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**HnRNPA2/B1 在乳腺癌细胞 MCF-7 中的  
作用与机制研究**

**The role and mechanism of hnRNP A2/B1 in MCF-7 Cells**

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## 摘要

核不均一核糖核蛋白 A2/B1 (hnRNPA2/B1) 在很多肿瘤中高表达, 但其在乳腺癌发生发展中的作用还不明确。为了探究其在乳腺癌中的具体作用与机制, 我们通过 CRISPR-Cas9 基因编辑技术构建敲除 hnRNPA2/B1 的 MCF-7 细胞系, 检测其对生物学功能的影响, 并研究 hnRNPA2/B1 对部分基因的可变剪接的作用。还通过亲和纯化技术结合液质联用质谱 (HPLC-MS), 构建与分析 hnRNPA2/B1 相互作用蛋白网络, 利用生物信息学方法对 hnRNPA2/B1 相互作用蛋白进行 KEGG 信号通路及功能分析, 进一步来研究 hnRNPA2/B1 在乳腺癌细胞中的作用与机制。

MTT 法检测发现敲除 hnRNPA2/B1 后细胞增殖会降低, transwell 检测发现细胞迁移和侵袭会增加, 并抑制细胞的凋亡和自噬。Western blotting 和定量 PCR 结果显示, 敲除 hnRNPA2/B1 后, 与增殖相关的基因 STAT3、P-STAT3、AKT、P-AKT、CDK4 表达减少, 细胞周期抑制因子 P27 增加。与 EMT 相关的基因 E-Cadherin 减少, Snail、Vimentin、Twist 增加, 并且 VEGF-A 增加, 促进了 EMT 的发生; 促凋亡相关的基因 Bax 减少, 裂解的 Caspase-3 减少, 与自噬相关的基因 LC3B II 减少, 自噬抑制因子 P62 增加。可变剪接研究结果显示 hnRNPA2/B1 参与了多个基因的可变剪接调控。敲除 hnRNPA2/B1 后, 促迁移的 RON 基因的外显子 11 的跳跃增加; Caspase-9 基因外显子 3 到 6 的跳跃增加, 全长的 Caspase-9 减少而抗凋亡的 Caspase-9B 增加; 抗凋亡的 C-FLIP 长的异构体增加。回复实验中恢复表达 hnRNPA2/B1 后 STAT3、E-Cadherin 水平增加, Snail、Vimentin、Twist 水平减少。

此外, hnRNPA2/B1 的相互作用蛋白网络的研究结果显示, hnRNPA2/B1 相互作用蛋白主要涉及到的 KEGG 信号通路有 RNA 运输、RNA 的降解、TCA 循环、细胞增殖、氧化磷酸化、丙酮酸代谢、阿尔兹海默氏病、蛋白酶体、细胞周期的进程、细胞粘附、细胞凋亡等。根据其所涉及到的信号通路结合我们生物学实验的结果, 我们从中找寻找到了重要的节点蛋白 STAT3 和 NPM, 并用 CO-IP 验证其与 hnRNPA2/B1 有相互作用, 激光共聚焦技术检测到其与 hnRNPA2/B1 存在共定位现象。

上述研究结果表明, 敲除 hnRNPA2/B1 会抑制乳腺癌细胞的增殖, 促进其迁移和侵袭, 抑制其凋亡和自噬, 而且 hnRNPA2/B1 与 STAT3、NPM 有相互作用。我们的结果提示 hnRNPA2/B1 可能在乳腺癌中的作用并不简单的是肿瘤抑制或促进作用。HnRNPA2/B1 在肿瘤的进程中可能起着双重的作用, 其促进了细胞的增殖, 但抑制了细胞的迁移和侵袭。HnRNPA2/B1 的表达水平可能成为乳腺癌进展及预后的一个潜在标志物。

**关键词:** CRISPR-Cas9; hnRNPA2/B1; 相互作用蛋白网络; 细胞迁移; 乳腺癌

## Abstraction

An emerging body of data shows the overexpression of hnRNPA2/B1 in many cancers, but its specific molecular mechanism in tumors is still poorly understood. In order to explore the mechanism of hnRNPA2/B1 and find valid therapeutic target in breast cancer. We used CRISPR-Cas9 to construct the stable MCF-7 cells in which hnRNPA2/B1 were knock-out, and explored the change of proliferation, migration, invasion, apoptosis, autophagy. Besides, affinity purification by flag magnetic beads and identified the separated proteins by HPLC-MS. Bioinformatics analysis of MS results using string database and constructed the interaction network of hnRNPA2/B1, the KEGG pathway of the interaction network of hnRNPA2/B1.

MTT assay showed knock-out of hnRNPA2/B1 reduce the proliferation of MCF-7 cells, transwell assay results showed knock-out of hnRNPA2/B1 increased the migration and invasion of MCF-7 cells, besides knock-out of hnRNPA2/B1 inhibited apoptosis and autophagy. Western blotting results showed that the expression of P-STAT3, AKT, P-AKT, CDK4 decreased and the expression of cell cycle inhibitor P27 increased. Some genes related to EMT, for example, the expression of E-Cadherin decreased, the expression of snail, vimentin, twist increased. Some genes related to apoptosis, for instance, the expression of Bax decreased, the expression of cleaved-caspase3 decreased. Some genes related to autophagy, the expression of LC3 II decreased, the expression of P62 increased. QPCR results showed that knock-out of hnRNPA2/B1 decreased the mRNA level of E-Cadherin, and increased the mRNA level of snail, vimentin, twist, VEGF-A. Alternative splicing results showed the skipping of exon 11 of RON increased, knock-out of hnRNPA2/B1 enhanced skipping of exons 3 to 6 generating the anti-apoptotic isoform caspase-9B, and increased the long isoform of c-FLIP. Restored the expression of hnRNPA2/B1 increased the mRNA expression of STAT3, E-Cadherin, and decreased the mRNA expression of Snail, vimentin, twist.

On the other hand, we obtained the interaction network of hnRNPB1, the KEGG pathway of the interaction network of hnRNPB1, including RNA transport, RNA degradation, TCA cycle, cell proliferation, oxidative phosphorylation, cell adhesion, cell apoptosis and so on. We searched the important node protein STAT3 and NPM, we found the interaction between hnRNPA2/B1 and them by CO-IP and the colocalization by immunofluorescence.

In conclusion, our results showed knock-out of hnRNPA2/B1 decreased the proliferation of MCF-7 cells, increased the migration and invasion ability and decreased the apoptosis and autophagy. Besides hnRNPA2/B1 had a interaction with NPM and STAT3. Our results implied that hnRNPA2/B1 was not only a tumor suppressor or oncogene. HnRNPA2/B1 may play dual role in breast tumor progression, it increased the proliferation ability of MCF-7, but decreased the migration and invasion ability of MCF-7 cells. The expression of hnRNPA2/B1 may be a potential markers for breast cancer progression and prognosis.

**Keywords:** CRISPR-Cas9; hnRNPA2/B1; interaction network; cell migration; breast cancer

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厦门大学博硕士论文摘要库

## 第一章 前言

### 1.1 肿瘤细胞特征

肿瘤有八大特征，这八大特点是肿瘤治疗复杂性的原因。

特征一：正常细胞只有在有外部刺激的情况下才会生长，而癌细胞却可以刺激自我生长。正常组织谨慎的控制产生和释放生长相关信号，维持正常组织的结构和功能。癌细胞能够通过多种方式维持细胞生长信号，能够自身产生生长因子受体，也能够传递信号给正常细胞，由正常细胞提供给它各种生长因子。

特征二：癌细胞可以使肿瘤抑制基因失效，无视令其停止生长的命令。DNA高通量测序结果在人类肿瘤细胞中一些生长生长因子受体被激活，使一些信号通路持续激活。人类肿瘤中40%的激活突变会影响B-Raf的结构，使Raf到MAPK信号通路被持续激活<sup>[1]</sup>。相似的，PI3K催化亚单位的突变在很多肿瘤中被发现，使得PI3K信号通路被持续激活<sup>[2,3]</sup>。

特征三：癌细胞拒绝对自己执行细胞凋亡程序，这本是可以让机体清除受损细胞和危险细胞的固定程序。凋亡引起的细胞程序性死亡是癌症发展的屏障，阐述肿瘤发生中细胞凋亡的机制对于癌症治疗有重大意义，细胞凋亡是由上游调控者和下游的受体组成<sup>[4]</sup>。调控者分两种，一种接收和呈递细胞外细胞死亡信号（外在的凋亡程序，例如Fas配体/Fas受体），另外一种整合细胞内来源的各种死亡信号，最终都激活caspases 8和caspases 9，最终引起受体蛋白水解酶去执行细胞凋亡，最终被临近的细胞和吞噬细胞解体和消化。

特征四：正常细胞分裂次数是有限的，而癌症细胞却可以无限分裂，也就是所谓的“长生不老”。有证据显示保护染色体末端的端粒对于癌细胞无限增殖有重要作用<sup>[5]</sup>，端粒由多个串联碱基组成，在非永生化细胞培养过程中会逐渐缩短，最终失去保护染色体末端避免融合的能力（这种融合会产生不稳定的染色体，进而形成不稳定的核型，最终影响细胞的活性），所以端粒的长度对细胞的增殖，避免细胞的衰老有重要作用。端粒酶是一种特殊的DNA聚合酶，对于端粒DNA

末端重复序列的增加是至关重要的，在大部分非永生化细胞中基本上不表达，但在非永生化细胞和癌细胞中基本上都有表达，通过延长端粒 DNA 的长度，端粒酶与抵抗衰老和凋亡有关系。

特征五：癌症细胞可以刺激新血管的生成（血管生成作用）来支持肿瘤的生长<sup>[6]</sup>。像正常组织一样，肿瘤需要持续的营养和氧的供应，也需要排泄代谢废物和 CO<sub>2</sub>，在成人中，只有伤口愈合和女性生殖循环血管发生才会启使，大部分的时候是静止的，相反的，在肿瘤进程中血管生成的开关是持续激活的，使静止的血管系统持续产生新的血管来供应肿瘤的生长。有证据显示血管发生是由诱导血管生成因子和抑制血管生成因子共同作用的结果<sup>[7, 8]</sup>，VEGF-A 基因编码的配体参与新生血管的发生，VEGF 信号是通过 3 个酪氨酸激酶（VEGFR-1 - 3）来调节。缺氧和致癌信号能够提高 VEGF 基因的表达<sup>[9-11]</sup>，另外 VEGF 配体可以和细胞外基质结合，能够被细胞外基质金属蛋白酶（MMP-9）释放和激活<sup>[12]</sup>。除此之外，另外的促肿瘤发生的信号，例如 FGF 家族参与肿瘤血管发生的维持<sup>[8]</sup>，TSP-1 结合血管内皮细胞的跨膜受体，引起抑制性的信号抵抗促血管生成<sup>[13]</sup>。

特征六：癌细胞可以脱离原本所在的位置或器官，侵犯周围组织或向身体远处扩散（转移）。在 2000 年的时候，侵袭和转移的机制任然是个谜，癌来源于上皮组织，向更高的病理学分级进化，伴随着局部的侵袭和远端的转移。癌细胞会发生形状改变，而且与其他细胞的联系和细胞外基质的联系也会改变，最显著的特征 E-Cadherin 减少，E-Cadherin 是一种主要的细胞粘附分子，介导细胞与细胞之间的粘附。在人类肿瘤中 E-Cadherin 的减少和突变失活说明他是肿瘤侵袭和转移的抑制因子<sup>[14, 15]</sup>。

特征七：癌细胞可以利用异常的代谢途径来产生能量。在有氧的情况下正常细胞进行糖代谢，第一步通过糖酵解产生丙酮酸，然后在线粒体产生 CO<sub>2</sub>，在无氧的条件下，更少的丙酮酸到好氧的线粒体。Otto Warburg 观察到了癌细胞异常的能量代谢，癌细胞即使在有氧的状态下也主要通过糖酵解来进行能量代谢。这种代谢产生的能量比有氧糖酵解产生的能量低 18 倍，癌细胞通过上调葡萄糖转运蛋白，最显著的是 GLUT1，它能够使糖输入到胞质中的量增加<sup>[16, 17]</sup>。确实很多肿瘤中糖吸收和利用确实增加，这可以通过放射性的葡萄糖的类似物 FDG 作为报告，PET 可以清楚的看到糖吸收。



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