低氧/厌氧产品案例——糖氧剥夺（OGD）模型

文章题目：LncRNA RMST-mediated miR-107 transcription promotes OGD-induced neuronal apoptosis via interacting with hnRNPK

LncRNA RMST 通过与异质性胞核核糖核酸蛋白 hnRNPK 相互作用调控 miR-107 转录促进 OGD 诱导的神经细胞凋亡

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使用气体浓度：厌氧（5% CO_2，95 % N_2）

主要内容：长链非编码 RNA LncRNA 横纹肌肉瘤相关转录本 RMST 沉默已被证明在体内保护缺血性脑损伤和在体外保护神经元损伤。然而，其在缺血性卒中的潜在机制尚不明确。本研究发现在 OGD 处理的海马神经元细胞 HT-22 中，RMST 呈现高表达。RMST 表达的改变导致 HT-22 细胞增殖和凋亡的显著改变。机制上，RMST 通过与异质性胞核核糖核蛋白 K (hnRNPK)相互作用，间接激活 p53/miR-107 信号通路，在 HT-22 细胞中实现其促凋亡功能。

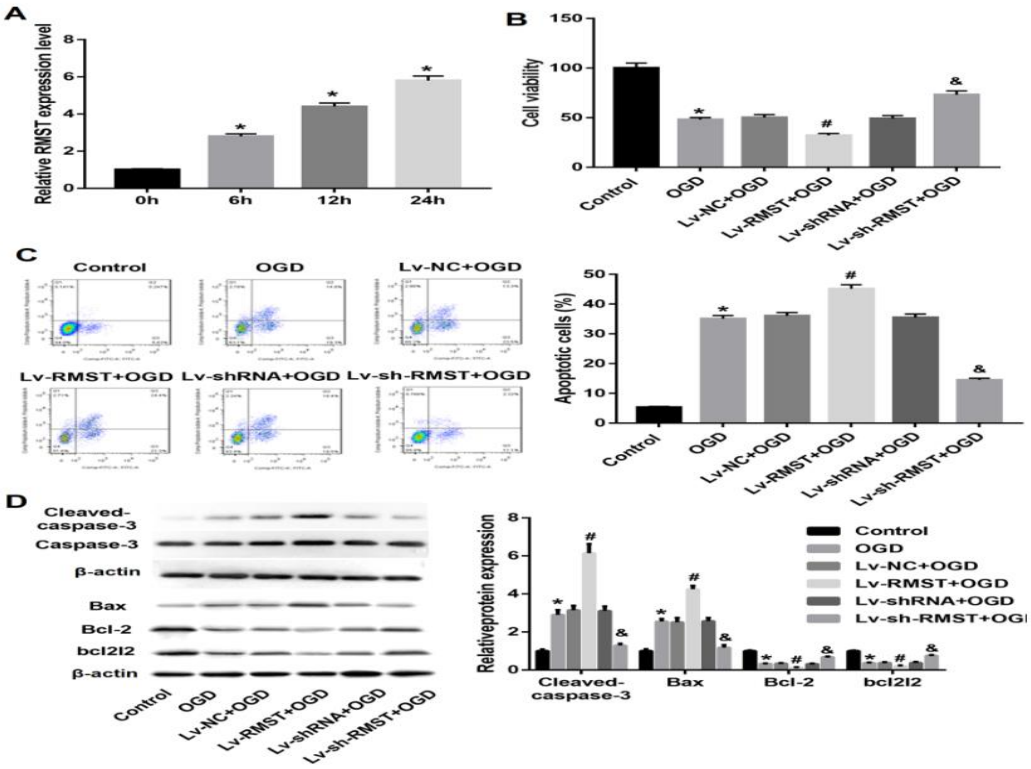

Fig1. RMST prmoted OGD-induced apoptosis of hippocampal neuron cell.(A) The expression of RMST using qRT-PCR in HT-22 hippocampal neuron cell line following treatment with OGD for 0, 6, 12 and 24 h. CCK-8 cell viability (B), cell apoptosis (C) using Annexin V-FITC and PI staining coupled with flow cytometry and the protein levels of cleaved caspase-3, caspase-3, Bax, Bcl2 and Bcl2l2 (D)determined by western blot in HT-22 cells transfected with Lv-NC, Lv-RMST,Lv-shRNA and Lv-sh-RMST before treatment with or without OGD for 24 h. * P<0.05 vs. Control group;# P<0.05 vs. Lv-NC+OGD; & P<0.05 vs. Lv-shRNA+ OGD. Eachexperiment had 3 replicates from 3 independent experiments (n = 3).

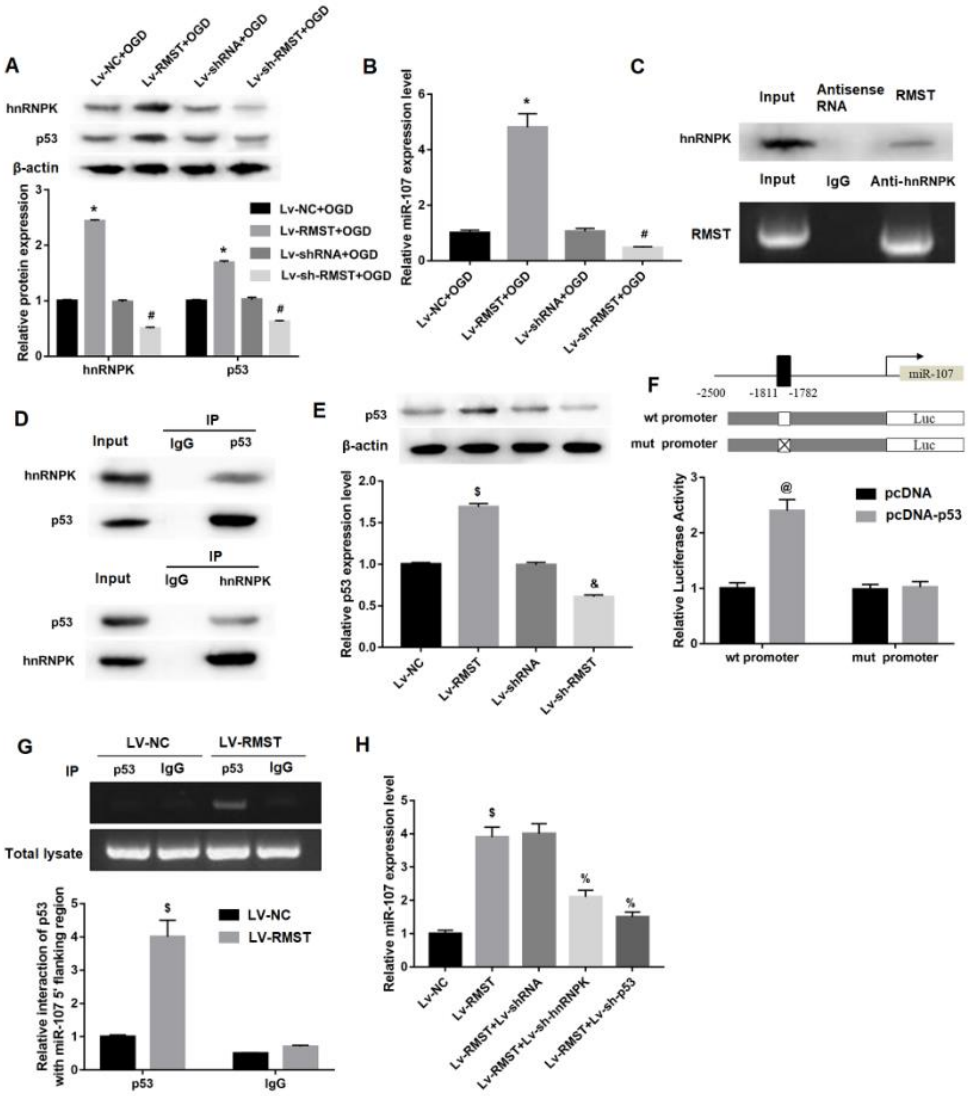

Fig2. RMST activated p53/miR-107 signaling pathway via interacting with hnRNPK.The expression levels of hnRNPK, p53 (A) and miR-107 (B) in HT-22 cells transfected with Lv-NC, Lv-RMST, Lv-shRNA or Lv-sh-RMST before treatment with OGD for 24 h; RNA pull-down assay along with western blotting to detect hnRNPK protein expression in HT-22 cells and RIP assay showing that hnRNPK interacted with RMST in HT-22 cells (C); Protein co-immunoprecipitation of hnRNPK via p53 (D); The expression levels of p53 (E) in HT-22 cells transfected with Lv-NC, Lv-RMST, Lv-shRNA or Lv-sh-RMST; Luciferase activity of different promoter constructs in HT-22 cells transfected with pcDNA-p53 or emptyvector (F); p53 interacted with miR-107 promoter in the region of the p53 binding site as shown by qRT-PCR analysis of ChIP in HT-22 cells transfected with Lv-NC or Lv-RMST (G);.The expression of miR-107 in HT-22 cells transfected with Lv-NC or Lv-RMST or together with Lv-shRNA, Lv-sh-hnRNPK, or Lv-sh-p53 (H);# $P<0.05$ vs. Lv-NC; & $P<0.05$ vs.Lv-RMST+Lv-shRNA. Each experiment had 3 replicates from 3 independent experiments (n = 3).

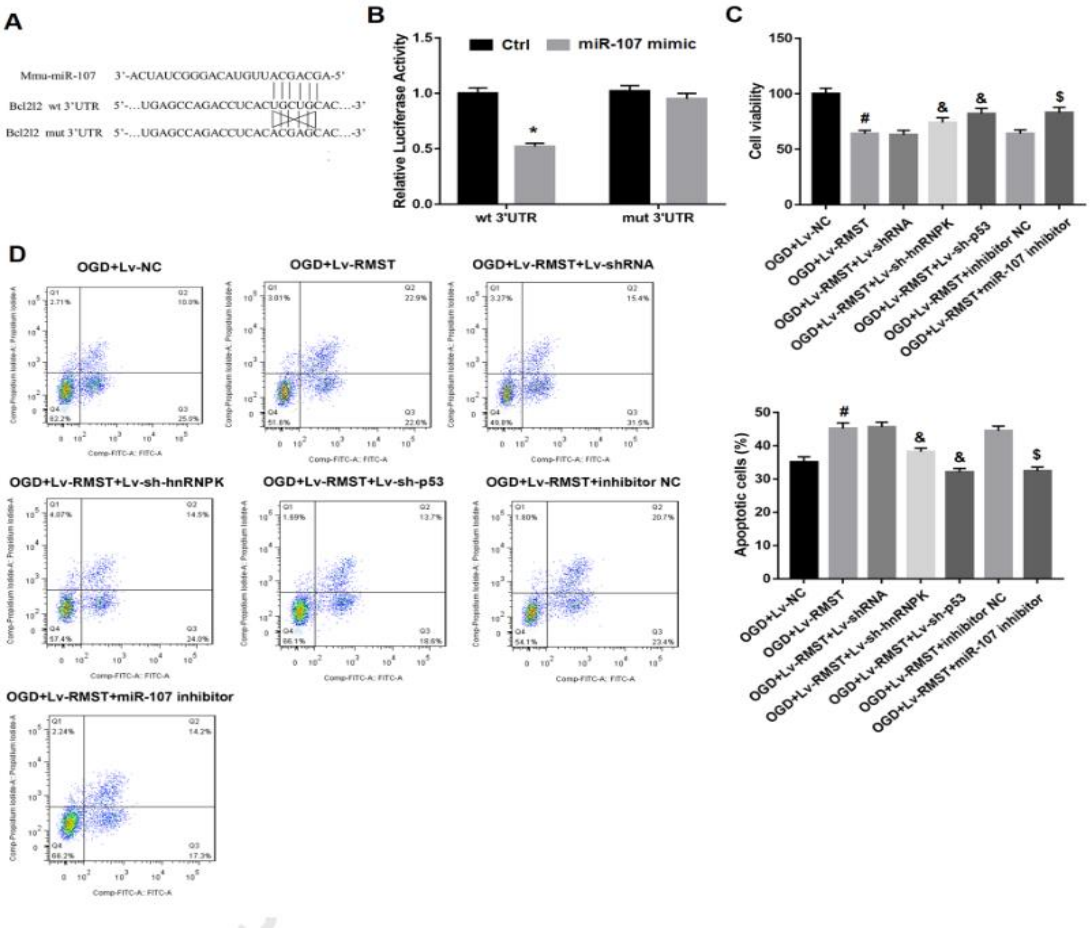

Fig3. RMST induced HT-22 cell apoptosis through targeting p53/miR-107/Bcl2l2 axis by interacting with hnRNPK.Schematic illustration of the predicted binding sites between Bcl2l2 and miR-107, and mutation of potential miR-107 binding sequence in Bcl2l2 (A); Luciferase assays in HT-22 cells transfected Bcl2l2 wild type or mutants with miR-107 (B); Cell viability(C), and cell apoptosis (D) in HT-22 cells transfected with Lv-NC or Lv-RMST or together with Lv-shRNA, Lv-sh-RMST, Lv-sh-p53, inhibitor NC or miR-107 inhibitor before treatment with OGD for 24 h.* P<0.05 vs. Ctrl group; # P<0.05 vs.OGD+ Lv-NC;& P<0.05vs. OGD+Lv-RMST+Lv-shRNA;$ P<0.05 vs.OGD+Lv-RMST+inhibitor NC. Each experiment had 3 replicates from 3 independent experiments (n = 3).

OGD 可诱导神经细胞凋亡，RMST 在 OGD 诱导的海马神经元 HT-22 凋亡细胞中呈现高表达，过表达 RMST 可促进 OGD 诱导的 HT-22 细胞凋亡，沉默 RMST 可抑制 OGD 诱导的 HT-22 细胞凋亡，其机制是 RMST 通过与 hnRNPK 相互作用激活 p53/miR-107 信号通路进而诱导 HT-22 细胞发生凋亡。

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