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Nucleostemin 和 HDAC1 在卵巢肿瘤中的表达及意义 *

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摘要 目的:研究核干细胞因子(Nucleostemin, NS)和组蛋白去乙酰化酶 1(HDAC1)在卵巢肿瘤及正常卵巢组织中的表达,并分析其表达与临床指标的关系及两者间的表达相关性。**方法:**选择 2010 年至 2017 年我院卵巢石蜡标本 60 例,其中卵巢肿瘤 50 例,正常卵巢组织 10 例。应用免疫组化法检测 NS 与 HDAC1 的表达,并分析它们与年龄、肿瘤分期等临床指标的相关性。**结果:**在 30 例卵巢恶性肿瘤、10 例卵巢交界性肿瘤、10 例卵巢良性肿瘤组织中,NS 表达的阳性率分别为 93.3%、40%、20%,HDAC1 的阳性率分别为 90%、60%、20%。在正常卵巢组织中未见 NS 及 HDAC1 表达。在卵巢恶性肿瘤组中,NS 和 HDAC1 的阳性表达率显著高于其他三组($P<0.05$),两者表达呈正相关($r=0.56, P<0.05$),且均与分化程度呈负相关($r=-0.76, P<0.001$; $r=-0.53, P<0.01$),而与患者年龄、术前血清 CA125 水平、临床分期和病理类型无关。**结论:**NS 和 HDAC1 倾向于卵巢恶性肿瘤中表达,且与卵巢恶性肿瘤的分化程度负相关。

关键词:脑核干细胞因子;组蛋白去乙酰化酶 1;卵巢肿瘤

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Expression and Significance of Nucleostemin and HDAC1 in the Ovarian Tumor*

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ABSTRACT Objective: To study the expression of Nucleostemin (NS) and HDAC1 in ovarian tumor and normal ovarian tissues, and to analyze their relation with clinical indicators and the correlation of their expressions. **Methods:** From 2010 to 2017, 60 ovarian paraffin specimens were selected in our hospital, including 50 ovarian tumors and 10 normal ovarian tissues. Immunostaining was performed using antibodies for NS and HDAC1. Spearman rank correlation was employed for their relations with clinical indicators such as age and clinical stage and the correlation of their expression. **Results:** In 30 cases of malignant ovarian tumors, 10 cases of borderline ovarian tumors and 10 cases of benign ovarian tumors, the positive rates of NS expression were 93.3%, 40%, 20%, and the positive rates of HDAC1 were 90%, 60% and 20%, respectively. There was no expression of NS and HDAC1 in normal ovarian tissues. The positive rates of NS and HDAC1 in malignant ovarian tumors were significantly higher than those in the other three groups ($P<0.05$). There was a positive correlation between the expression of NS and HDAC1 ($r=0.56, P<0.05$) and their expression were negatively correlated with the degree of differentiation and ($r=-0.76, P<0.001$; $r=-0.53, P<0.01$) in malignant ovarian tumors, but not with age, preoperative serum CA125 level, clinical stage and pathological type. **Conclusions:** NS and HDAC1 tend to be expressed and are negatively correlated with the differentiation in malignant ovarian tumors.

Key words: Nucleostemin; HDAC1; Ovarian tumor

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前言

核干细胞因子(Nucleostemin, NS)定位于细胞核仁中,是一种鸟苷三磷酸结合蛋白,可以维持干细胞的增殖并能抑制其向成熟细胞分化,主要参与维持干细胞和肿瘤细胞的增殖分化、周期进程及更新^[1,2]。NS 在乳腺癌、食管癌、脑胶质瘤、膀胱癌等十几种肿瘤组织标本及人胚胎肾细胞(Human embryo kid-

ney, HEK-293)、宫颈癌细胞(Hela 细胞)、人成骨肉瘤细胞(os-732)、肝癌细胞(HepG2)中高表达^[3-7]。研究表明,在卵巢恶性肿瘤组织中 NS 调控 cyclinD1 蛋白的调控,影响肿瘤细胞的增殖与分裂^[8]。NS 基因通过激活 Wnt/betacatenin 信号通路,影响肿瘤的发生、发展、凋亡、侵袭和转移^[9-12]。组蛋白乙酰化和去乙酰化是基因表达过程中重要的调控方式,在转录过程中起着重要的作用^[13],它影响着细胞代谢及生长,与肿瘤的发生同样存

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在密不可分的关系。实验表明,在乳腺恶性肿瘤中,HDAC1 通过 Wnt 信号通路对肿瘤细胞凋亡产生影响。HDAC1 在卵巢恶性肿瘤中的表达特点,以及其对卵巢恶性肿瘤的发生和发展是否起重要作用,尚无相关报道。本研究通过免疫组织化学方法检测卵巢肿瘤组织中的 NS 与 HDAC1 蛋白表达情况,并分析二者在卵巢恶性肿瘤组织中表达的临床意义及对其是否存在相关性进行探讨。

1 资料与方法

1.1 一般资料

1.1.1 组织样本 从本试验收集哈尔滨医科大学附属第四医院 2010~2017 年石蜡标本及组织标本共计 60 例。临床病例资料齐全,术后病理诊断明确,年龄 27~78 岁,平均年龄 54.7 岁。其中正常卵巢组织 10 例。良性卵巢肿瘤组织 10 例。交界性卵巢肿瘤组织 10 例。卵巢恶性肿瘤组织 30 例,卵巢恶性肿瘤组织中卵巢浆液性囊腺癌 10 例,粘液性囊腺癌 8 例,卵巢子宫内膜样癌 8 例,性索间质肿瘤 2 例,生殖细胞瘤 2 例。术前患者未接受过任何化疗与放疗。其中高分化 6 例,中分化 7 例,低分化 17 例。FIGO 分期 I 期 12 例,II 期 3 例,III 期 15 例,IV 期 0 例。

1.1.2 主要试剂 羊抗人 NS 特异性抗体(稀释浓度 1:400)购于美国 R&D Systems, Inc 公司,生物素化兔抗羊二抗及正常兔封闭血清及羊抗人 HDAC1 特异性抗体(稀释浓度 1:200)购于武汉博士德生物工程有限公司,辣根酶标记链霉卵白素购于北京中杉金桥生物公司。免疫组化 S-P 染色即用型试剂盒购自武汉博士德公司。

1.2 实验方法

所有标本常规 10%中性福尔马林固 24 小时,酒精逐级脱水,二甲苯透明,石蜡包埋,3 μm 连续切片分别进行组织学染色和免疫组化染色。H.E.染色再次确认原始组织学诊断。采用免疫组织化学 S-P 法,操作步骤严格按试剂盒说明书进行,阳性对照采用已知阳性片为标准,阴性对照采用 PBS 取代第一抗体,其余步骤相同。该实验采用的显微镜为 Leica DM 2500 显

微镜,电子采集系统选择 Motic 6.0。H.E.片子和免疫组织化学片子由两位病理专家分别阅片后记录免疫组化染色结果。阅片时隐去该病理切片所属患者全部临床信息。卵巢组织和卵巢肿瘤组织的免疫组化结果评价标准如下:在高倍镜下,一张病理切片随机选取 10 个视野。每个视野内取 1000 个细胞进行计数。将染色细胞按照染色程度分三个等级:0,无染色;1,染色程度弱;2,中等强度染色或染色呈强阳性。将病理切片按染色细胞所占百分比进行分级:1,<10%;2,10%~50%;3,>50%。低表达(-)包括细胞染色比例<10%且染色程度为 0、1、2 的病理切片和染色细胞所占比例<50%的染色程度为 0、1 的病理切片;阳性表达(+)包括细胞比例为 10-50%的染色程度为 2 分的病理切片和细胞所占比例>50%的染色程度为 1 分的病理切片。强阳性表达(++)包括染色细胞所占比例>50%的染色程度为 2 分的病理切片。

1.3 统计学分析

采用统计学软件 R (version 3.3.1) 对数据进行处理。计数资料采用卡方检验或费舍尔精确检验,以 $P<0.05$ 为差异有统计学意义;两组间的差异分析采用 Mann-Whitney U-test 方法,相关分析采用 Spearman 相关法,统计学意义的标准是 $P<0.05$ 。

2 结果

2.1 NS 蛋白在卵巢肿瘤及正常卵巢组织中的表达

NS 蛋白在卵巢恶性肿瘤中表达阳性率为 93.3%(28/30),在交界性肿瘤中表达阳性率为 40%(4/10),在良性肿瘤中表达的阳性率为 20%(2/10)。NS 蛋白主要在卵巢肿瘤的细胞核中表达,卵巢恶性肿瘤组中的 NS 阳性表达率显著高于其他三组($P<0.05$)。在卵巢恶性肿瘤组中,NS 的表达与分化程度呈负相关($r=-0.76$, $P<0.001$),且在低分化组中的表达显著高于中、高分化组($P<0.05$, $P<0.01$),而与患者年龄、术前血清 CA125 水平、临床分期和病理类型均无关。

表 1 卵巢恶性肿瘤中 NS 的免疫组化表达

Table 1 Immunohistochemical expression of NS in malignant ovarian tumor

	n	NS		
		-	+	++
FIGO Staging	30			
I	12	1	5	6
II	3	0	1	2
III	15	1	2	12
Differentiation	30			
Well#	5	2	2	1
Moderate*	7	0	4	3
Poor	18	0	2	16
Pathological classification	30			
Epithelial tumor	26	2	7	17
Sex cord-stromal tumors	2	0	0	2
Germ cell tumor	2	0	1	1

Note: Immunohistochemical reactivity is defined by negative (-), weak (+) to moderate positive and strong positive (++); * and # represent statistical significance $P<0.05$ and $P<0.01$.

2.2 HDAC1 蛋白在卵巢肿瘤及正常卵巢组织中的表达

在 30 例卵巢恶性肿瘤中,HDAC1 蛋白表达的阳性率为 90%(27/30);10 例卵巢交界性肿瘤中,HDAC1 的阳性率为 60%(6/10);10 例卵巢良性肿瘤组织中 HDAC1 的阳性率为 20%(2/10),在卵巢正常组织中无表达。卵巢恶性肿瘤组中的 HDAC1 阳性表达率显著高于其他三组($P<0.05$)。在卵巢恶性肿瘤组中,HDAC1 的表达与分化程度呈负相关($r=-0.53$, $P<0.01$),且在低分化组中的表达显著高于中、高分化组($P<0.05$, $P<0.05$),但与患者年龄、术前血清 CA125 水平、临床分期和病理类型无关。

2.3 NS 和 HDAC1 蛋白在卵巢恶性肿瘤中表达的相关性

Spearman 相关分析表明,在卵巢恶性肿瘤组织中 NS 和 HDAC1 的表达呈正相关($r=0.56$, $P<0.05$),见表 3。

卵巢癌死亡率居妇科恶性肿瘤首位,成为严重威胁妇女生命和健康的主要肿瘤。卵巢癌在女性生殖器官浸润性恶性肿瘤中几乎占 1/3。并且,卵巢癌起病隐匿,病人很少表现出特异性的症状,且目前极其缺乏有效的早期筛查和诊断手段,70%以上的病人确诊时为晚期,5 年生存率一直浮动在 30%~40%。因此,迫切需要明确卵巢癌的病因同时积极寻找卵巢恶性肿瘤分子治疗的靶点。

近年来越来越多的研究人员关注卵巢恶性肿瘤的病因,试图从根本上控制卵巢癌的发生并寻找卵巢癌有效的治疗方法。大量研究表明,恶性肿瘤的发生发展是多种基因结构和功能出现改变所致,卵巢恶性肿瘤也不例外。越来越多的科研工作者关注并投入到卵巢恶性肿瘤的分子生物学特点的研究中。寻找更多与卵巢恶性肿瘤发生发展密切相关的癌基因与抑癌基因。

3 讨论

表 2 卵巢 malignant 肿瘤中 HDAC1 的免疫组化表达

Table 2 Immunohistochemical expression of HDAC1 in malignant ovarian tumor

	n	HDAC1		
		-	+	++
FIGO Staging	30			
I	12	2	4	6
II	3	0	0	3
III	15	1	7	7
Differentiation	30			
Well*	5	2	2	1
Moderate*	7	1	4	2
Poor	18	0	5	13
Pathological classification	30			
Epithelial tumor	26	3	10	13
Sex cord-stromal tumors	2	0	0	2
Germ cell tumor	2	0	1	1

Note: Immunohistochemical reactivity is defined by negative (-), weak (+) to moderate positive and strong positive (++); * represents statistically significant $P<0.01$.

表 3 NS 和 HDAC1 在卵巢恶性肿瘤中的表达相关性

Table 3 Immunohistochemical expression and correlation of NS and HDAC1 in malignant ovarian tumor

HDAC1	NS			r	P
	-	+	++		
-	1	2	0	0.56	$P<0.05$
+	1	4	6		
++	0	2	14		

Note: Immunohistochemical reactivity is defined by negative (-), weak (+) to moderate positive and strong positive (++).

NS 是 Tsia 等人发现的 p53 结合蛋白,它在多种肿瘤组织及肿瘤细胞系中高表达,并在促进细胞恶性转变、癌进展及细胞重组^[14]中也起着关键作用。在恶性食管癌术后化疗患者中,NS 高表达组较低表达组的无病生存时间明显缩短^[15]。NS 通过

调控细胞周期对肿瘤细胞和干细胞进行调控的证据已经越来越明显,NS 基因的缺失对不同类型细胞周期进展存在不同影响。敲出除 NS,可致急性粒细胞白血病细胞(Molt-4)停滞在 G₀/G₁ 期^[16],也可导致人乳腺癌细胞(MDA-MB-231)大部分停

滞于S期^[9,17]。在小鼠胚胎干细胞中,通过使NS缺失造成细胞周期阻滞,促成细胞的衰老及凋亡^[18]。Wang^[19]等人的研究发现敲除NS基因使卵巢癌细胞系细胞周期发生停滞。Hu等人也通过研究表明NS基因对细胞周期存在调控作用^[20]。Liu等通过RNAi技术,敲除Hela细胞系中的NS基因,大量的Hela细胞不能完成S期的DNA复制^[21]。G₀/G₁期细胞百分比明显增加,S期细胞数量明显减少,充分说明NS基因作用于细胞周期的位点可能在S期或G₀期。S期细胞的减少以及G₀/G₁细胞数量显著增多是细胞增殖减慢的一个标志。最近的研究表明,NS的表达是乳腺癌侵袭性表型和不良预后的标志,可能作为乳腺癌干细胞相关治疗的潜在靶点^[22]。

NS在恶性肿瘤中高表达,敲掉NS可使细胞增殖减慢,是一种潜在的治疗恶性肿瘤的基因靶点。研究表明,在恶性脑胶质瘤细胞中NS基因可通过激活Wnt通路加快细胞增殖,从而在肿瘤进展中发挥重要作用^[23,24]。NS蛋白在HL60细胞中对细胞传导通路及细胞凋亡产生影响^[25-27]。本实验通过免疫组化方法证实NS蛋白在高分化卵巢恶性肿瘤中的表达显著低于低分化和中分化的卵巢恶性肿瘤,与其恶性程度呈正相关,随着卵巢肿瘤恶性程度的加重,NS蛋白表达的百分比及染色程度均明显升高。由以上结果推断NS参与了卵巢癌恶变进程中细胞增殖等生物学行为。NS蛋白在卵巢恶性肿瘤、交界性肿瘤及良性肿瘤中表达阳性率的差异,也印证了NS在肿瘤发生与进展过程中存在作用。如果在卵巢恶性肿瘤中NS也特异性地激活Wnt/betacatenin通路,那么抑制NS与Wnt通路之间的相互作用,促进肿瘤细胞分化,也可能成为治疗肿瘤的新途径。

HDAC1在多种肿瘤组织中具有高表达的特点,而在非小细胞肺癌中敲低HDAC1的表达可抑制细胞增殖,阻止细胞迁移,减少细胞侵袭,减少肿瘤血管生成,诱导细胞凋亡^[28]。HDAC1可能参与细胞周期的调控并且与肿瘤的发生发展有关。研究表明,HDAC1与Wnt存在一定的潜在关系,对于细胞增殖、凋亡有一定的调控作用^[29-31]。本研究中,在恶性卵巢肿瘤、交界性卵巢肿瘤、良性卵巢肿瘤中,HDAC1的阳性表达率分别为90%、60%、20%。HDAC1在正常卵巢组织中同样不表达。HDAC1蛋白在恶性卵巢肿瘤中的表达阳性率明显高于良性卵巢肿瘤和交界性卵巢肿瘤,这与HDAC1既往研究结果保持一致。实验通过分析发现,在卵巢恶性肿瘤中,NS和HDAC1表达呈正相关,由此推断两者在功能上可能通过Wnt通路存在一定的联系。Liu^[32]等沉默NS基因在前列腺癌中的表达后,HDAC1的表达明显下降,这一结果证明NS和HDAC1具有一定的协同作用,可能在肿瘤细胞中共同调控细胞周期,影响细胞增殖,且NS可能通过调控HDAC1影响细胞周期。综上推断,联合抑制NS与HDAC1的表达,可能成为治疗卵巢恶性肿瘤的新方法。

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