上皮性卵巢癌组织中 miR-199a-3p、COL12A1 的表达及作用机制研究

王方方[1]　韩宝生[2]
1 华北理工大学研究生院　2 唐山市妇幼保健院
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[摘　要] 卵巢恶性肿瘤的发病率及死亡率均较高。上皮性卵巢癌(epithelial ovarian cancer,EOC)占卵巢恶性肿瘤的85%-90%,其恶性程度高、病情发展迅速,仍然是妇科恶性肿瘤患者死亡的主要原因[1,2],由于其原发于盆腔深部,以前超声和糖链抗原125(CA125)检测是国内EOC常见的筛查手段[3],但超声诊断灵敏度以及特异度低,(CA)125在其他非卵巢恶性肿瘤及非肿瘤相关疾病中也有一定的阳性率,70%患者发现时已处于癌症晚期失去了最佳手术时机。最新研究显示,上皮性卵巢癌患者病死率仍然较高,5年生存率仅为40%-50%[4]。因其早期症状不明显,且进展迅速、易于播散等特点,通常确诊时大多数已进入中晚期阶段[5]。到目前为止,EOC的发病机制尚未研究透彻,近年来EOC的研究热点是分子机制的研究,并寻找其治疗EOC的方法。
[关键词] 上皮性卵巢癌；miRNA-199a-3p；COL12A1
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Study on the Expression and Mechanism of miR-199a-3p and COL12A1 in Epithelial Ovarian Cancer

Fangfang Wang[1]　Baosheng Han[2]
1 North China University of Science and Technology　2 Tangshan Maternal and Child Health Hospital
[Abstract] The morbidity and mortality of the ovarian malignant tumors are relatively high. epithelial ovarian cancer (EOC) accounts for 85%-90% of ovarian malignancies. Its high degree of malignancy and rapid disease progression are still the main cause of death in patients with gynecological malignancies. It occurs in deep part of the pelvis, and the ultrasound and sugar chain antigen 125(CA125) detection were common screening methods for EOC in the past in China. But due to the low sensitivity and diagnosis, and a certain positive rate in related disease when (CA)125 is used in other non-ovarian malignant tumors and non-tumors. 70% of patients are in advanced stage of cancer when they are discovered,and have lost the best opportunity for surgery. The latest research shows that the mortality rate of patients with epithelial ovarian cancer is still high, with the 5-year survival rate is only 40%-50%. Because of its not obvious early symptoms, rapid progress, and easy spread, most of them have entered the middle and late stage when they are diagnosed. So far, the pathogenesis of EOC has not been thoroughly studied. In recent years, the research hot spot of EOC has been the study of molecular mechanisms and the search for its treatment of EOC.
[Key words] epithelial ovarian cancer; miRNA-199a-3p; COL12A1

1 研究的现状

1.1 miRNA的概念。miRNA是一类非编码微小RNA分子,它是天然存在的。成熟的miRNA分子是一个19-25个核苷酸组成的小分子,是由70-100个核苷酸的发卡结构的pre-miRNA裂解而来[6]。pre-miRNA经细胞质核糖核酸酶III-Dicer切割,形成约22个核苷酸的miRNA双链:其中的一条链被降解,另一条链则形成成熟的小miRNA分子。在动物体中,成熟的miRNA通过与特定的mRNA的3’-UTR(3’untranslatedregion,3’非编码区)结合,从而抑制目标mRNA的翻译过程,甚至降解目标mRNA,从而调节目标基因的表达[7]。经生物信息学分析预测,超过60%的编码蛋白基因的mRNA可成为miRNA的结合靶点[8]。miRNA通过与特定的mRNA的3’-UTR结合调节相应的基因的表达,参与到多种细胞生物学功能,包括细胞生长、分化、死亡等[9]。研究发现,miRNA的异常表达或突变存在多种肿瘤中,作为肿瘤细胞

的重要的调控因子参与肿瘤的发生、发展以及侵袭转移等过程[10,11]。目前,miRNA在EOC中的研究开始受到人们的重视。

1.2 miRNA参与卵巢癌的诊断。现阶段,(CA)125主要用于EOC诊断和预后的肿瘤标志物,但是,其缺点较多,灵敏度、特异性较差,且易受外界干扰,因此,miRNA若能成为诊断肿瘤的手段,会受到广泛应用,其优点很多,不易受到外界影响。Resnick等[12]发现miRNA-21、miRNA-29a、miRNA-92、miRNA-93、miRNA-126相对于正常样本在卵巢癌血清中显著高表达,而miRNA-155、miRNA-99b、miRNA-127则显著低表达,且发现miRNA-21、miRNA-93、miRNA-92在CA125正常水平的卵巢癌患者中显著高表达,提示miRNA可作为卵巢癌诊断的潜在生物标记物。徐立坚[13]通过小RNA测序及RT-PCR方法,发现卵巢癌患者血清中4条miRNA(miRNA-22、miRNA-93、miRNA-106b、miRNA-451)在正常妇女血清中和卵巢恶性病变血清中有显著性表达差异,并且在不同CA125指标(≤35U/ml和>35U/ml)中,miRNA-106b和miRNA-93显著差异表达,随着CA125指标增大miRNA-106b和miRNA-93表达呈现逐渐下降趋势,说明对术前CA125水平正常的患者而言,miRNA-106b和miRNA-93可以作为卵巢癌的早期诊断的潜在生物标记物。

1.3 miRNA参与卵巢癌的治疗。miRNA本身也可能成为卵巢癌治疗的靶位。因此,可以改变miRNA的表达以控制靶基因以治疗卵巢疾病。miRNA治疗卵巢疾病的关键方法有两种:一种是减少在卵巢疾病中过度表达的miRNA的水平,另一种是增加在卵巢疾病中降低的miRNA表达的水平。Xu等[14]发现卵巢癌组织中miRNA-145表达下调,并通过与其靶标P70S6K1结合抑制P70S6K1表达,而P70S6K1可调控下游血管生成因子缺氧诱导因子1(HIF-1)和血管内皮生长因子(VEGF)表达,从而抑制肿瘤生长和血管生成,因此,卵巢癌患者可通过恢复miRNA-145表达达到治疗目的。let-7家族成员于卵巢癌通常有明显下调[15],Ras基因是let-7家族的靶标基因[16],并且let-7还调控着其他癌基因如HMGA2、CDK6、cMYC、CDC25A和cylinD2,提示let-7可能参与了调控癌基因的表达,从而介导卵巢癌的发生、分化。Yang等[17]研究发现,miRNA-130b在耐药卵巢癌中低表达,而增强其表达可以部分逆转耐药性,说明miRNA-130b表达与卵巢癌化疗耐药相关,且miRNA-130b可特异性与集落刺激因子(CSF)-1mRNA结合抑制其表达。

1.4 miRNA参与卵巢癌的预后。miRNA对于EOC的预后也有重要作用。娄艳辉等[18]研究发现miRNA-21在上皮性卵巢癌组织高表达,与卵巢癌组织分级、淋巴结转移及分期密切相关,并且抑癌基因(PTEN)蛋白在上皮性卵癌组织表达降低,与miRNA-21的表达呈显著负相关,从而推测高表达miRNA-21可能通过与PTEN结合来调控卵巢细胞凋亡等行为,此外miRNA-130a、miRNA-93、miRNA-214都是通过与PTEN结合诱导卵巢癌顺铂耐药性[19],故检测这些miRNA表达水平可评估患者对化疗的敏感性和预后。Mez-zanzanica和Lu等[20,21]发现经过紫杉醇化疗的上皮性卵巢癌患者组织let-7a表达量显著降低,而生存率也降低,let-7a与肿瘤分级、疾病阶段和组织学定位无关联;并且证实let-7a的靶标为凋亡因子caspase-3,其表达异常会导致caspase-3表达水平降低,提示let-7a可能是卵巢癌患者选择化疗药物及监测化疗效果的有效标记物。此外,Marchini等[22]发现Ⅰ期卵巢癌患者与无复发患者比,miRNA200c在复发患者体内呈明显低表达,且其靶标Fas相关磷酸酯酶明显低表达,提示miRNA200c可作为卵巢癌复发的潜在生物标志物。Kim等[23]用miRNA微阵列芯片技术检测发现miRNA-153、miRNA-519a、miRNA-18b、miRNA-485p、miRNA-511在良性肿瘤、恶性肿瘤和交界性肿瘤中表达明显不同,并且与卵巢癌分期明显相关,故认为miRNA对判断预后有重要作用。

1.5 COL12A1概述。胶原十二型α1链(COL12A1)由染色体定位为6q12-q13的基因编码,是含有中断的三螺旋胶原结构域的纤维相关胶原家族的成员[24]。COL12A1作为纤维之间的桥梁,其突变据报道与肌病有关[25]。COL12A1,由于其在人类癌症中的重要作用而受到越来越多的关注,因为它在几种不同的癌症类型中都有过表达,与几种癌症类型预后不良密切相关[26-28]。COL12A1与多种肿瘤有关,如肾癌、胃癌、乳腺癌和结肠癌,提示COL12A1可能是一种新的潜在肿瘤生物标志物[29-32]。COL12A1与COL6A3、COL8A1、COL1A2、COL5A2、COL10A1、COL11A1、COL2A1作为FACIT(三螺旋间纤维相关胶原)胶原家族的一员,具有细胞外基质结构成分的分子功能。这些FACIT成员参与了胶原纤维组织的生物学过程,并构建了胶原三聚体的细胞成分。尽管COL12A在多种肿瘤组织中均有表达,但其确切功能仍不清楚。Januchowski等人[33]发现COL12A1在肿瘤细胞的耐药性和肿瘤进展中起作用。COL12A1可能是卵巢癌的一个新的潜在标志物和预后因子,其途径可能是潜在的治疗靶点。

2 研究目的及意义

卵巢癌的发生和发展是多环节、多因素调控的复杂的生物学过程。对卵巢癌发病机制和治疗的研究仍需进一步深化。通过在线预测软件预测：COL12A1具有miR-199a-3p的靶向结合位点,此次实验希望通过探讨miR-199a-3p靶向调节COL12A1来探讨卵巢癌的发生及发展,验证miR-199a-3p调节COL12A1基因的表达,分析卵巢癌患者组织中miR-199a-3p和COL12A1的表达与临床病理特征的关系,为卵巢癌的研究及治疗开辟了一条新的途径。

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通讯作者：

韩宝生(1963--),男,汉族,河北省唐山市人,硕士研究生,主任医师,研究方向：妇产科学。