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热休克蛋白 gp96 3′UTR 作为 ceRNA 通过 miR-642a 调控 DOHH 的表达 *

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摘要 热休克蛋白 gp96 在肝脏等多种肿瘤中过量表达，与肿瘤的恶性程度和患者不良预后呈显著相关性，其在肿瘤发生发展中作用机制有待深入探讨．通过生物信息学技术预测、荧光素酶报告基因检测、免疫印迹分析、实时定量 PCR、RNA 干扰，研究 gp96 3′UTR 作为 ceRNA (competing endogenous RNA) 对 miR-642a 和脱氧羟腐氨酸羟化酶 (deoxyhypusine hydroxylase，DOHH)表达的影响．研究结果显示，miR-642a 特异性靶向的野生型 gp96 3′UTR，而不是 miR-642a 结合位点突变的 gp96 3′UTR，可吸附下调 miR-642a 并同时上调 miR-642a 靶基因 DOHH 的表达，进一步研究发现 gp96 3′UTR 对 DOHH 的调控依赖于 miR-642a．实验还发现 DOHH 并不影响 gp96 的表达．Gp96 通过 ceRNA 上调 DOHH 的表达，为研究 gp96 促进肝癌等肿瘤的发生发展提供了新思路．

关键词 热休克蛋白 gp96，miR-642a，脱氧羟腐氨酸羟化酶，ceRNA
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热休克蛋白 gp96 (又称 GRP94)，作为内质网中最主要的蛋白之一，是细胞质热休克蛋白 HSP90 的同源蛋白质[1]，gp96 作为分子伴侣参与新生蛋白质的折叠和变性蛋白质的降解[2]．我们和他人的研究均发现 gp96 在肝癌、乳腺癌、骨髓瘤等多种肿瘤高表达，已经查明 gp96 与 Wnt 共受体 LRP6、胰岛素样生长因子、整合蛋白、HER2、uPAR 等相互作用，对于维持这些肿瘤蛋白的稳定性和功能发挥重要调节作用[3-7]，然而 gp96 在调节肿瘤发生、发展的作用机制仍需进一步深入研究．

microRNAs(miRNA)是一类由约 22 个核苷酸组成的非编码 RNAs，可与靶 mRNA 的 3′UTR 不完全配对结合，从而导致靶基因的翻译抑制或降解[8-9]，参与细胞的生长、凋亡、分化等的调节[10-12]．最近的研究发现，长链非编码 RNA、假基因编码的 RNA 以及部分 mRNA、甚至病毒 mRNA 可作为“海绵”吸附、隔离与其相互作用的 miRNA，从而抑制 miRNA 的功能，进而导致 miRNA 其他靶基因的表达上调[13-17]．竞争性内源 RNAs (competing endogenous RNA，ceRNAs) 首先由 Pandolfi 等提出，他们发现细胞中的长非编码 RNA、假基因 RNA 和环状 RNA 与编码蛋白的 mRNA 包含有共有的 miRNA 结合序列，通过诱饵或“海绵”吸附的机制可竞争性结合 miRNA，因此这些不同类型的 RNA 之间形成相互调控的网络，可能在生物发育和诸如肿瘤等疾病发生、发展中发挥重要作用[13,15]．

鉴于 gp96 蛋白、mRNA 的丰度在细胞中尤其是在肿瘤细胞中均很高，本研究将探索 gp96 mRNA 是否作为 ceRNA 发挥调节功能，研究 gp96 3′UTR 作为 ceRNA 对 miR-642a 和脱氧羟腐氨酸羟化酶(deoxyhypusine hydroxylase，DOHH)表达的影响．结果表明：gp96 3′UTR 可通过抑制 miR-642a 的表达，上调 miR-642a 靶基因 DOHH 的表达．

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1　材料与方法

1.1　材料

1.1.1　质粒、siRNA 和细胞株

通过 PCR 扩增含有 miR-642a 结合序列的 gp96 3′UTR，特异性引物序列如下：上游引物，5′ TTATACTCTCACCATTTGGATC 3′；下游引物 5′ TGACAAGATTTTACATCAAGAA 3′，将得到的 PCR 产物克隆到 pGL3-control 荧光素酶报告载体上，得到的重组载体被命名为 gp96-wt. 将 gp96-wt 质粒中 miR-642a 结合的种子序列通过试剂盒突变后得到的质粒命名为 gp96-mut. pcDNA3.1-gp96 为本实验室构建，pGL3-control(Promega 公司)、pcDNA3.1(+) (Invitrogen 公司)和海肾萤光素酶对照报告基因载体 pRL-TK 由本实验室保存.

gp96 的 siRNA 序列：5′ AAGUUGAUGAAC-UGAGAGUACUUCC 3′，由广州市锐博生物科技有限公司合成.

293T 购自美国模式菌种收集中心(ATCC)，人肝癌细胞系 Huh-7 购自上海协和细胞库.

1.1.2　试剂与抗体

小鼠抗人 gp96 抗体和小鼠抗人 DOHH 抗体购自 Santa Cruz Biotechnology；小鼠抗人 β-actin 抗体和辣根过氧化物酶标记的二抗，购自北京中杉金桥生物技术有限公司. 双荧光素酶报告基因检测试剂盒购自 Promega 公司，转染试剂 Lipofectamine 2000 和 opti-MEM 购自 Invitrogen 公司. RNA 提取试剂 Trizol 购自天根生化科技有限公司. DMEM 培养基及胎牛血清购自 Gibco 公司. SYBR Green Premix 试剂、反转录试剂盒，购自 TaKaRa 公司. ECL 超敏发光液，购自北京普利莱基因技术有限公司. miR-642a mimics， miR-642a inhibitor 均由广州锐博生物公司合成.

1.2　方法

1.2.1　RNA 提取、反转录和荧光定量 PCR

按 Trizol 试剂操作说明提取总 RNA，取 500 ng RNA 作为模板，进行反转录. 采用 Takara 公司的 SYBR® Premix Ex Taq™ Ⅱ (Perfect Real Time) 检测试剂盒(GAPDH 作为内参)进行荧光定量 PCR (Real-time PCR)检测 mRNA 的表达；所用的引物序列为： gp96 (forward)， 5′ CAGTTTTGGATC-TTGCTGTGG 3′；gp96(reverse)，5′ TACAGCAA-CTGTCGCCACC 3′；DOHH(forward)，5′ TGTCA-TCGCTTGTGTCTTGC 3′；DOHH(reverse)，5′ G-GAGTCACAGCACAAC 3′； GAPDH (forward)，5′ GGTGAAGGTCGGTGTGAACG 3′； GAPDH (reverse)，5′ CTCGCTCCTGGAAGATGGTG 3′.

miR-642a 表达水平的检测采用 ABI 公司的 TaqMan miRNA 试剂盒进行 Real-time PCR 检测 (U6 作为内参).

1.2.2　细胞培养与转染

所有细胞均用含 10%胎牛血清的 DMEM 培养基(100 U/ml 青霉素、100 mg/L 链霉素)在 37℃、5% CO_2 培养箱中培养. 转染前一天消化细胞，按照合适细胞数接种于 6 孔板或 12 孔板中，按 Lipofectamine 2000 说明书进行转染.

1.2.3　双荧光检测

在 Huh7 细胞中共转染 pRL-TK、miR-642a 或 mimic control (mock)、 gp96-wt 或 gp96-mut 质粒 48 h 后，将细胞裂解后按照说明书进行细胞双荧光素酶检测. 通过荧光发光仪分别检测萤火虫荧光素酶的活性和海肾荧光素酶活性，并以海肾荧光素酶活性作为内参分析荧光素酶的活性.

1.2.4　蛋白质免疫印记

细胞转染 48 h 后，在裂解液(0.05 mol/L Tris, pH 7.6，含 0.15 mol/L NaCl，2 mmol/L EDTA，1% Nonidet P-40)中加入蛋白酶抑制剂(德国 Roche 公司)对细胞进行裂解. 用 BCA 法测蛋白质浓度，每个样品取等量蛋白质用 10% SDS-PAGE 电泳分离；将蛋白转移到 PVDF 膜上(美国 Millipore 公司)；用含有 5%脱脂奶粉的 TBST 封闭 2 h，加入一抗(1： 1 000) 4℃孵育过夜，以 TBST 洗 5 次，每次 10 min，然后加入辣根过氧化物酶标记的二抗(1： 2 000) 室温孵育 2 h 后，同样用 TBST 洗去二抗，最后用 ECL 超敏发光液显影检测.

1.2.5　统计学分析

每个试验组有 3 个复孔，所有的试验都重复 2～3 次. 采用 t-test 和 Pearson's χ 计算各组之间的差异，$P < 0.05$ 认为具有差异，$P < 0.01$ 认为具有显著差异. 结果以 $\bar{x} \pm s$ 表示.

2　结　　果

2.1　gp96 3′UTR 含有 miR-642a 的靶点

使用 Microcosm Targets (http://www.ebi.ac.uk/enright-srv/microcosm/cgi-bin/targets/v5/search.pl) 预测 gp96 3′UTR 上 miRNA 潜在作用靶点，按分值由高到低依次为： miR-513-3p、 miR-642a、miR-152、miR-578、miR-215、miR-192、miR-454、

miR-640、miR-99a、miR-613、miR-186、miR-100等．由于Michael等发现在前列腺癌细胞中miR-642a靶向脱氧羟腐氨酸羟化酶(DOHH)，下调DOHH从而影响细胞增殖[18]．因此本研究进一步验证miR-642a与gp96 3′UTR之间的相互作用．首先使用PGL3-control构建了含有gp96 3′UTR的野生型(gp96-wt)和miR-642a结合位点种子序列突变(gp96-mut)的荧光素酶报告质粒(图1a)，将gp96-wt、gp96-mut质粒和pRL-TK分别与miR-642a、mimic control(mock)在Huh7细胞系中共转染，48 h后用双荧光素酶报告基因检测试剂盒检测荧光活性．结果显示转染miR-642a可明显降低gp96-wt质粒的荧光值，而对gp96-mut没有作用(图1b)．同时Western blot结果显示转染miR-642a能明显下调gp96蛋白质水平(图1c)，以上结果表明miR-642a靶向gp96 3′UTR．

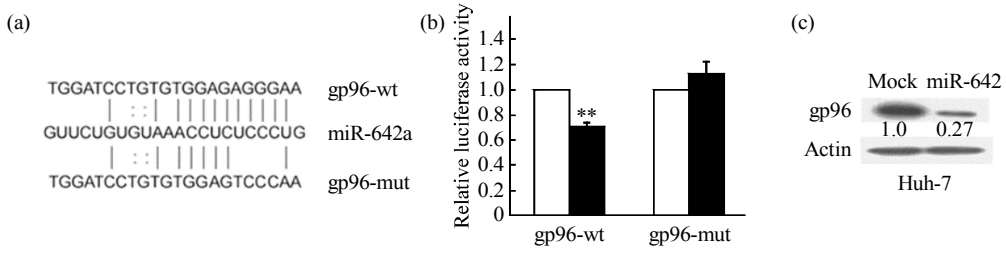

Fig. 1　MiR-642a down-regulates gp96 expression through binding to its 3′UTR

Predicted miR-642a binding sequence located in the gp96 3′UTR at 2612nt to 2613nt. Perfect matches are indicated by a line. Mutations were made in the seed region of the miR-642a binding sites (a). Huh7 cells were co-transfected with a miR-642a mimic or a mimic control (mock) and luciferase reporter containing wild type (gp96-wt) or mutated (gp96-mut) gp96 3′UTR. At 48 h post-transfection, the firefly luciferase and renilla luciferase activities were measured using a dual-luciferase assay kit. $^{**}P < 0.01$ (b). □: Mock; ■: miR-642a. Huh7 cells were transfected with miR-642 mimic or a mimic control as mock. At 48 h after transfection, gp96 protein levels were measured by Western blotting. Actin was used as a loading control. (c).

2.2　gp96 3′UTR上调DOHH的表达

在Huh-7细胞中过表达野生型gp96 3′UTR显著下调miR-642a的水平，而miR-642a结合位点突变型3′UTR则对miR-642a没有影响(图2a)，说明gp96 3′UTR上与miR-642a结合的位点引起miR-642a的下调．反之，干扰gp96 mRNA的表达

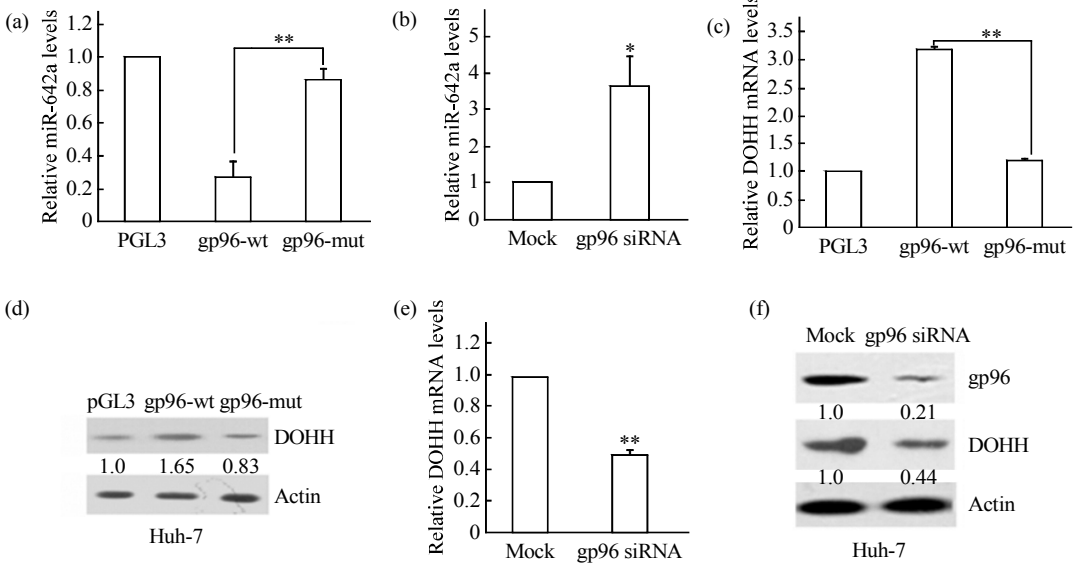

Fig. 2　Gp96 3′UTR up-regulates DOHH expression

At 24 h after Huh7 cells were transfected with gp96-wt, gp96-mut or PGL3 as a control, miR-642a (a) and DOHH mRNA (c) levels was quantified with real-time PCR. The protein levels of DOHH were detected at 48 h by Western blotting. Actin was used as a loading control (d). Huh-7 cells were transfected with gp96 siRNA or the control siRNA as a mock, miR-642a (b) and DOHH mRNA (e) levels was quantified with real-time PCR at 24h. Western blotting analysis of gp96 and DOHH protein levels at 48 h (f). $^{*}P<0.05$, $^{**}P<0.01$.

则上调 miR-642a 的水平(图 2b)．鉴于前期研究发现 DOHH 的 mRNA 是 miR-642a 的靶分子[18]，因此进一步检测 gp96 3′UTR 对 DOHH 的影响．正如所预期，与转染空载体和 gp96-mut 相对比，转染 gp96-wt 可显著上调 DOHH 的 mRNA(图 2c)和蛋白质水平(图 2d)($P < 0.01$)，同时利用 RNAi 敲低 gp96 的表达引起 DOHH mRNA(图 2e)和蛋白质水平(图 2f)的降低．

为了进一步排除 gp96 蛋白质对 DOHH 表达产生影响的可能性，构建表达 gp96 的 pcDNA3.1 的载体 pcDNA3.1-gp96，表达载体不含 gp96 3′UTR. 转染 pcDNA3.1-gp96 并不明显影响细胞中 miR-642a 的水平(图 3a)，也不影响 DOHH 的 mRNA(图 3b)和蛋白质水平(图 3c)．以上结果提示 gp96 3′UTR 作为 ceRNA 调节 DOHH 的表达．

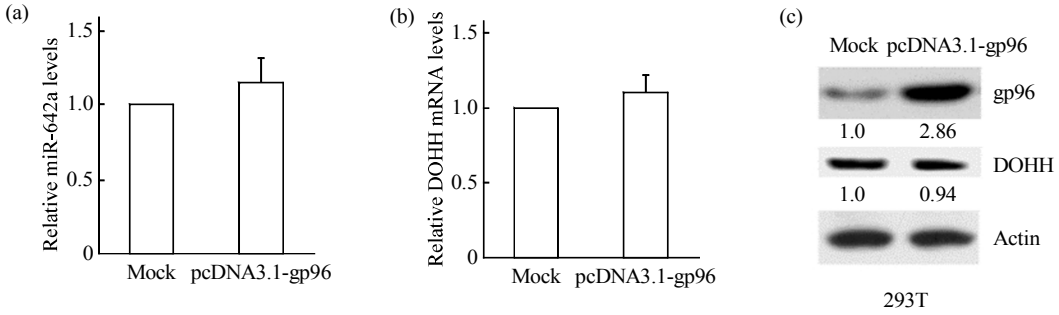

Fig. 3　Overexpression of gp96 protien does not affect miR-642a and DOHH levels

293T cells were transfected with the gp96 expression vector pcDNA3.1-gp96 or pcDNA3.1 as a mock. The miR-642a (a) and DOHH mRNA (b) levels were determined by real-time PCR at 24 h after transfection. At 48 h after transfection, gp96 and DOHH protein levels were assessed by Western blotting. Actin was used as a loading control (c).

2.3　gp96 通过 miR-642a 参与调控 DOHH 的表达

为进一步探讨 gp96 3′UTR 是否通过 miR-642a 调节 DOHH 的表达，人工合成 miR-642a 的反义抑制剂(miR-642a inhibitor) 如图 4a 和图 4b 所示，与对照组相比，干扰 gp96 表达能显著下调 DOHH 的 mRNA 和蛋白质水平($P < 0.01$)，但如果同时转染 miR-642a 抑制剂则 gp96 失去对 DOHH 的调节作用，说明 gp96 调控 DOHH 的表达依赖于 miR-642a．

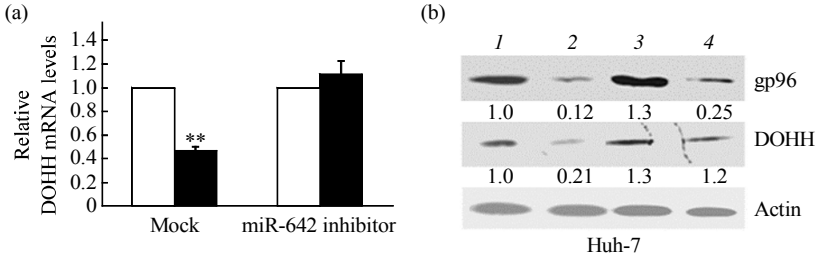

Fig. 4　Gp96 regulates DOHH expression through miR-642a

Huh7 cells were co-transfected with gp96 siRNA or control siRNA and miR-642 inhibitor or an inhibitor control (mock). At 24 h after transfection, DOHH mRNA levels were quantified by real-time PCR (a). □: Mock; ■: gp96 siRNA. At 48 h after transfection, the expression of gp96 and DOHH protein was detected by Western blotting (b). **$P < 0.01$. *1*: Mock; *2*: gp96 siRNA; *3*: miR-642a inhibitor; *4*: gp96 siRNA + miR-642a inhibitor.

2.4　DOHH 不影响 gp96 表达

进一步研究 DOHH 是否对 gp96 表达有调控作用．与对照组相比，干扰 DOHH 基因表达后 Huh-7 细胞中 miR-642a(图 5a)，gp96 mRNA(图 5b)和蛋白质(图 5c)水平均没有明显变化，说明 DOHH 不影响 gp96 表达．

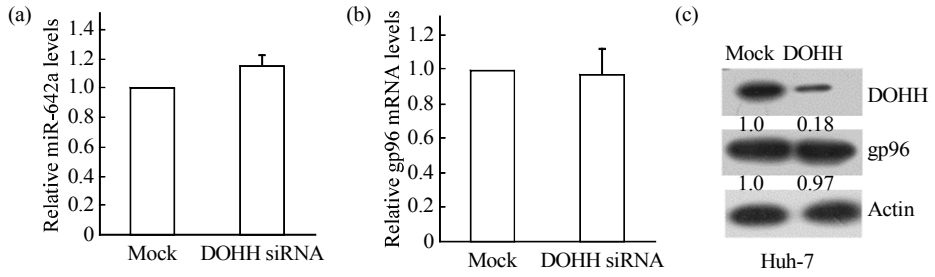

Fig. 5　DOHH does not affect gp96 expression

Huh7 cells were transfected with DOHH siRNA or the control siRNA as mock. At 24 h after transfection, the miR-642a (a) and gp96 (b) mRNA levels were determined by real-time PCR. At 48 h after transfection, gp96 and DOHH protein levels were detected by Western blotting. Actin was used as a loading control (c).

3　讨　　论

我们及他人的研究均发现，ceRNA 的调控依赖于具有相同 miRNA 结合位点的 mRNA 的丰度、miRNA 的水平以及 miRNA 与靶 mRNA 之间的结合能力等[19-20]．本研究发现，gp96 3′UTR 可调控 miR-642a 和 DOHH 的表达，反之 DOHH则不能调控 gp96 的表达，推测这是由于在肝癌细胞中 gp96 mRNA 的丰度远大于 DOHH mRNA 的水平，gp96 与 DOHH mRNA 之间的调控机制有待进一步深入探讨．

DOHH 又称 NHLRC1，是一个参与羟腐氨酸(hypusine)合成的金属酶．它包含 8 个串联 HEAT 重复序列，4 个重复序列在 N 端和 4 个在 C 端[21]．DOHH 介导真核细胞翻译起始因子 5A(eIF5A)的翻译后修饰，eIF5A 翻译后第 49 位的赖氨酸被修饰为一个特殊氨基酸 hypusine，对其发挥功能必需．hypusine 残基合成分二步：a．由脱氧羟腐氨酸合成酶(deoxyhypusinesynthase，DHS)催化赖氨酸残基变成脱氧羟腐氨酸；b．脱氧羟腐氨酸残基被 DOHH 羟基化，完成 hypusine[22]．由于蛋白质合成异常是导致细胞癌变和细胞异常增殖的标志性特征，因此 DOHH 以及受其调控的 eIF5A 与多种肿瘤的发生、发展、转移密切相关[23-25]．

本研究通过生物信息预测和靶点验证、实验分析发现热休克蛋白 gp96 3′UTR 作为 ceRNA 正向调控 DOHH 的表达，这种调控作用依赖于 miR-642a、不依赖于 gp96 的蛋白质表达．我们实验室以及其他人的研究均发现乙肝慢性感染显著上调 gp96 的 mRNA 和蛋白质表达[26-29]，gp96 的表达水平与慢性乙肝疾病进展密切相关，gp96 在肝肿瘤中过量表达与肿瘤的恶性程度和不良预后呈显著相关性[30]．目前对 gp96 在肿瘤发生发展中的作用机制全部集中在其作为分子伴侣蛋白的功能，并且我们的研究发现 gp96 mRNA 的 3′UTR 作为 ceRNA 可上调肿瘤基因 DOHH 的表达，这为深入研究 gp96 如何参与调节肝癌等肿瘤的发生发展提供了一种新思路．需要进一步对包括 DOHH 在内的可能受 gp96 3′UTR 调控的基因在肿瘤中的表达水平及信号通路进行深入研究，这将为全面分析 gp96 促进肿瘤细胞生长的机制以及靶向治疗提供理论依据．

参　考　文　献

[1] Wu S, Hong F, Gewirth D, *et al*. The molecular chaperone gp96/GRP94 interacts with Toll-like receptors and integrins via its C-terminal hydrophobic domain. The Journal of Biological Chemistry, 2012, **287**(9): 6735-6742

[2] Weekes M P, Antrobus R, Talbot S, *et al*. Proteomic plasma membrane profiling reveals an essential role for gp96 in the cell surface expression of LDLR family members, including the LDL receptor and LRP6. Journal of Proteome Research, 2012, **11** (3): 1475-1484

[3] Rachidi S, Sun S, Wu B X, *et al*. Endoplasmic reticulum heat shock protein gp96 maintains liver homeostasis and promotes hepatocellular carcinogenesis. Journal of Hepatology, 2015, **62**(4): 879-888

[4] Chhabra S, Jain S, Wallace C, *et al*. High expression of endoplasmic reticulum chaperone grp94 is a novel molecular hallmark of malignant plasma cells in multiple myeloma. Journal of Hematology & Oncology, 2015, **8**: 77

[5] Li X, Sun L, Hou J, *et al*. Cell membrane gp96 facilitates HER2 dimerization and serves as a novel target in breast cancer.

International Journal of Cancer Journal International du Cancer, 2015, **137**(3): 512−524

[6] Hou J, Li X, Li C, *et al*. Plasma membrane gp96 enhances invasion and metastatic potential of liver cancer *via* regulation of uPAR. Molecular Oncology, 2015, **9**(7): 1312−1323

[7] Li X, Wang B, Liu W, *et al*. Blockage of conformational changes of heat shock protein gp96 on cell membrane by a alpha-helix peptide inhibits HER2 dimerization and signaling in breast cancer. PloS One, 2015, **10**(4): e0124647

[8] Bartel D P. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004, **116**(2): 281−297

[9] Humphreys D T, Westman B J, Martin D I, *et al*. MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. Proc Natl Acad Sci USA, 2005, **102**(47): 16961−16966

[10] Chen J F, Mandel E M, Thomson J M, *et al*. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetics, 2006, **38**(2): 228−233

[11] Cheng A M, Byrom M W, Shelton J, *et al*. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Research, 2005, **33**(4): 1290−1297

[12] Carleton M, Cleary M A, Linsley P S. MicroRNAs and cell cycle regulation. Cell Cycle, 2014, **6**(17): 2127−2132

[13] Karreth F A, Reschke M, Ruocco A, *et al*. The BRAF pseudogene functions as a competitive endogenous RNA and induces lymphoma *in vivo*. Cell, 2015, **161**(2): 319−332

[14] Li C, Hu J, Hao J, *et al*. Competitive virus and host RNAs: the interplay of a hidden virus and host interaction. Protein & Cell, 2014, **5**(5): 348−356

[15] Tay Y, Rinn J, Pandolfi P P. The multilayered complexity of ceRNA crosstalk and competition. Nature, 2014, **505** (7483): 344−352

[16] Xu J, Li Y, Lu J, *et al*. The mRNA related ceRNA-ceRNA landscape and significance across 20 major cancer types. Nucleic Acids Research, 2015, **43**(17): 8169−8182

[17] Jens M, Rajewsky N. Competition between target sites of regulators shapes post-transcriptional gene regulation. Nature Reviews Genetics, 2015, **16**(2): 113−126

[18] Epis M R, Giles K M, Kalinowski F C, *et al*. Regulation of expression of deoxyhypusine hydroxylase (DOHH), the enzyme that catalyzes the activation of eIF5A, by miR-331-3p and miR-642-5p in prostate cancer cells. The Journal of Biological Chemistry, 2012, **287**(42): 35251−35259

[19] Ala U, Karreth F A, Bosia C, *et al*. Integrated transcriptional and competitive endogenous RNA networks are cross-regulated in permissive molecular environments. Proc Natl Acad Sci USA, 2013, **110**(18): 7154−7159

[20] Hu J, Xu Y, Li C, *et al*. A cross-talk between Hepatitis B virus and host mRNAs confers viral adaptation to liver. Scientific Reports, 2015, **5**: 10572

[21] Park J H, Aravind L, Wolff E C, *et al*. Molecular cloning, expression, and structural prediction of deoxyhypusine hydroxylase: a HEAT-repeat-containing metalloenzyme. Proc Natl Acad Sci USA, 2006, **103**(1): 51−56

[22] Chamot D, Kuhlemeier C. Differential expression of genes encoding the hypusine-containing translation initiation factor, eIF-5A, in tobacco. Nucleic Acids Research, 1992, **20**(4): 665−669

[23] Fujimura K, Wright T, Strnadel J, *et al*. A hypusine-eIF5A-PEAK1 switch regulates the pathogenesis of pancreatic cancer. Cancer Res, 2014, **74**(22): 6671−6681

[24] Memin E, Hoque M, Jain M R, *et al*. Blocking eIF5A modification in cervical cancer cells alters the expression of cancer-related genes and suppresses cell proliferation. Cancer Res, 2014, **74**(2): 552−562

[25] Sievert H, Pallmann N, Miller K K, *et al*. A novel mouse model for inhibition of DOHH-mediated hypusine modification reveals a crucial function in embryonic development, proliferation and oncogenic transformation. Disease Models & Mechanisms, 2014, **7**(8): 963−976

[26] Yao D-F, Wu X-H, Su X-Q, *et al*. Abnormal expression of HSP gp96 associated with HBV replication in human hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int, 2006, **5**(3): 381−386

[27] Liu Z, Li X, Qiu L, *et al*. Treg suppress CTL responses upon immunization with HSP gp96. European Journal of Immunology, 2009, **39**(11): 3110−3120

[28] Zhu X-D, Li C-L, Lang Z-W, *et al*. Significant correlation between expression level of HSP gp96 and progression of hepatitis B virus induced diseases. World Journal of Gastroenterology, 2004, **10**(8): 1141−1145

[29] Lim S O, Park S G, Yoo J-H, *et al*. Expression of heat shock proteins (HSP27, HSP60, HSP70, HSP90, GRP78, GRP94) in hepatitis B virus-related hepatocellular carcinomas and dysplastic nodules. World Journal of Gastroenterology, 2005, **11**(14): 2072−2079

[30] Wu B, Chu X, Feng C, *et al*. Heat shock protein gp96 decreases p53 stability by regulating Mdm2 E3 ligase activity in liver cancer. Cancer Letters, 2015, **359**(2): 325−334

Heat Shock Protein gp96 3′UTR Functions as A ceRNA in Promoting DOHH Expression via miR-642a*

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Abstract Heat shock protein gp96 is overexpressed in many kinds of tumors including hepatic tumors, and its overexpression is significantly correlated with tumor malignant degree and poor prognosis in patients. The mechanisms of heat shock protein gp96 in the development of tumors need to be further investigated. The effects of gp96 3′UTR as a ceRNA (competing endogenous RNA) on miR-642a and DOHH expression was studied through bioinformatics prediction, luciferase reporter assay, Western blotting, real-time PCR, RNA interference. MiR-642a specifically targets gp96 3′UTR. The wild type but not the mutant gp96 3′UTR in miR-642a binding site could sequester and downregulate miR-642a levels, which led to increased expression of the miR-642a target DOHH. Further studies showed that regulation of DOHH expression by gp96 3′UTR was miR-642a dependent. It was also found that DOHH does not affect the expression of gp96. Heat shock protein gp96 promotes DOHH expression *via* its 3′UTR as a ceRNA, providing new insights into the role of gp96 on the development of hepatic tumors and other tumors.

Key words Gp96, miR-642a, DOHH, ceRNA

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