中文摘要

SULT2B1 在卵巢癌组织中表达及其生物信息学分析

背景和目的：

卵巢癌是妇科恶性肿瘤中最主要的致死原因，因为早期临床症状不特异，多数患者确诊时已为中晚期。手术结合以铂类为基础的化学药物治疗构成卵巢癌常规临床治疗路径，还可根据基因检测结果后续补充靶向药物治疗。但多数患者仍面临复发、转移和耐药，预后情况整体较差。卵巢癌的发病机制仍未完全明确，大量研究表明卵巢癌中存在多种复杂的基因突变，与卵巢癌的发生、进展、转移和耐药密切相关，因此急需找到新的基因靶点，揭示相关的分子机制，指导早期诊断、预后评估和临床靶向治疗。

羟基类固醇硫酸基转移酶 2B1（hydroxysteroid sulfotransferase 2B1，SULT2B1)是一种介导羟基类固醇硫酸化的酶，参与儿茶酚胺、甲状腺激素及类固醇激素等多种关键内源性化合物及各种药物和其他外来物质的代谢。大量文献表明，SULT2B1 参与多种恶性肿瘤的发生、发展且与不良预后相关，而关于 SULT2B1 在卵巢癌中的作用少有报道。

本研究旨在：（1）探究 SULT2B1 在正常卵巢组织及卵巢癌组织中的表达情况；（2）探究卵巢癌中 SULT2B1 表达差异与临床病理特征的关系；（3）评估 SULT2B1 在卵巢癌诊断及预后评估方面的价值；（4）探索可能与 SULT2B1 参与卵巢癌发生发展相关的信号通路。

方法：

（1）从 TCGA 数据库及 GTEx 数据库中获取 379 例卵巢癌组织和 180 例正常卵巢组织的转录组数据，使用 R 语言“limma”包对转录组信息进行均一化及差异分析，使用 GEPIA 数据库验证上述结果并探究 SULT2B1 表达差异与卵巢癌病理分期的关系。

（2）使用 R 语言“pROC”包绘制 SULT2B1 表达量对卵巢癌诊断价值的 ROC 曲线。

（3）利用 TCGA 数据库中卵巢癌患者的临床数据及 SULT2B1 的表达情况通过 Kaplan-Meier 方法进行生存分析并绘制生存曲线，并使用 GEPIA 数据库验证上述结果。

（4）临床收集 40 例上皮性卵巢癌组织，15 例卵巢良性肿瘤组织及 10 例正常卵巢组织的石蜡病理切片及相应患者的临床病理信息，进行免疫组化染色，并分析 SULT2B1 表达量差异与上皮性卵巢癌临床病理特征的关系。

（5）使用 6 例正常卵巢组织及 7 例上皮性卵巢癌组织标本进行 Western Blot 实验以确定 SULT2B1 在正常卵巢组织与卵巢癌组织中的差异性表达情况。

（6）以 SULT2B1 表达量中位数为临界值将 TCGA 数据库中卵巢癌组织转录组数据分为 SULT2B1 高表达组与低表达组，对二者进行差异分析以寻找与 SULT2B1 表达相关的基因，使用 DAVID 数据库对上述差异基因进行 GO 和 KEGG 富集分析，寻找 SULT2B1 影响卵巢癌发生发展的相关生物学进程或信号通路及可能下游靶点。

（7）使用 TIMER 数据库分析 SULT2B1 参与卵巢癌肿瘤免疫细胞浸润的情况。

结果：

（1）TCGA 与 GTEx 数据库联合分析及 GEPIA 数据库分析结果均显示，SULT2B1 在卵巢癌组织中高表达。GEPIA 数据库分析表明，SULT2B1 的表达差异与卵巢癌的病理分期相关（F=7.09，P＜0.01）。

（2）SULT2B1 高表达对卵巢癌具有很高的诊断价值（AUC=97.6%，P＜0.05）。

（3）TCGA 与 GTEx 数据库联合分析及 GEPIA 数据库分析结果均显示，SULT2B1 与卵巢癌的不良预后相关（P＜0.05）。

（4）免疫组化染色实验结果表明，SULT2B1 在正常卵巢组织中低表达，在卵巢癌组织中高表达，在亚细胞水平主要定位于细胞质。未发现 SULT2B1 与患者年龄、FIGO 分期、病理类型、术前 CA125 及脱落细胞之间的关系。

（5）Western Blot 实验结果确定 SULT2B1 在卵巢癌组织中较正常卵巢组织高表达（P＜0.05）。

（6）以 SULT2B1 表达量高低为分组依据的 TCGA 数据库中卵巢癌组织的差异分析结果显示 SULT2B1 表达上调可能与核质运输、雌激素代谢、VEGF 等信号通路相关。SULT2B1 与雌激素信号通路相关基因 HBEGF 在卵巢癌中的表达呈正相关（R=0.24，

P=1.1E-08）。

（7）TIMER 数据库分析结果显示 SULT2B1 与卵巢癌中 B 细胞、巨噬细胞、中性粒细胞、树突状细胞、CD4+细胞、CD8+细胞均无明显相关性。

结论：

（1）SULT2B1 在正常卵巢组织中低表达，在卵巢癌组织中的高表达，在亚细胞水平主要定位于细胞质，提示其可能与卵巢癌的发生发展相关。

（2）SULT2B1 对卵巢癌具有较高的特异性诊断价值，且 SULT2B1 高表达与卵巢癌不良预后相关，可作为潜在的卵巢癌诊断及预后评估工具。

关键词：

SULT2B1，卵巢癌，TCGA，GTEx

Abstract

Expression and a bioinformatics analysis of SULT2B1 in ovarian cancer tissue

Background and objective:

Ovarian cancer is the main cause of death in gynecological malignancies, because the early clinical symptoms are not specific, most patients are diagnosed in the middle and advanced stage. Surgery combined with platinum-based chemotherapy constitutes the standard clinical treatment pathway for ovarian cancer, and targeted drug therapy can also be supplemented based on the results of genetic testing. However, most patients still face relapse, metastasis and drug resistance, and the overall prognosis is poor. The pathogenesis of ovarian cancer is still not completely clear. A large number of studies have shown that there are a variety of complex gene mutations in ovarian cancer, which are closely related to the occurrence, progression, metastasis and drug resistance of ovarian cancer. Therefore, it is urgent to find new gene targets, reveal related molecular mechanisms to guide the early diagnosis, prognosis assessment and clinical targeted therapy.

Hydroxysteroid sulfotransferase 2B1 (SULT2B1) is a class of enzymes mediating hydroxysteroid sulfation that is involved in the metabolism of many key endogenous compounds such as catecholamines, thyroid hormones and steroid hormones, as well as various drugs and other foreign substances. A large number of literatures have reported that SULT2B1 is involved in the occurrence and development of various malignant tumors and is associated with poor prognosis, while the role of SULT2B1 in ovarian cancer is rarely reported.

The objectives of this study were: (1) to explore the expression of SULT2B1 in normal ovarian tissue and ovarian cancer tissue; (2) to explore the relationship between SULT2B1 expression and clinicopathologic features in ovarian cancer; (3) to evaluate the value of

SULT2B1 in the diagnosis and prognosis of ovarian cancer; (4) to explore signaling pathways that may be related to SULT2B1 involved in the production and development of ovarian cancer.

Methods:

(1) The transcriptome data of 379 cases of ovarian cancer and 180 cases of normal ovaries were obtained from TCGA database and GTEx database, and the transcriptome information was homogenized and analyzed using “limma” package of R language. The GEPIA database was used to verify the above results and explore the relationship between the expression of SULT2B1 and the pathological stage of ovarian cancer.

(2) The ROC curve of SULT2B1 expression in the diagnosis of ovarian cancer was plotted using the R language “pROC” package.

(3) The clinical data of ovarian cancer patients and the expression of SULT2B1 in the TCGA database were used to perform survival analysis and plot survival curves by Kaplan-Meier method, and the above results were verified by GEPIA database.

(4) 40 cases of epithelial ovarian cancer tissue, 15 cases of benign ovarian tumor tissue and 10 cases of normal ovarian tissue were collected, and the clinicopathological information of the corresponding patients was collected. Immunohistochemical staining was performed to analyze the relationship between the expression of SULT2B1 and the clinicopathological characteristics of epithelial ovarian cancer.

(5) Western Blot analysis was performed using tissue samples from 6 cases of normal ovarian tissue and 7 cases of epithelial ovarian cancer tissue to determine differential expression of SULT2B1 in normal ovarian tissue and ovarian cancer tissue.

(6) The transcriptome data of ovarian cancer tissue in the TCGA database were divided into SULT2B1 high expression group and low expression group according to the median expression of SULT2B1. The differences between the two groups were analyzed to find the genes related to SULT2B1 expression. GO and KEGG enrichment analysis of the above differential genes were performed using the DAVID database to find the relevant biological

processes or signaling pathways and possible downstream targets of SULT2B1 affecting the occurrence and development of ovarian cancer.

(7) The TIMER database was used to analyze the involvement of SULT2B1 in the infiltration of ovarian cancer immune cells.

Results:

(1) The combined analysis of TCGA and GTEx databases and GEPIA database analysis showed that SULT2B1 was highly expressed in ovarian cancer tissue. GEPIA database analysis showed that the difference of SULT2B1 expression was correlated with the pathological stage of ovarian cancer (F=7.09, P<0.01)

(2) The high expression of SULT2B1 had a high diagnostic value for ovarian cancer (AUC=97.6%, $P < 0.05$).

(3) The combined analysis of TCGA and GTEx databases and GEPIA database analysis showed SULT2B1 was correlated with poor prognosis（P＜0.05）.

(4) Immunohistochemical staining showed low expression of SULT2B1 in normal ovarian tissue and high expression of SULT2B1 in ovarian cancer tissue, and SULT2B1 was mainly localized in the cytoplasm at the subcellular level. No relationship was found between SULT2B1 and patients' age, FIGO stage, pathological type, preoperative CA125 and exfoliated cells.

(5) Western Blot results showed that SULT2B1 was more highly expressed in ovarian cancer tissue than in normal ovarian tissue ($P < 0.05$).

(6) The results of the differential analysis of ovarian cancer tissue from TCGA database based on the expression level of SULT2B1 showed that the upregulation of SULT2B1 expression may be related to nucleocytoplasmic transport, estrogen metabolism, VEGF and other signaling pathways. SULT2B1 was positively correlated with the expression of the estrogen signaling pathway-related gene HBEGF in ovarian cancer (R=0.24, P=1.1E-08).

(7) The analysis of TIMER database showed that SULT2B1 was not significantly correlated with B cells, macrophages, neutrophils, dendritic cells, CD4+ cells and CD8+

cells in ovarian cancer.

Conclusion:

(1) The expression of SULT2B1 is low in normal ovarian tissue and high in ovarian cancer tissue, and SULT2B1 is mainly located in the cytoplasm at the subcellular level, suggesting that SULT2B1 may be related to the occurrence and development of ovarian cancer.

(2) SULT2B1 has high specific diagnostic value for ovarian cancer, and high expression of SULT2B1 is associated with poor prognosis of ovarian cancer, which can be used as a potential diagnostic and prognostic evaluation tool for ovarian cancer.

Key words:

SULT2B1, Ovarian cancer, TCGA, GTEx

目　录

中英文缩略词对照表

英文缩写	英文全称	中文全称
SULT2B1	hydroxysteroid sulfotransferase 2B1	羟基类固醇硫酸基转移酶 2B1
SULTs	cytosolic sulfotransferases	胞质硫酸基团转移酶
CS	cholesterol sulfate	胆固醇硫酸盐
DHEA	dehydroepiandrosterone	脱氧表雄酮
EMT	Epithelial-to-Mesenchymal Transition	上皮间充质转化
ESCC	esophageal squamous cell carcinoma	食管鳞状细胞癌
VEGF-A	vascular endothelial growth factor-A	血管内皮生长因子-A
CRC	colorectal cancer	结直肠癌
SNPs	single-nucleotide polymorphisms	单核苷酸多态性
HCC	hepatocellular carcinoma	肝细胞癌
DOCK2	dedicator of cytokinesis 2	细胞分裂蛋白 2
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	3 羟基 3 甲基戊二酰辅酶 A 还原酶
PC	prostate cancer	前列腺癌
ER-β	estrogen receptor-β	雌激素受体-β
AR	androgen receptor	雄激素受体
TNFα	tumor necrosis factor α	肿瘤坏死因子 α
LXR	liver X receptor	肝脏 X 受体
PR	progesterone receptor	孕激素受体
HER2	human epidermal growth factor receptor 2	人类表皮生长因子受体 2
Tam	tamoxifen	他莫昔芬
AEBS	microsomal antiestrogen binding site	微粒体抗雌激素结合位点
ChEH	cholesterol-5,6-epoxide hydrolase	胆固醇-5，6-环氧化物水解酶
NSCLC	non-small cell lung cancer	非小细胞肺癌
ccRCC	clear cell renal carcinoma	透明细胞肾细胞癌
EC	endometrial cancer	子宫内膜癌

续表：

英文缩写	英文全称	中文全称
STS	steroid sulfatase	类固醇硫酸酯酶
HPV	human papillomavirus	人乳头瘤病毒
VP	verteporfin	维替泊芬
TCGA	The Cancer Genome Alats	癌症基因组
GTEx	Genotype-Tissue Expression	基因型-组织表达
FC	fold change	差异倍数
adj.P value	adjust P value	校正后的 P 值
GEPIA	Gene Expression Profilling Interactive Analysis	基因表达谱交互式分析
ROC	receiver operating characteristics	受试者工作特征
AUC	area under the curve	曲线下面积
DAVID	Database for Annotation, Visualization and Integrated Discovery	数据库注释，可视化和集成发现
GO	Gene Ontology	基因本体论
KEGG	Kyoto Encyclopedia of Genes and Genomes	京都基因和基因组百科全书
TIMER	Tumor Immune Estimation Resource	肿瘤免疫评估资源
BP	biological process	生物学过程
CC	cellular component	细胞组成
MF	molecular function	分子功能
HBEGF	heparin-binding epidermal growth factor	肝素结合表皮生长因子

第 1 章 绪 论

卵巢癌是导致女性死亡的第五大癌症，也是妇科肿瘤中的主要死因[1]。卵巢癌的高死亡率主要来源于其早期诊断的困难性，卵巢癌早期在盆腹腔内隐匿进展，大约 70%的卵巢癌患者确诊时已为 III-IV 期，5 年生存率低于 50%[2-3]。上皮性卵巢癌占全部卵巢癌的主要部分，非上皮细胞类型卵巢癌仅占 10%，上皮性卵巢癌主要包括浆液性癌、子宫内膜样癌、透明细胞癌及黏液性癌，而高级别浆液性卵巢癌是上皮性卵巢癌最常见的组织学亚型[4-5]。除少数早期患者仅需进行手术治疗外，铂类联合紫杉醇药物治疗适用于大多数上皮性卵巢癌患者，在药物治疗前，早期患者通常先行全面分期手术、晚期患者行肿瘤细胞减灭术治疗减少肿瘤负荷，还可结合是否存在 BRCA1/2 基因变异及同源重组修复缺陷选择性联合 PARP 抑制剂和免疫检查点抑制物综合治疗，尽管如此，大多数患者仍面临复发和耐药[6-8]。因此，寻找新的精准预测卵巢癌发生及预后水平的标志物，揭示其相应作用的分子机理，确定相应的临床治疗新靶点，对卵巢癌患者预后改善尤为重要。

羟基类固醇硫酸基转移酶 2B1（hydroxysteroid sulfotransferase 2B1，SULT2B1）是人类胞质硫酸基团转移酶（cytosolic sulfotransferases，SULTs）基因超家族的成员之一，通过介导活性硫酸根的转移催化羟基类固醇硫酸盐的形成，参与包括胆固醇在内的多种内源性类固醇激素、神经递质及外源性化合物的代谢，与多种代谢相关疾病及恶性肿瘤密切相关[9-10]。有研究表明，SULT2B1 在具有更强增殖能力和耐药性表型的卵巢癌细胞中表达上调，但仍需要进一步探索 SULT2B1 在卵巢癌发生、发展、耐药等机制中发挥的作用[11]。

本文利用生物信息学分析的方法探究了 SULT2B1 在正常卵巢组织和卵巢癌组织中的表达情况，探究了 SULT2B1 与卵巢癌临床病理特征的关系，评估了 SULT2B1 在卵巢癌诊断及预后方面的价值，并发掘了可能与 SULT2B1 参与诱发卵巢癌相关的信号通路，为进一步发掘卵巢癌病因及发病机制，探索新型诊断及预后评估工具，确定卵巢癌的新型分子治疗靶点提供了参考。