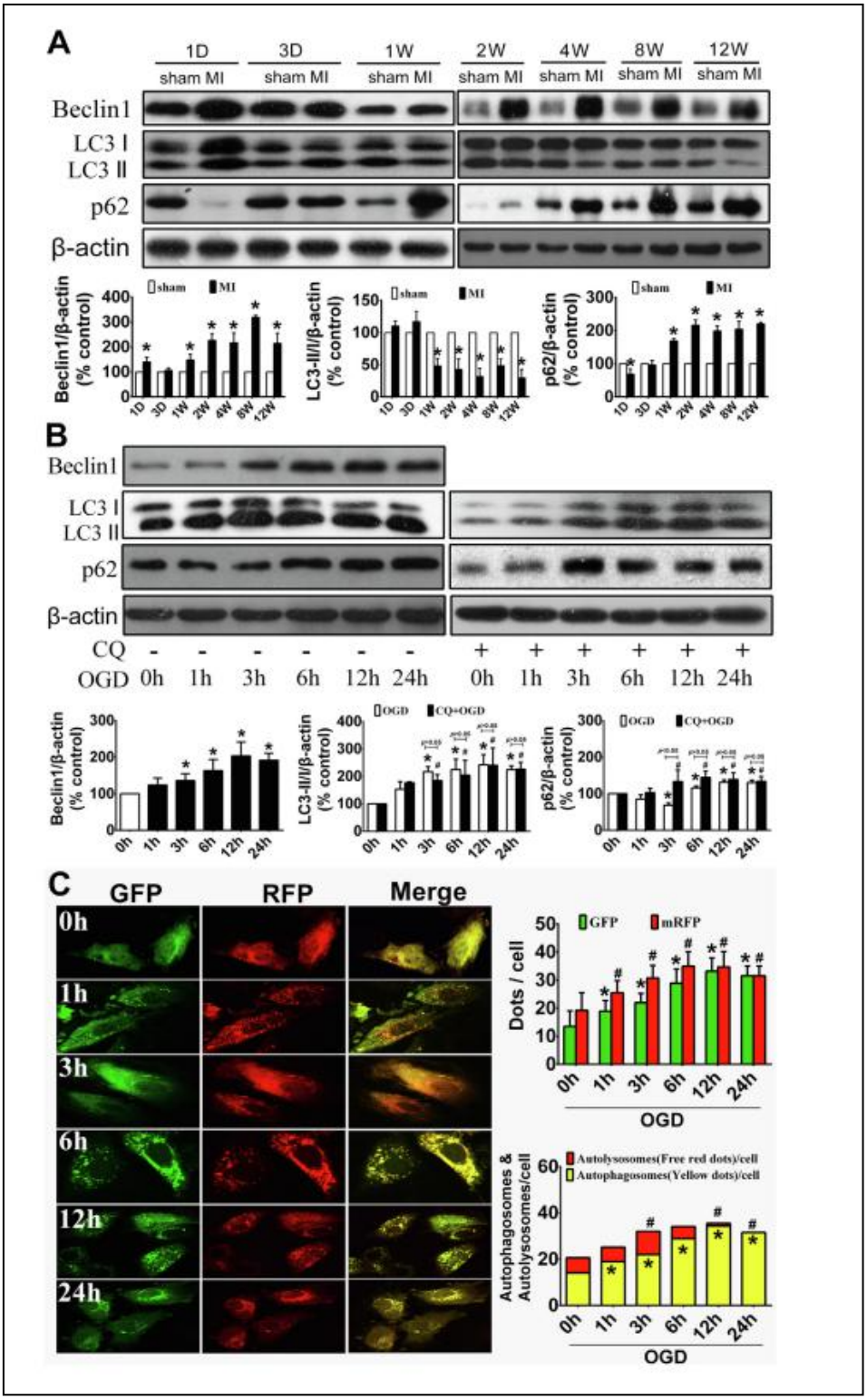

Fig. 2. Autophagy flux was impaired by myocardial ischemia in vivo and by OGD in vitro.(A) Autophagic markers in the border zone of infarct myocardium were determined by western blotting at indicated time point after MI (Student t test, n = 3, *P < 0.05 vs. sham. The expression of proteins in MI mice was normalized to that of the sham mice in each time point, and β-actin served as a loading control). (B) After pretreated with or without CQ for 2 h, H9C2 cells were subjected to OGD and the expression of beclin1, LC3 and p62 was examined by western blotting at indicated time point (one-way ANOVA, n = 5–6, *P < 0.05 vs. 0 h in OGD, #P < 0.05 vs. 0 h in CQ + OGD).(C) After infected with mRFP-GFP-LC3 adenoviral particles for 24 h, the cells were subjected to OGD. Fluorescent signals were captured with the confocal laser scanning microscopy at indicated time point and the number of autolysosomes and autophagosomes was determined by counting of red puncta or yellow puncta, respectively (one-way ANOVA, *P < 0.05 or #P < 0.05 vs. 0 h in yellow puncta or red puncta, respectively. Thirty randomly selected cells per experimental group were analyzed). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

用左前降支(LAD)冠状动脉的永久结扎来诱导小鼠的心肌梗塞，通过小鼠心肌梗塞诱导心功能不全和心力衰竭（图 1）；

自噬活性在小鼠体内心肌梗塞的早期就被诱导，但是自噬流(自噬的整个过程)随着心脏功能障碍的进展而受到阻碍（图 2a）;1% O_2 诱导 H9C2 OGD 模型，结果显示 OGD 处理时间依赖性上调 beclin1 和 LC3II/I 的表达，p62 的表达在 OGD 后 3 小时内下调，但在 6 小时后出现明显的累积(图 2b)，通过串联 RFP-GFP-LC3 荧光分析，OGD 治疗时间依赖性地增加了黄色斑点的数量，红色斑点(自溶体)的数量在 OGD 后 3 小时内增加，但随后在 OGD 后 12 小时减少(图 2c)，表明自噬是由 OGD 在短时间内诱导的，12h 后是自噬受损降解。