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**HnRNP A2/B1 在乳腺癌中的作用与
机制研究**

The role and mechanism of hnRNP A2/B1 in breast cancer

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摘要

核不均一核糖核蛋白A2/B1 (hnRNP A2/B1) 是hnRNP蛋白家族成员, 在肿瘤细胞增殖与凋亡过程中具有重要作用。本实验为了探究hnRNP A2/B1蛋白在乳腺癌组织中的表达及其在乳腺癌细胞中的定位。研究hnRNP A2/B1在乳腺癌细胞中所涉及的主要功能、信号通路以及可变剪接和蛋白融合过程, 并探究hnRNP A2/B1对细胞增殖、凋亡、迁移等生物功能的影响。从而阐明hnRNP A2/B1在乳腺癌中的作用机制, 为肿瘤的机理研究、临床诊断和治疗提供一个新靶点。本实验用免疫组化方法检测hnRNP A2/B1在大鼠乳腺组织中的变化, 并结合免疫荧光检测hnRNP A2/B1在姜黄素诱导人乳腺癌细胞凋亡过程中的定位变化。通过构建hnRNP A2/B1蛋白稳定沉默的乳腺癌细胞株, 进而进行转录组测序并通过生物信息分析hnRNP A2/B1所参与的生物功能、信号通路、对其他基因可变剪接的影响以及融合蛋白的形成过程。通过MTT、流式细胞、划痕和Transwell小室迁移实验分别检测了hnRNP A2/B1沉默对细胞增殖、凋亡、迁移的影响。并用免疫印迹和real-time PCR检测了增殖、凋亡、迁移相关的基因变化。利用裸鼠实验进一步验证hnRNP A2/B1在体内的生物功能。

实验结果显示hnRNP A2/B1在大鼠乳腺癌组织中表达增加, 其主要定位在人乳腺癌细胞MDA-MB-231的核内, 经姜黄素诱导凋亡后发生向胞质转移的现象。转录组测序发现, hnRNP A2/B1沉默后, 蛋白结合、序列特异性DNA结合、钙离子结合、转录因子TFIID复合物、细胞黏附、生物黏附相关功能发生变化, 酪氨酸代谢、基质细胞癌、癌症、Wnt、MAPK等通路发生变化, 基因EFCAB4A、WASH7P、CHID1发生可变剪接变化并参与一些基因融合。此外, 细胞实验检测发现在乳腺癌细胞系MDA-MB-231中沉默hnRNP A2/B1后激活了ERK1/2通路, 促进细胞增殖但效果不明显; 促进细胞凋亡, 抗凋亡基因Bcl-2表达下降, 促凋亡基因p53表达上升。hnRNP A2/B1沉默后, 促进细胞迁移, 细胞间粘附性减弱。EMT相关基因E-cadherin表达下降, Twist、Snail、Vimentin表达上升, 侵袭相关基因MMP-9表达增加。体内实验进一步证实沉默hnRNP A2/B1后促进肿瘤生长。

研究结果表明hnRNP A2/B1在乳腺癌中表达增加, 主要定位在细胞核内。

hnRNP A2/B1抑制乳腺癌细胞MDA-MB-231的迁移以及细胞凋亡。hnRNP A2/B1参与蛋白结合、序列特异性DNA结合、钙离子结合、转录因子TFIID复合物、细胞黏附、生物黏附等生物功能以及酪氨酸代谢、基质细胞癌、癌症、Wnt、MAPK等信号通路。hnRNP A2/B1参与基因EFCAB4A、WASH7P、CHID1的可变剪接。

关键词：hnRNP A2/B1；增殖；凋亡

厦门大学博硕士论文摘要库

Abstraction

Heterogeneous nuclear ribonucleoproteins(hnRNP A2/B1),a member of the hnRNP family, plays a key role in cell life process, such as differentiation, apoptosis.

To investigate the difference of expression of hnRNP A2/B1 between the normal and cancer tissue and the change of location of hnRNP A2/B1 during the apoptosis procession. To research main biological function, pathways, splicing and gene fusion in which hnRNP A2/B1 participated and further testified the results of transcriptome sequencing. Illustrating hnRNP A2/B1 involved splicing procession, gene fusion, and the mechanism in breast cancer provides a new target for mechanism research, clinical diagnosis and treatment in cancer.Using immunohistochemical to detect the difference of expression of hnRNP A2/B1 between the normal and cancer tissue, and using the immunofluorescence to observe the change of location of hnRNP A2/B1 during the apoptosis procession. By constructing stable silence cell lines and then was transcriptome sequenced to detect the biological function, pathways, splicing and gene fusion that hnRNP A2/B1 participated in. Using MTT, flow cytometry, scratching, transwell to identify the role of hnRNP A2/B1 in the proliferation, apoptosis, and migration, respectively. Using western blotting and real-time PCR to detect the changes of proliferation, apoptosis, EMT and migration related genes after silencing hnRNP A2/B1. We further verified the biological function of hnRNP A2/B1 through tumor xenograft.

The expression of hnRNP A2/B1 increased in cancer tissue compared with normal and adjacent tissue. HnRNP A2/B1 mainly located in nuclear, it transferred to cytoplasm after induced by curcumin. Transcriptome sequencing indicated that the function of hnRNP A2/B1 mainly involved in specially associating with DNA, proteins, Ca⁺, transcription factor compounds and had a significant influence on cell adhesion and biological adhesion. It also took part in various pathways, for example, Tyrosine metabolism, Basal cell carcinoma, Pathways in cancer, Wnt signaling

pathway, MAPK signaling pathway. And it played a role in splicing and gene fusion procession. After silencing hnRNP A2/B1, The gene EFCAB4A、WASH7P、CHID1 had different splicing. Besides, cell experiments further verified that silencing hnRNP A2/B1 induced proliferation through ERK1/2 pathway and inhibited apoptosis in MDA-MB-231. After silencing hnRNP A2/B1, the adhesion among cells decreased significantly due to changes of the expression of EMT and migration related genes. The expression of E-cadherin decreased while its inhibitor snail,twist, vimentin increased. The expression of invasion related gene also increased. Tumor xenograft futher identified that silencing hnRNP A2/B1 induced proliferation.

Our study manifested that the expression of hnRNP A2/B1 increased in cancer tissue and it mainly located in nuclear, it transferred to cytoplasm after induced by curcumin. HnRNP A2/B1 inhibited proliferation, migration, apoptosis. HnRNP A2/B1 mainly involved in associating with DNA, proteins, Ca⁺, transcription factor compounds and had a significant influence on cell adhesion and biological adhesion. It also took part in various pathways, for example, Tyrosine metabolism, Basal cell carcinoma, Pathways in cancer, Wnt signaling pathway, MAPK signaling pathway. And it played a role in splicing and gene fusion procession.

Key Words: hnRNP A2/B1; proliferation; apoptosis

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第一章 前言

1.1 细胞癌变机理研究的理论及实践意义

细胞癌变是机体的正常细胞在机体自身遗传因素及外界环境条件共同作用下,导致细胞发生连续并且不受机体控制的异常增殖^[1];发生癌变的细胞不仅会发生形态结构、核型以及细胞排列的变化,而且细胞表面的物质成份也会有明显改变,癌细胞的转移便是基于其表面的糖蛋白成份的减少,从而减弱了细胞间的黏附作用,使癌细胞通过体液的循环便于向身体其他位置转移^[2]。

正常细胞在具有正常完整的遗传物质前提下,并通过自身严密的分子及功能调控网络来保证自己沿正常的生命周期轨迹增殖、分化与凋亡。然而,机体并不是一直处于稳态环境中,在外界各种致癌因素的诱导下,细胞核内遗传物质会发生突变、扩增、移位、外源或内源基因的插入,如亚硝酸类致癌物与 DNA 鸟嘌呤的 O6 结合后形成了 O6-甲基鸟嘌呤抑制了该鸟嘌呤与正常胞嘧啶的配对,从而发生了碱基置换^[3, 4];动物实验证明用二乙基亚硝胺(diethylnitrosamine, DEN)处理两周龄小鼠,可以使小鼠细胞的遗传物质发生畸变从而导致肝癌形成^[5, 6];一些物理因素如紫外线造成 DNA 链上两个相邻的胸腺嘧啶形成二聚体等。这些致癌因子通过改变细胞遗传物质可以使细胞中抑癌基因失活和原癌基因的激活,抑癌基因失活后无法行使其抗癌功能,如:调控细胞周期、与癌蛋白形成复合物抑制癌蛋白活性、参与信号转导及细胞粘连调控等,最终导致细胞癌变。除了基因组成和结构本身发生改变的原因外,基因表达失调也是引起细胞癌变的另一重要因素,例如一些调节基因表达的蛋白发生了变化进而影响基因的正常表达,特别是位于某些关键部位,细胞会发生癌变^[7]。细胞的癌变机理的研究和阐明为我们寻找肿瘤的治疗靶点提供了有效的理论依据。

1.2 乳腺癌细胞癌变机理研究

乳腺癌是危害女性健康的重要杀手之一,它的发生发展涉及多种原因。研究表明雌孕激素水平的异常增高与乳腺的非典型增生和癌变有密切关系,雌孕激素

受体在雌孕激素诱导乳腺癌的发生过程中起了不可分割的介导作用,雌激素受体(ER)通过与雌激素结合后构象发生改变形成二聚体,将胞外信号传递到胞内,启动特定的靶基因表达合成有关酶和蛋白并能激活一些癌基因的表达而直接致癌,或者刺激一些转化生长因子(TGF- α)和上皮生长因子(EGF)等间接发挥致癌作用^[8]。雌激素受体由 ER α 和 ER β 两种受体组成,两者的作用相反,ER α 与共激活因子结合后激活转录因子(AP-1,c-jun),从而影响细胞的增殖分化,ER β 则抑制转录。临床数据表明大部分乳腺癌患者的 ER α /ER β 的阳性率显著增加^[9,10]。

BRCA1 和 BRCA2 是乳腺癌的两个易感基因,大部分遗传性乳腺癌的发生与他们相关。当遗传或后诱发原因使两条染色体上的 BRCA1 和 BRCA2 等位基因均发生突变后,它们会丧失抑制肿瘤生长的作用,从而导致乳腺癌的发生于发展^[11,12]。研究表明一些癌基因的表达在乳腺非典型增生阶段会明显升高,如 Ras, c-Myc, c-erbB-2 等。原癌基因 HER-2 与乳腺癌的发生发展过程密切相关,并作为乳腺癌转移和预后的重要标记物。HER-2 是一类具有酪氨酸激酶活性的蛋白家族,它可以调节正常乳腺的发育,但其过度表达会导致乳腺癌的发生。它通过 Ras-Raf-Mek-MAPK、PI3K-AKT 等信号通路来发挥促进肿瘤细胞生长、抑制细胞凋亡、增加肿瘤细胞侵袭的作用。临床数据表明,20%-30%的乳腺癌患者的 HER-2 基因表达增高,这些乳腺癌患者一般表现为生存率低、恶性程度高而且易发生淋巴结转移^[13]。Ki-67 是一种和 G0 期相关的核抗原,是目前乳腺癌病变程度的重要临床指标。Ki-67 表达越高,则乳腺癌的恶性程度越高^[14]。

在第 29 届圣安东尼奥乳腺癌研讨会上汇集了不同类型乳腺癌的治疗方法以及基础研究的新进展。用拉帕替尼联合卡培他滨或曲妥珠单克隆抗体治疗乳腺癌 HER-2 阳性乳腺癌、他莫昔芬联合依维莫司治疗激素受体阳性乳腺癌、用卡培他滨或吉西他滨联合顺铂治疗转移三阴性乳腺癌分别取得了显著的效果。揭示乳腺癌的不同病变机理对我们设定针对特定类型的乳腺癌的治疗方法提供了有效的途径。

1.3 肿瘤细胞的增殖与凋亡的调控网络

1.3.1 细胞增殖调控与肿瘤形成机理的研究

1.3.1.1 细胞增殖机理研究

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