

· 论 著 ·

HJURP 在人脑胶质瘤中的表达及意义：
基于 CGGA 数据库的生信分析

孙玉晨 杨生伟 王惠娟

【摘要】目的 探讨 Holliday 交叉识别蛋白(HJURP)在人脑胶质瘤中的表达及其与临床、病理特征及预后的关系。**方法** 计算机检索中国脑胶质瘤基因组图谱(CGGA)数据库获取 mRNAseq-325 数据集中 325 例胶质瘤的转录组测序(RNA-seq)数据及临床资料,应用 mRNAseq-693 数据集中 693 例脑胶质瘤数据进行验证。利用 R 语言软件分析 HJURP 的表达和脑胶质瘤临床、病理特征的关系。使用 Kaplan-Meier 生存分析评估 HJURP 表达对人脑胶质瘤生存的影响。**结果** 胶质瘤 HJURP 的表达水平明显上调($P<0.05$);其表达水平与胶质瘤 IDH 基因型、1p19q 共缺失状态、WHO 病理分级有关($P<0.0001$),与胶质瘤 MGMT 启动子甲基化无明显相关性($P>0.05$)。Kaplan-Meier 生存曲线分析显示,与 HJURP 高表达组相比,HJURP 低表达组显示出显著的生存优势($P<0.0001$)。GO、KEGG 及 GSVA 分析显示 HJURP 参与脑胶质瘤细胞的有丝分裂调控、细胞周期、染色体分离等。**结论** HJURP 在人脑胶质瘤中异常高表达,并与不良预后相关。这提示 HJURP 可作为人脑胶质瘤的生物标志物。

【关键词】 脑胶质瘤;Holliday 交叉识别蛋白(HJURP);临床特征;病理特征;预后

【文章编号】 1009-153X(2024)12-0724-05 **【文献标志码】** A **【中国图书资料分类号】** R 739.41; Q 786

Expression and significance of HJURP in human glioma: a bioinformatics analysis based on the CGGA database

SUN Yu-chen¹, YANG Sheng-wei², WANG Hui-juan³. 1. Department of Neurosurgery, The Second Hospital of Hebei Medical University, Shijiazhuang 050000, China; 2. Department of Neurosurgery, Zhangjiajie People's Hospital, Zhangjiajie 427000, China; 3. Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang 050000, China

【Abstract】 Objective To explore the expression of Holliday Junction Recognition Protein (HJURP) in human glioma and its association with clinical, pathological characteristics, and prognosis of patients. **Methods** The mRNAseq-325 dataset containing RNA-seq data and clinical information of 325 glioma cases was retrieved from the Chinese Glioma Genome Atlas (CGGA) database by computer. The dataset of 693 glioma cases in mRNAseq-693 was utilized for validation. The relationship between the expression of HJURP and the clinical and pathological characteristics of glioma was analyzed using R language software. Kaplan-Meier survival analysis was employed to assess the influence of HJURP expression on the survival of human glioma. **Results** The expression level of HJURP in glioma was significantly upregulated ($P<0.05$); its expression level was correlated with the IDH genotype of glioma, 1p19q co-deletion status, and WHO pathological grade ($P<0.0001$), but not significantly related to MGMT promoter methylation ($P>0.05$). Kaplan-Meier survival curve analysis demonstrated that the HJURP low-expression group exhibited a significant survival advantage compared to the HJURP high-expression group ($P<0.0001$). GO, KEGG, and GSVA analyses revealed that HJURP was involved in the regulation of mitosis, cell cycle, and chromosome segregation in glioma cells. **Conclusion** HJURP is aberrantly highly expressed in human glioma and is associated with an unfavorable prognosis. This indicates that HJURP can serve as a biomarker for human glioma.

【Key words】 Glioma; Holliday junction recognition protein; Clinical characteristics; Pathological characteristics; Prognosis

胶质瘤为颅内最常见的恶性肿瘤,具有侵袭性强、易复发、预后差等特点,即使采用手术切除联合术后放化疗的积极治疗,5 年生存率仍极低^[1-5],其中胶质母细胞瘤(glioblastoma multiforme, GBM; WHO

分级Ⅳ级)5 年生存率仅 6%^[6]。探索脑胶质瘤进展的调控机制以及潜在生物标志物,对其诊断、预后评估和治疗至关重要。

Holliday 交叉识别蛋白(Holliday junction recognition protein, HJURP)参与有丝分裂调控和染色体分离^[7],其异常激活与肿瘤细胞无限增殖相关^[8],在肝癌、前列腺癌、膀胱癌、乳腺癌、肺癌等恶性肿瘤中异常表达^[9-11]。本文检索中国脑胶质瘤基因组图谱(Chinese Glioma Genome Atlas, CGGA)数据库,应用生物信息学方法分析 HJURP 在人脑胶质瘤中表达特征及其对病人预后的影响。

doi:10.13798/j.issn.1009-153X.2024.12.005

基金项目:河北省自然科学基金(H2022206078);河北省卫生健康委科研基金(20230047)

作者单位:050000 石家庄,河北医科大学第二医院神经外科(孙玉晨),神经内科(王惠娟);427000 湖南,张家界市人民医院神经外科(杨生伟)

1 资料与方法

1.1 数据获取 通过UALCAN(<https://ualcan.path.uab.edu/>)基于TCGA数据库(The Cancer Genome Atlas Program)对HJURP在胶质母细胞瘤和正常对照组之间表达情况进行预分析。从CGGA数据库(<http://www.cgga.org.cn/>)下载mRNAseq-325数据集^[12-15]($n=325$)及mRNAseq-693数据集^[12,15-17]($n=693$),包括胶质瘤转录组测序(RNA-seq)数据及相应临床信息。

1.2 数据处理及可视化 应用R语言(版本4.3)以及Excel、GraphpadPrism等软件处理。临床信息缺失和生存时间为0 d的样本予以删除,受试者工作特征(receiver operating characteristic, ROC)曲线删除mRNAseq-325数据集中无WHO分级的4例病人信息,mRNAseq-693数据集删除1例无WHO分级信息病人。通过Pearson相关分析,在mRNAseq-325和mRNAseq-693两个数据集中筛选出与HJURP最相关的基因($R>0.5$; $P<0.05$),上传至DAVID数据库(Database for Annotation, Visualization, and Integrated Discovery, DAVID; v6.8)进行GO和KEGG分析,并根据P值进行可视化。从AmiGO2网站(<http://amigo.geneontology.org/amigo>)获取与HJURP相关的生物学过程的基因集,采用Pearson相关分析HJURP与相关生物学过程的相关性,并应用R语言进行可视化处理。

1.3 预后分析 删除mRNAseq-325数据集12例没有生存信息病人数据,删除mRNAseq-693数据集36例没有生存信息病人数据,应用X-tile软件计算出最佳截断值,根据HJURP表达量分为高表达组和低表达组,应用Graphpad软件绘制Kaplan-Meier生存曲线。

1.4 统计学方法 应用SPSS 23.0软件进行分析;定量资料以 $\bar{x}\pm s$ 表示,采用t检验和单因素方差分析; $P<0.05$ 为差异具有统计学意义。

2 结果

2.1 胶质瘤HJURP表达水平与病人临床、病理特征的关系 通过UALCAN基于TCGA数据库预分析结果显示GBM组织HJURP表达显著高于正常对照组($P<0.05$;图1)。基于CGGA数据库两个数据集的结果显示,不同HJURP表达水平的胶质瘤病人具有不同的临床病理学特征(图2)。mRNAseq-325数据集分析结果显示,WHO分级Ⅳ级胶质瘤($n=139$)HJURP表达水平显著高于WHO分级Ⅱ级($n=103$)

和Ⅲ级胶质瘤($n=79$),并且随着胶质瘤WHO级别的增高,HJURP表达水平也随之升高($P<0.0001$)。此外,HJURP在无1p/19q联合共缺失的胶质瘤中表达水平显著升高($P<0.0001$),在异柠檬酸脱氢酶(isocitrate dehydrogenase, IDH)野生型胶质瘤中高度富集($P=0.0003$)。HJURP表达水平在MGMT启动子甲基化和非甲基化两组之间无统计学差异($P=0.8652$)。这些结果均在mRNAseq-693数据集得到验证(图2)。这提示恶性程度较高的胶质瘤中HJURP表达水平更高。ROC曲线评价HJURP在胶质母细胞瘤中的表达特异性发现在mRNAseq-325数据集中,曲线下面积(area under the curve, AUC)为76.1%($P<0.0001$),mRNAseq-693数据集AUC为70.9%($P<0.0001$)。这表明HJURP可作为胶质瘤的潜在生物标志物。

2.2 胶质瘤HJURP表达水平与病人生存预后的关系 应用X-tile软件计算出mRNAseq-325数据集最佳截断值为3.6,定义HJURP表达量 ≥ 3.6 为高表达组, <3.6 为低表达组;mRNAseq-693数据集最佳截断值为3.8,定义HJURP表达量 ≥ 3.8 为高表达组, <3.8 为低表达组。Kaplan-Meier生存曲线分析显示,与HJURP高表达组相比,HJURP低表达组显示出显著的生存优势($P<0.0001$;图3)。

2.3 GO、KEGG及GSVA分析 GO和KEGG分析结果发现两个数据集所获取的结果相似,在人脑胶质瘤中与HJURP最相关的生物学过程包括细胞分裂、DNA复制、DNA修复、细胞周期、有丝分裂姐妹染色体分离等。此外,与HJURP最相关的细胞组分是:细胞核、着丝粒、染色体等。相关分子功能为:蛋白质结合、RNA结合、染色质结合、DNA结合、单链

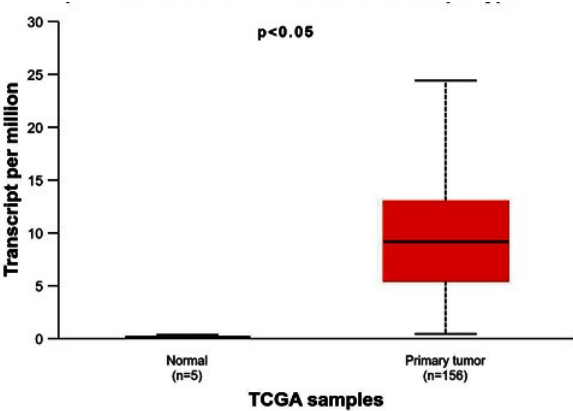


图1 TCGA数据库预分析显示胶质母细胞瘤HJURP的表达明显上调
Figure 1 Pre-analysis of the TCGA database indicates up-regulation of HJURP in glioblastoma

DNA结合、ATP结合等。HJURP最相关的信号通路包括细胞周期及DNA复制等相关通路。这些结果提示HJURP可能在人脑胶质瘤细胞有丝分裂调控、细胞周期、染色体分离中发挥重要作用。mRNAseq-325和mRNAseq-693两个数据集的GSVA确定HJURP在细胞分裂、细胞周期、有丝分裂、DNA复制等过程的富集评分。富集评分与HJURP表达的相关性分析显示,HJURP表达与大多数细胞分裂、细胞周期、有丝分裂、DNA复制等过程均呈正相关。

3 讨论

HJURP在有丝分裂中对丝粒蛋白A(CENP-A)合成和维持功能起着关键作用^[18]。CENP-A位于着丝粒染色质的中心位置,对染色体的正常分离起着重要作用^[19]。HJURP可作为CENP-A的分子伴侣,在染色体分离和细胞周期调节中起着关键作用,其在非小细胞肺癌、膀胱癌、肝癌、乳腺癌等多种恶性肿瘤中表达异常上调,且与不良预后密切相关^[20]。

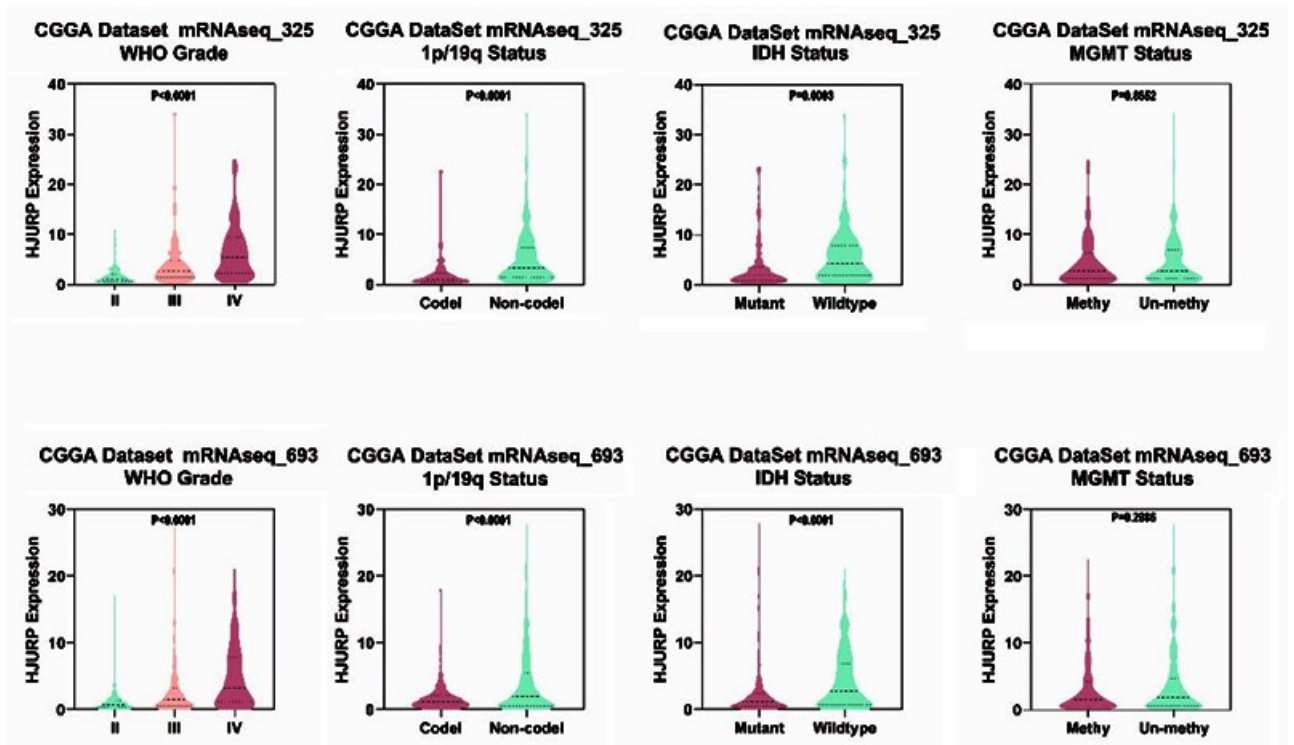


图2 胶质瘤HJURP表达水平与病人临床病理特征的关系

Figure 2 Relationship between the HJURP expression levels of in glioma tissues and the clinical and pathological characteristics of glioma patients

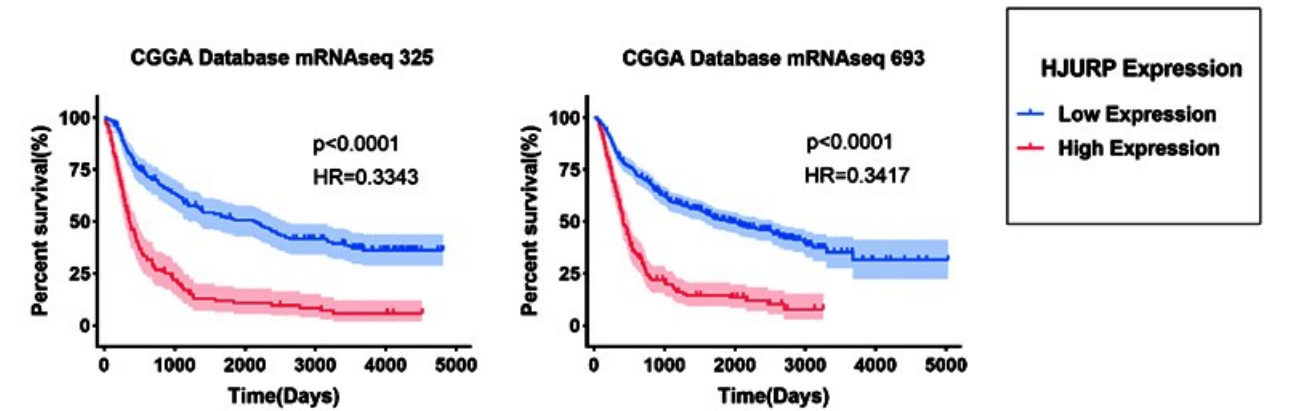


图3 Kaplan-Meier 生存曲线分析胶质瘤HJURP表达水平与病人生存预后的关系

Figure 3 Kaplan-Meier survival curve analysis of the relationship between the expression levels of HJURP in glioma tissues and the survival prognosis of glioma patients

我们通过UALCAN基于TCGA数据库对HJURP基因进行预分析发现,HJURP在GBM中的表达显著高于正常对照组。本文基于CGGA数据库的两个胶质瘤mRNA表达谱及相应临床数据集,分析胶质瘤HJURP的表达水平,发现HJURP与人脑胶质瘤临床病理学特征密切相关,HJURP的表达水平与病人预后负相关。这提示其在人脑胶质瘤中的起着“促癌基因”的作用,这与Valente等^[21]和Serafim等^[22]体外细胞实验结果相一致。

HJURP可能通过参与细胞周期过程影响胶质瘤的发生、发展。本研究GO分析、KEGG分析以及GSVA分析发现HJURP在人脑胶质瘤的细胞有丝分裂调控、细胞周期、染色体分离、DNA修复过程中发挥重要作用。这也与先前文献报道的HJURP在其他肿瘤中的作用相似^[20]。HJURP过表达可通过参与细胞周期等过程促进胶质瘤细胞增殖。此外,其可能通过参与DNA修复过程来维持肿瘤基因组稳定性,导致了胶质瘤耐药性^[20]。据报道,通过沉默HJURP基因可诱导肿瘤细胞凋亡、阻滞肿瘤细胞周期,同时提高肿瘤的放化疗敏感性,进而产生抗肿瘤效果^[20,23]。HJURP在不同的肿瘤细胞类型显示出不同的分子功能,可能与其表达的亚细胞定位有关,对此仍有争议。例如在U-2OS细胞、浆液性卵巢癌细胞中,HJURP定位于细胞核^[19,24];而在前列腺癌等多种癌细胞中,HJURP主要表达于细胞质^[8]。有报道表明,HJURP在人脑胶质瘤中主要于细胞核表达,且随着胶质瘤级别的升高而增强^[23,25]。这在一定程度上和本文研究结果一致,而其具体作用机制有待进一步研究。

但是,我们仅通过数据库的挖掘对HJURP基因在人脑胶质瘤组织中作用进行分析,具有一定局限性,有待开展体内外实验进一步验证;同时,针对HJURP的靶向药物研究也需探索。

总之,HJURP在人脑胶质瘤中异常高表达,并与不良预后相关。这提示HJURP可作为脑胶质瘤的潜在标志物。

【伦理学声明】:本研究方案于2022年2月28日经河北医科大学第二医院科研伦理委员会审批,批号为2022-AE016。

【利益冲突声明】:本文不存在任何利益冲突。

【作者贡献声明】:孙玉晨负责数据下载、数据分析、可视化分析、文章撰写;杨生伟负责数据分析、文章框架构建、修改文章;王惠娟负责思路指导、修改文

章以及技术支持等。
【致谢】:感谢毛博轩同志在数据分析及绘图中的技术支持。同时感谢中国脑胶质瘤基因组图谱(Chinese Glioma Genome Atlas,CGGA)数据库提供的公开原始数据资料。

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(2023–11–29 收稿, 2024–07–24 修回)

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(2023–07–02 收稿, 2024–04–02 修回)