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·基础研究·

小鼠缺血性脑损伤模型急性期海马 SIRT3 与自噬相关蛋白表达水平的相关性分析 *

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摘要 目的：观察小鼠缺血性脑损伤模型急性期海马区域沉默信息因子 3(SIRT3)和自噬相关蛋白的表达，并探讨两者的相关性。**方法**：选择 C57BL/6J 小鼠，采用大脑中动脉阻塞(MCAO)法建立缺血性脑损伤模型，并将小鼠随机分为假手术组(sham)和模型组(MCAO)。缺血性脑损伤急性期(6h)，采用间接免疫荧光法观察小鼠海马 CA1、CA3 和 DG 区域 SIRT3 的表达，应用蛋白印迹法检测 SIRT3、自噬相关蛋白 LC3 I/II 和 Beclin-1 的表达，而后用 Spearman 相关性分析明确 SIRT3 和 LC3-II、Beclin-1 表达的相关性。**结果**：海马各区域 SIRT3 阳性细胞数量在损伤后明显增多(P<0.05)，且 SIRT3 蛋白表达也相对上调(P<0.05)；损伤后自噬相关蛋白 LC3-II 和 Beclin-1 表达亦增高 (P<0.05)；Spearman 相关性分析发现 SIRT3 与自噬相关蛋白 LC3-II、Beclin-1 表达均呈显著正相关(P<0.05)。**结论**：小鼠缺血性脑损伤模型急性期海马区域 SIRT3 和自噬相关蛋白的表达具有显著相关性，SIRT3 对海马区域自噬的调节可能有重要作用。

关键词：沉默信息因子 3；自噬；缺血性脑损伤；海马

中图分类号：R-33；R651.15 **文献标识码**：A **文章编号**：1673-6273(2018)23-4401-04

Correlative Analysis of Hippocampal SIRT3 with Autophagy-related Protein Expression in the Acute Period of Mouse Model of Cerebral Ischemia*

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ABSTRACT Objective: To explore the expression and correlation of hippocampal NAD dependent deacetylase sirtuin-3 (SIRT3) with autophagy-related proteins in the acute period in a mouse model of cerebral ischemia. **Methods:** Cerebral ischemia was established using middle cerebral artery occlusion (MCAO) in C57BL/6J mice and randomly divided into the sham group and the MCAO group. In the acute period (6 h) of cerebral ischemia, immunofluorescence was used to observe the changes of SIRT3 expression in hippocampal CA1, CA3 and DG areas, and western blotting was used to analyze the relative protein expression of SIRT3 and autophagy-related proteins LC3 I/II and Beclin-1. Spearman correlative analysis was used to validate the relationship between SIRT3 and autophagy-related proteins expression. **Results:** SIRT3-positive cells were increased in the hippocampal subareas of MCAO groups compared with that of the sham group(P<0.05), and so do the protein levels of SIRT3(P<0.05); the protein expressions of LC3-II and Beclin-1 were also up-regulated in the MCAO group (P<0.05); Spearman correlational analysis presented the expression of SIRT3 and autophagy-related proteins (LC3-II and Beclin-1) were positively correlated(P<0.05). **Conclusion:** The expression of hippocampal SIRT3 and autophagy-related proteins were significantly correlated, indicating the essential role of SIRT3 in autophagy regulation.

Key words: SIRT3; Autophagy; Cerebral ischemia injury; Hippocampus

Chinese Library Classification(CLC): R-33; R651.15 Document Code: A

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前言

缺血性脑卒中(Ischemic Stroke，IS)后海马区域细胞不仅发生凋亡与坏死，还可发生自噬。目前研究显示 IS 后自噬相关蛋白 LC3II、Beclin-1 和 p62 等表达增高，提示着自噬水平的上调[1-3]。沉默信息调节因子 3(Silent information regulator，SIRT3)是线粒体的 NAD 依赖的去乙酰化酶家族成员，广泛存在于线粒体基质和细胞核中[4,5]。我们推测 SIRT3 可能通过调控线粒体自噬影响 IS 后细胞转归[6]。SIRT3 定位于线粒体，在肝脏、心肌、大脑等组织中呈高表达状态[7,8]，海马区域自噬水平的高低将直接影

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响到动物认知和情感反应。然而,SIRT3 在缺血性脑卒中如何发挥作用及其机制目前尚未完全明确。

为进一步探讨 SIRT3 对小鼠缺血性脑损伤模型海马区域细胞自噬及凋亡的影响，本研究采用 MCAO 法建立缺血性脑损伤模型,通过免疫荧光和蛋白印迹法观察急性期自噬相关蛋白和 SIRT3 的表达情况,并分析两者的相关性,结果报道如下。

1 材料与方法

1.1 实验动物与分组

选择健康成年 C57BL/6J 小鼠(20-22g)32 只,由第四军医大学实验动物中心提供。实验动物随机分为 2 组,即假手术组(sham)和模型组(MCAO),每组 8 只。实验过程中如遇死亡则另行补齐。

1.2 方法

1.2.1 小鼠脑缺血再灌注模型的建立 术前 12 h 禁食不禁水,用腹腔麻醉(10%水合氯醛,3 mg/kg)麻醉小鼠,游离左侧颈总动脉(CCA)、颈外动脉(ECA)和颈内动脉(ICA),然后结扎 E-CA 和 CCA 近心端,动脉夹夹闭 CCA 远端,在 CCA 结扎与夹闭两处之间用动脉剪剪一小口,将浸于肝素生理盐水中的尼龙线栓(头端直径为 0.23 mm)沿 CCA 插入 ICA [9, 10],约 12 mm,然后结扎颈总动脉固定线栓,然后缝合皮肤。整个手术过程中室温保持在 22～25℃。术后将动物置于放有清洁垫料的饲养盒中,自由饮水、进食。

1.2.2 间接免疫荧光染色 每组各取 3 只进行染色。无痛麻醉后剪开腹腔暴露心脏，以 200 mL 生理盐水灌注后用 4%多聚甲醛灌注固定。常规石蜡包埋,冠状切片(厚度为 2 mm)。石蜡切片常规脱蜡止水,pH 6.0 的柠檬酸修复液进行抗原修复;磷酸盐缓冲液(Phosphate buffered saline,PBS)漂洗 3 次,每次 6 min;加入 0.3% H_2O_2 溶液封闭内源性过氧化酶 15 min;PBS 漂洗 3 次,每次 6min;加入驴血清封闭 1h 后甩去血清加入兔抗 SIRT3(1:500,Abcam,美国)抗体,在 4 ℃冰箱中赋予过夜,洗脱。滴加荧光二抗(Invitrogen,1:2000, 山羊抗兔和山羊抗小鼠)孵育 3 h,洗去二抗并加入 DAPI 染色液暗室孵育 10 min,在荧光显微镜下观察分析损伤区域海马各区域 SIRT3 阳性细胞数量。观察采用单盲双人法,观察单个低倍镜下(20×)阳性细胞个数(取 10 个切片× 10 个视野的平均数)[11]。

1.2.3 海马区域蛋白样品制备和蛋白印迹检测 提取缺血区海马(即损伤半侧海马组织)蛋白样品,蛋白裂解液冰上裂解 15 min 后,4℃ 12000 转离心 30 min。BCA 蛋白定量后 -20℃保存待用。分别取 60 μg 各组蛋白样品进行蛋白电泳(10% SDS-PAGE) 和转模 (NC 膜)。与兔抗 LC3 (1:2000)、SIRT3(1:400)、Beclin-1(1:2000), β-Actin(1:2000), (Abcam,USA)抗体结合过夜,然后与辣根过氧化物酶标记的二抗(1:20000)结合孵育 1.5 h,显色压片后使用 Gel-Pro analyzer 软件进行图像分析。

1.3 统计学分析

应用 SPSS19.0 统计软件对数据进行统计分析，所有定量数据均以均数± 标准差(Mean± SD)表示,两组间比较采用 Student-t 检验,以 P<0.05 为差异有统计学意义。

2 结果

2.1 损伤侧海马 3 区域 SIRT3, LC3 间接免疫荧光染色

如图 1,2 所示,脑损伤急性期即模型建立后 6h,小鼠损伤侧海马 CA1、CA3、DG 等区域 SIRT3 和 LC3 表达(SIRT3,LC3 阳性细胞个数)均明显上调。其中,MCAO 组 CA1 区阳性细胞最多,为 SIRT3(33± 5.5)和 LC3 (27± 7.2),比 sham 组明显增多(P<0.05);MCAO 组 CA3 区域阳性细胞数居中,为 SIRT3 (12± 2.9)和 LC3(7± 1.9),但比 sham 组明显增多(P<0.05);DG 区域阳性细胞数最少,为 SIRT3(7± 1.7)和 LC3(12± 5.3),亦相比 sham 组显著增多(P<0.05)。

2.2 损伤侧海马区域 SIRT3、LC3 I/II 及 Beclin-1 蛋白表达

如图 3 所示,缺血性脑损伤急性期(即 6h),相比 sham 组,MCAO 组海马区域 SIRT3、自噬相关蛋白 LC3II 和 Beclin-1 的表达均在损伤后上调(P<0.05)。

2.3 SIRT3 阳性细胞数和 LC3-II, Beclin-1 蛋白表达的相关性分析

各组小鼠损伤侧海马 SIRT3 蛋白表达和 LC3-II, Beclin-1 蛋白表达进行 Spearman 相关性分析结果显示:LC3-II:r=0.738,*P<0.05;Beclin-1:r=0.639,P<0.05。

3 讨论

75%以上脑卒中患者为 IS,幸存者往往伴有不同程度的神经功能障碍[12,13]。近 30 年来,IS 后脑缺血损伤机制及脑保护作用研究已成为世界性的难点和热点问题[14]。大量基于电镜、免疫组化、免疫荧光的形态学证据表明在体或体外培养神经元在类 IS 损伤后急性期出现自噬激活[15]。在原代培养的神经元损伤模型中,伤后 1、3、6、12 和 24 h,自噬相关分子的表达随时间变化逐渐增高[2],而自噬的标志分子 LC3-Ⅱ、Beclin-1 的表达的高低与发生自噬的程度成正比[6]。本研究中,急性期小鼠缺血性脑损伤时,LC3-II 以及 Beclin-1 表达量显著上调,提示自噬被激活。

众所周知,自噬可能对 IS 后神经元损伤具有保护作用。以往研究显示自噬基因 Atg5、Atg7 敲除的小鼠中,皮层神经元不发生自噬,但仍出现大量神经元死亡[16]。有文献报道 SIRT3 介导的线粒体自噬在脑炎症以及退行性神经病动物模型中通过抑制自体吞噬泡和溶酶体的结合,减轻脑水肿并改善神经功能可能起到积极作用[12,17-19]。本研究中,SIRT3 与自噬相关蛋白表达成显著正相关,提示 SIRT3 可能参与到自噬相关的神经元保护机制之中。Sirt3 主要在线粒体中集聚,作为正性因素发挥着保护神经元的重要作用，并且 Sirt3 介导的保护线粒体功能和氧化还原内稳态被证明是为多个神经保护药物的保护机制和策略[20]。如 Mai[16]等发现白藜芦醇显著增加 SIRT3 蛋白表达,SIRT3 的抑制会影响到其对 HT22 神经元的氧化应激以及内质网 ROS 清除的药物功能。

近来研究亦表明缺血性损伤中,细胞会启动自噬机制来清除受损线粒体，此时自噬可避免大量凋亡因子释放进入胞质,对细胞起一定保护作用[2,21]。线粒体自噬是神经元自噬的主要形式,在神经元转归中扮演重要角色。而目前研究显示 SIRT3 可以通过多种途径调控自噬水平[6,22]对细胞发挥保护作用:① SIRT3 可通过去乙酰化 FOXO3a 进而上调众多抗氧化酶,包括过氧化氢酶(catalase, CAT)和 MnSOD 等,增加 ROS 清除率[23,24];也可通过激活异柠檬酸脱氢酶 IDH2 和谷氨酸脱氢酶(GDH),

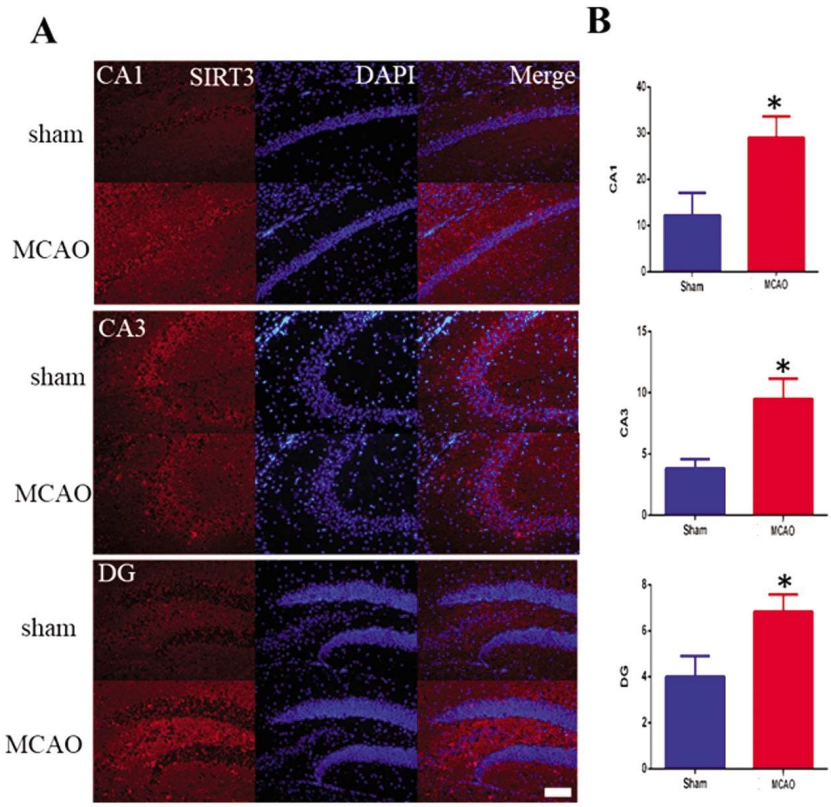

图 1 损伤区域海马各区 SIRT3 表达的间接免疫荧光染色及分析

Fig.1 The expression of SIRT3 in the ipsilateral mouse hippocampus using immunofluorescence staining. (A)The expression of SIRT3 in damaged hippocampus including CA1, CA3 and DG. (B) the quantification of the SIRT3 expression between the two groups. The horizontal bar=50 μm. P <0.05 vs. sham group

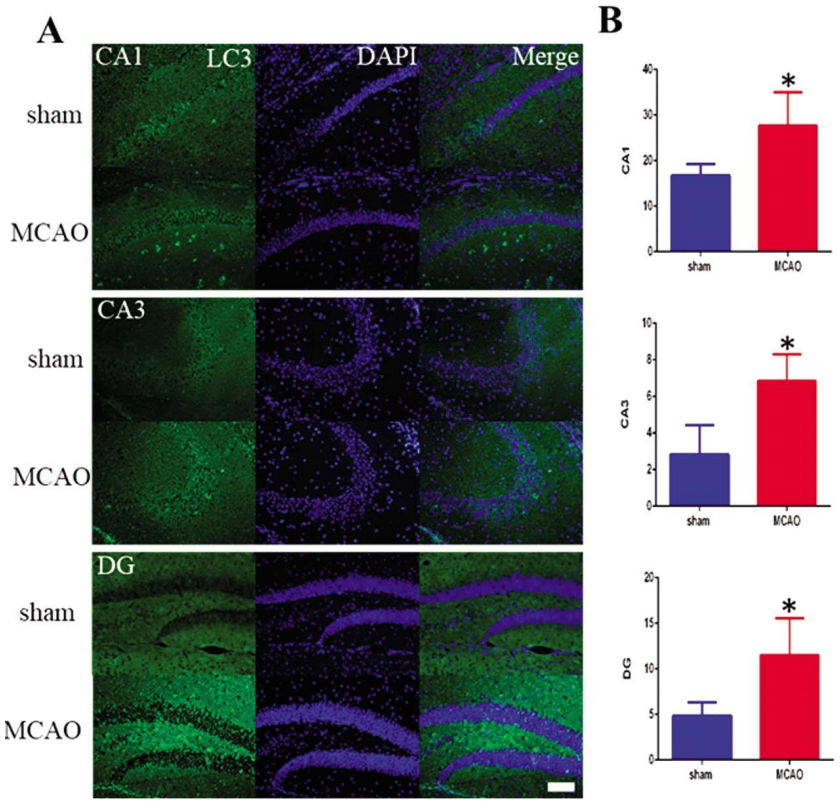

图 2 损伤区域海马各区 SIRT3 表达的间接免疫荧光染色及分析

Fig.2 The expression of LC3 in the ipsilateral mouse hippocampus using immunofluorescence staining. (A)The expression of LC3 in damaged hippocampus including CA1, CA3 and DG. (B) the quantification of the LC3 expression between the two groups. The horizontal bar=50 μm. P <0.05 vs. sham group

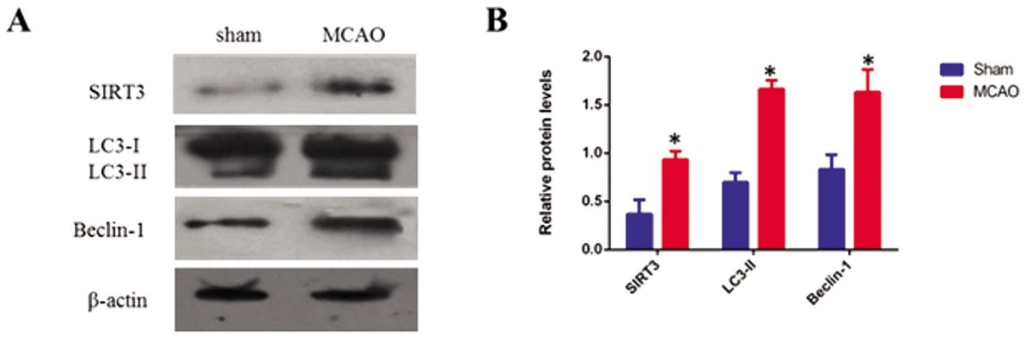

图 3 损伤侧海马总 SIRT3，LC3 I/II 和 Beclin-1 表达的蛋白印迹分析

Fig.3 The relative protein expression of SIRT3, LC3 I/II andBeclin-1 in the ipsilateral mouse hippocampus using western blotting. (A)The expression of SIRT3, LC3 I/II andBeclin-1; (B) the quantification of the expression and the data were normalized to beta-actin. P <0.05 vs. sham group.

促进 NADPH 生成，NADPH 最终通过还原谷型胱甘肽清除 ROS；SIRT3 可去乙酰化线粒体电子传递链中的组分，包括复合辅 I(NADH 脱氢酶)、复合物 II(琥珀酸脱氢酶)及复合物 III 的成分，从而调节 ROS 的产生[25]。再者，在原代培养大鼠海马神经元中，SIRT3 通过抑制线粒体钙摄取和促进线粒体生物发生发挥神经保护作用，SIRT3 增加海马神经元对氧化性急性损伤的抵抗能力[23]。本研究结果提示明 MCAO 急性期海马区域 SIRT3 对自噬可能具有促进作用，进而对细胞产生保护作用。

总之，本研究结果表明小鼠缺血性脑损伤模型急性期海马区域 SIRT3 表达上调，可能通过促进细胞自噬保护神经细胞。但 SIRT3 和自噬相关分子之间如何联系尚未可知。下一步实验阐明两者的潜在的联系和分子信号通路。

参 考 文 献(References)

[1] Nixon R A, Yang D S. Autophagy and neuronal cell death in neurological disorders[J]. Cold Spring Harb Perspect Biol, 2012, 4(10)

[2] Balduini W, Carloni S, Buonocore G. Autophagy in hypoxia-ischemia induced brain injury: evidence and speculations[J]. Autophagy, 2009, 5(2): 221-223

[3] van der Kooij M A, Groenendaal F, Kavelaars A, et al. Combination of deferoxamine and erythropoietin: therapy for hypoxia-ischemia-induced brain injury in the neonatal rat?[J]. Neurosci Lett, 2009, 451(2): 109-113

[4] Anamika, Khanna A, Acharjee P, et al. Mitochondrial SIRT3 and neurodegenerative brain disorders [J]. J Chem Neuroanat, 2017. pii: S0891-0618(17)30123-0

[5] Su J, Liu J, Yan X Y, et al. Cytoprotective Effect of the UCP2-SIRT3 Signaling Pathway by Decreasing Mitochondrial Oxidative Stress on Cerebral Ischemia Reperfusion Injury[J]. Int J Mol Sci, 2017, 18(7)

[6] Dai S H, Chen T, Wang Y H, et al. Sirt3 protects cortical neurons against oxidative stress via regulating mitochondrial Ca^{2+} and mitochondrial biogenesis[J]. Int J Mol Sci, 2014, 15(8): 14591-14609

[7] Bian H, Shan H, Chen T. Resveratrol ameliorates hypoxia/ischemia-induced brain injury in the neonatal rat via the miR-96/Bax axis [J]. Childs Nerv Syst, 2017, 33(11): 1937-1945

[8] Jin L, Galonek H, Israelian K, et al. Biochemical characterization, localization, and tissue distribution of the longer form of mouse SIRT3 [J]. Protein Sci, 2009, 18(3): 514-525

[9] Yang X, Geng K Y, Zhang Y S, et al. Sirt3 deficiency impairs neurovascular recovery in ischemic stroke [J]. CNS Neurosci Ther, 2018 [Epub ahead of print]

[10] Chauhan A, Hudobenko J, Al M A, et al. Myeloid-specific TAK1 deletion results in reduced brain monocyte infiltration and improved outcomes after stroke[J]. J Neuroinflammation, 2018, 15(1): 148

[11] Long Q, Qiu B, Wang K, et al. Genetically engineered bone marrow mesenchymal stem cells improve functional outcome in a rat model of epilepsy[J]. Brain Research, 2013, 1532: 1-13

[12] Jesko H, Wencel P, Strosznajder R P, et al. Sirtuins and Their Roles in Brain Aging and Neurodegenerative Disorders[J]. Neurochem Res, 2017, 42(3): 876-890

[13] Maaijwee N A, Rutten-Jacobs L C, Schaapsmeerders P, et al. Ischaemic stroke in young adults: risk factors and long-term consequences[J]. Nat Rev Neurol, 2014, 10(6): 315-325

[14] Chavez J C, Hurko O, Barone F C, et al. Pharmacologic interventions for stroke: looking beyond the thrombolysis time window into the penumbra with biomarkers, not a stopwatch[J]. Stroke, 2009, 40(10): e558-e563

[15] Carloni S, Buonocore G, Balduini W. Protective role of autophagy in neonatal hypoxia-ischemia induced brain injury [J]. Neurobiol Dis, 2008, 32(3): 329-339

[16] Mai S, Muster B, Bereiter-Hahn J, et al. Autophagy proteins LC3B, ATG5 and ATG12 participate in quality control after mitochondrial damage and influence lifespan[J]. Autophagy, 2012, 8(1): 47-62

[17] Yu W, Gao B, Li N, et al. Sirt3 deficiency exacerbates diabetic cardiac dysfunction: Role of Foxo3A-Parkin-mediated mitophagy[J]. Biochim Biophys Acta, 2017, 1863(8): 1973-1983

[18] Yan W J,Liu R B, Wang L K, et al. Sirt3-Mediated Autophagy Contributes to Resveratrol-Induced Protection against ER Stress in HT22 Cells[J]. Front Neurosci, 2018, 12: 116

[19] Zhao W, Li Y, Jia L, et al. Atg5 deficiency-mediated mitophagy aggravates cardiac inflammation and injury in response to angiotensin II[J]. Free Radic Biol Med, 2014, 69: 108-115

[20] Cheng A, Yang Y, Zhou Y, et al. Mitochondrial SIRT3 Mediates Adaptive Responses of Neurons to Exercise and Metabolic and Excitatory Challenges[J]. Cell Metab, 2016, 23(1): 128-142

[21] Rugarli E I, Langer T. Mitochondrial quality control: a matter of life and death for neurons[J]. EMBO J, 2012, 31(6): 1336-1349

[22] Onyango P, Celic I, McCaffery J M, et al. SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria [J]. Proc Natl Acad Sci U S A, 2002, 99(21): 13653-13658

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[3] Berek J S, Bast R C. Ovarian cancer screening. The use of serial complementary tumor markers to improve sensitivity and specificity for early detection[J]. Cancer, 2015, 76(S10): 2092-2096

[4] Varia M, Rosenman J, Venkatraman S, et al. Intraperitoneal chromic phosphate therapy after second-look laparotomy for ovarian cancer[J]. Cancer, 2015, 61(5): 919-927

[5] Fang W, Rong X X, Xie R Y, et al. Cytokine-induced killer cells efficiently kill stem-like cancer cells of nasopharyngeal carcinoma via the NKG2D-ligands recognition[J]. Oncotarget, 2015, 6(33): 35023-35039

[6] Lin T, Song C, Chuo D Y, et al. Clinical effects of autologous dendritic cells combined with cytokine-induced killer cells followed by chemotherapy in treating patients with advanced colorectal cancer: a prospective study[J]. Tumor Biology, 2016, 37(4): 4367-4372

[7] Bristow R E, Lagasse L D, Karlan B Y. Secondary surgical cytoreduction for advanced epithelial ovarian cancer. Patient selection and review of the literature[J]. Cancer, 2015, 78(10): 2049-2062

[8] Averette H E, Janicek M F, Menck H R. The National Cancer Data Base report on ovarian cancer. American College of Surgeons Commission on Cancer and the American Cancer Society [J]. Cancer, 2015, 76(6): 1096-1103

[9] Oza A M, Cibula D, Benzaquen A O, et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial[J]. Lancet Oncology, 2015, 16(1): 87-97

[10] Skates S J, Horick N, Yu Y, et al. Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA 15-3, CA 72-4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions [J]. Journal of Clinical Oncology, 2016, 22(20): 4059-4066

[11] Corn B W, Lanciano R M, Boente M, et al. Recurrent ovarian cancer. Effective radiotherapeutic palliation after chemotherapy failure [J]. Cancer, 2015, 74(11): 2979-2983

[12] Luo G, Xiao Z, Long J, et al. CA125, Pancreatic Cancer[J]. Journal of Gastrointestinal Surgery, 2013, 17(12): 2092-2098

[13] Romagnolo C, Leon A E, Fabricio A S, et al. HE4, CA125 and risk of ovarian malignancy algorithm (ROMA) as diagnostic tools for ovarian cancer in patients with a pelvic mass: An Italian multicenter study[J]. Gynecologic Oncology, 2016, 141(2): 303-311

[14] Lan Z, Fu D, Yu X, et al. Diagnostic values of osteopontin combined with CA125 for ovarian cancer: a meta-analysis [J]. Familial Cancer, 2016, 15(2): 221-230

[15] Xu Y, Zhong R, He J, et al. Modification of cut-off values for HE4, CA125 and the ROMA algorithm for early-stage epithelial ovarian cancer detection: Results from 1021 cases in South China[J]. Clinical Biochemistry, 2016, 49(1-2): 32-40

[16] Liu J, Li H, Cao S, et al. Maintenance therapy with autologous cytokine-induced killer cells in patients with advanced epithelial ovarian cancer after first-line treatment [J]. Journal of Immunotherapy, 2015, 37(2): 115-122

[17] Gammaitoni L, Giraudo L, Macagno M, et al. Abstract 2290: Effective immunotherapy with cytokine-induced killer cells against autologous melanoma cancer stem cells [J]. Cancer Research, 2016, 76(14 Supplement): 2290-2290

[18] Liu J, Wang L, Wang Y, et al. Phenotypic characterization and anticancer capacity of CD^{8+} cytokine-induced killer cells after antigen-induced expansion[J]. Plos One, 2017, 12(4): e0175704

[19] Zhang Q, Wang C R, Yu J P, et al. The Establishment of an HE4-CLIA Method and the Combined Analysis of HE4 and CA125 in Ovarian Cancer [J]. Journal of Clinical Laboratory Analysis, 2016, 30 (5): 709-718

[20] Zhao X, Zhang Z, Li H, et al. Cytokine induced killer cells-based immunotherapies in patients with different stages of renal cell carcinoma [J]. Cancer Letters, 2015, 362(2): 192-198

[21] Yelei Guo, Weidong Han. Cytokine-induced killer (CIK) cells: from basic research to clinical translation [J]. Chinese Journal of Cancer, 2015, 34(3): 1-9

[22] Wang S, Wang Z. Efficacy and safety of dendritic cells co-cultured with cytokine-induced killer cells immunotherapy for non-small-cell lung cancer [J]. International Immunopharmacology, 2015, 28 (1): 22-28

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[23] Dai S H, Chen T, Wang Y H, et al. Sirt3 attenuates hydrogen peroxide-induced oxidative stress through the preservation of mitochondrial function in HT22 cells[J]. Int J Mol Med, 2014, 34(4): 1159-1168

[24] Das S, Mitrovsky G, Vasanthi H R, et al. Antiaging properties of a grape-derived antioxidant are regulated by mitochondrial balance of fusion and fission leading to mitophagy triggered by a signaling network of Sirt1-Sirt3-Foxo3-PINK1-PARKIN [J]. Oxid Med Cell Longev, 2014, 2014: 345105

[25] Finkel T, Deng C X, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins[J]. Nature, 2009, 460(7255): 587-591