低氧/厌氧产品应用案例——心肌细胞研究

文章题目：Up-regulation of miR-138 inhibits hypoxia-induced cardiomyocyte apoptosis via down-regulating lipocalin-2 expression

过表达 miR-138 通过下调 Lcn-2 的表达抑制缺氧诱导的心肌细胞凋亡

文章出处：Exp Biol Med (Maywood). 2016 Jan, 241(1): 25-30.南方医科大学南方医院心内科

使用气体浓度:低氧（1% O_2、5%CO_2、94%N_2）,常氧（21%O_2）

主要内容：缺氧诱导的心肌细胞凋亡显著促进许多心脏疾病的发展，例如缺血性心脏病，心力衰竭等。通过抑制细胞凋亡来促进细胞存活是减轻由心肌功能障碍引起的心肌细胞丢失的有效策略之一。过表达 miR-138 通过下调促凋亡基因 Lcn2 的表达来抑制缺氧诱导的心肌细胞凋亡。

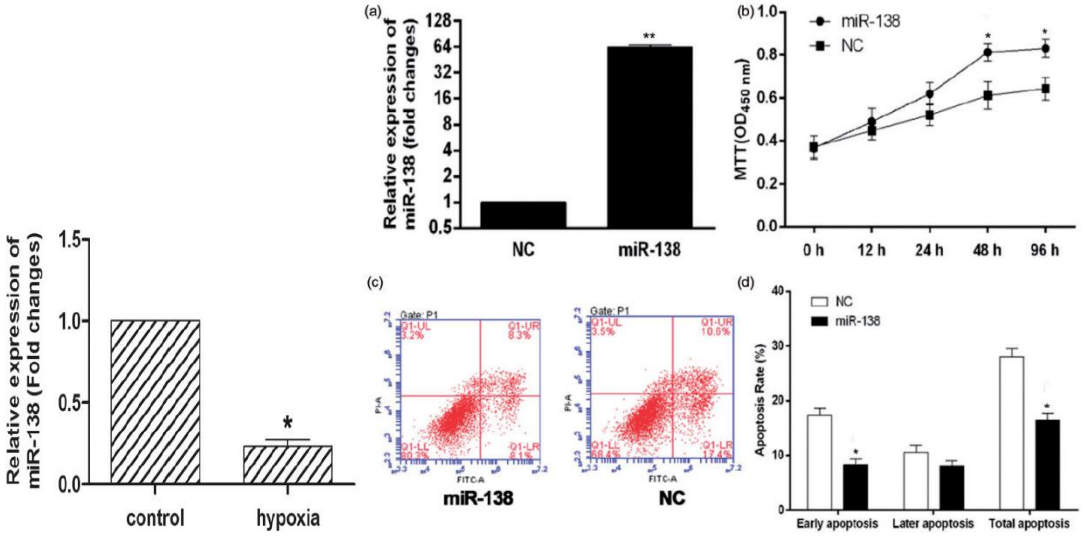

Figure 1. Expression of miR-138 in hypoxic cardiomyocytes

Figure 2. Effect of miR-138 over-expression on cell growth and apoptosis in hypoxic cardiomyocytes

(a) The over-expression of miR-138 in miR-138 upon mimetic transfection was validated using qRT-PCR. HL-1 cells transfected with empty plasmid were used as a negative control (NC).

(b) After transfected with miR-138 mimics, HL-1 cells were cultured in 1% O2 and 5% CO2 (hypoxia) for different duration, and cell survival curve was measured by MTT.

(c) Exposed to hypoxia for 48 h, cell apoptosis was tested by Annexin V-FITC/PI flow cytometry, and the proportion of apoptosis cells was measured.

(d) Cells treated with miR-138 mimics versus cells treated with miR-NC.

在 1%氧浓度下缺氧诱导 24 小时后，小鼠 HL-1 细胞中 miR-138 的表达显著降低，表明心肌细胞中的 miR-138 被低氧调节。而过表达 miR-138 显著促进 HL-1 细胞的增殖，且显著降低了缺氧诱导的细胞凋亡，特别是早期细胞凋亡。

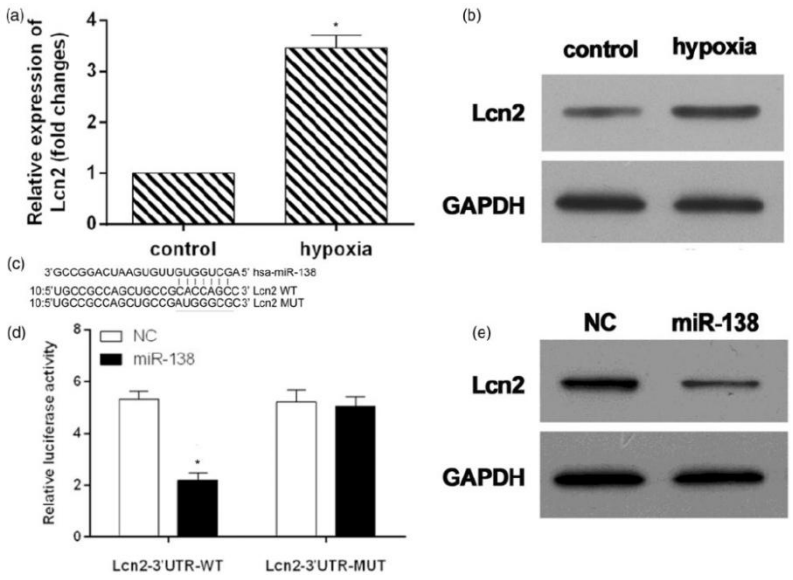

Figure 3. Lcn2 was target gene of miR-138

The mRNA (a) and protein (b) expressions of Lcn2 in hypoxic cardiomyocytes.

(c) Sequence alignment of miR-138 and 3'UTR of Lcn2 using TargetScan algorithm.

(d) HL-1 cells were co-transfected with miR-138 mimics and a luciferase reporter containing a fragment of the Lcn2 3'UTR harboring either the miR-138 binding site (Lcn2-3′UTR-WT) or a mutant (Lcn2-3′UTR-MUT). The assays showed that luciferase activity in the Lcn2-3′UTR-WT group was significantly decreased compared to the luciferase activity of the mutant groups.

(e) Western blot detection of Lcn2 expression in HL-1 cells treated with miR-138-overexpression or NC constructs..

在缺氧（1% O_2）条件下培养 48 h，Lcn2（促凋亡蛋白）的转录及蛋白水平显著增加。双荧光素酶实验验证了 miR-138 与 Lcn2的靶向结合，过表达 miR-138可显著下调 HL-1 细胞中 Lcn2 蛋白表达，进一步表明Lcn2是miR-138的靶基因。

其他的应用文章

文章题目： miR-9 knockdown inhibits hypoxia-induced cardiomyocyte apoptosis by targeting Yap1（敲除 miR-9 通过靶向 Yap1 抑制缺氧诱导的心肌细胞凋亡。）

文章出处：Life Sci. 2019 Feb 15;219:129-135. 河南省人民医院儿童心脏科，福建省阜外中心心血管病医院