CD-DST 药敏指导高级别胶质瘤术后辅助化疗的疗效研究

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摘　要:[目的]探讨胶滴肿瘤药敏检测技术(CD-DST)指导高级别胶质瘤术后辅助化疗的疗效,以及体外敏感性与 1p/19q 杂合性缺失基因变异相关性。[方法]2014 年 1 月至 2017 年 3 月确诊高级别脑胶质瘤患者 63 例,手术肿瘤标本经 CD-DST 法标准处理后分别对替莫唑胺等 5 种化疗药物及组合进行检测,根据 CD-DST 结果指导规范术后辅助化疗方案;FISH 探针法测定肿瘤组织 1p/19q 杂合性缺失基因变异。[结果]63 例高级别脑胶质瘤患者体外 CD-DST 检测结果显示替莫唑胺、顺铂、VP-16、卡铂和卡莫司汀的 T/C 值分别为 68.97%、75.43%、68.96%、79.59%和 78.14%;联合方案 T/C 值分别为 70.80%、62.23%、72.30%、63.97%和 72.13%。CD-DST 指导的行规范治疗与未行规范治疗患者的中位总生存期分别为 14 个月和 6 个月,差异有统计学意义(P<0.05)。1p/19q 杂合性缺失 19 例(30.2%),1p/19q 杂合性缺失组行规范治疗者中位总生存期显著优于未行规范治疗者(15 个月 *vs* 5 个月,P<0.001)。[结论]CD-DST 指导术后辅助化疗方案显著改善高级别脑胶质瘤的总生存,结合 1p/19q 杂合性缺失对于胶质瘤的治疗具有预后和治疗预测价值。

主题词:胶质瘤;胶滴肿瘤药敏检测技术;替莫唑胺;1p/19q
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Collagen Gel Droplet-embedded Culture Drug Sensitivity Test to Predict Responses in Postoperative Adjuvant Chemotherapy for High-grade Gliomas

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Abstract: [Objective] To evaluate the application of collagen gel droplet-embedded culture drug sensitivity test (CD-DST) in predicting responses of postoperative adjuvant chemotherapy for patients with high-grade gliomas. [Methods] From January 2014 to March 2017, 63 patients with high-grade gilomas were enrolled in the study. The surgical specimens were collected and the CD-DST was performed for testing drug sensitivity. Among 63 patients, 26 received chemotherapy guided by CD-DST(pilot group) and 37 were not (control group). And 1p/19q co-deletion in glioma cells were detected by FISH method. [Results] CD-DST showed that the sensitivity of glioma to temozolomide, cisplatin, VP-16, carboplatin and carmustine was 68.97%, 75.43%, 68.96%, 79.59% and 78.14%, and the sensitivity to combined regimes was 70.80%, 62.23%, 72.30%, 63.97% and 72.13%, respectively. The median overall survival (mOS) was 14 months in pilot group and 6 months in control group(P<0.05). 1p/19q co-deletion was detected in 19 of 63 cases(30.2%). The mOS of patients with 1p/19q co-deletion in pilot group was significantly higher than that in control group (15months *vs.* 5months, P<0.001). [Conclusion] Postoperative adjuvant chemotherapy guided by CD-DST significantly improves the overall survival in patients with high-grade gliomas, particularly for those with 1p/19q co-deletion.

Subject words: glioma; CD-DST; temozolomide; 1p/19q

胶滴肿瘤药敏检测技术 (collagen gel droplet-embedded culture drug sensitivity test,CD-DST)是一种三维立体的化疗药物体外药敏检测技术,比传统 MTT 法检测准确率更高,广泛应用于肺癌、乳腺癌等实体肿瘤的体外药敏指导临床化疗策略[1,2]。神经胶质瘤(gliomas)简称胶质瘤,是发生于神经外胚层的

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肿瘤，约占所有颅内肿瘤的45%左右，年发病率约3.5/10万~4.5/10万，但总体生存获益仍不乐观，尤其是高级别胶质瘤的临床诊疗水平总体上还滞后于发达国家[3,4]。目前尚无CD-DST技术用于高级别胶质瘤的临床应用数据，本研究旨在通过63例高级别胶质瘤患者体外CD-DST药敏试验指导术后辅助化疗方案，评估规范治疗和非规范治疗的疗效差异，并结合1p/19q杂合性缺失检测，以期建立高级别胶质瘤个体化规范化治疗体系。

1 材料与方法

1.1 标本来源

自2014年1月至2017年3月于中国医科大学肿瘤医院/辽宁省肿瘤医院就诊的高级别胶质瘤患者63例，手术切取癌组织块体外CD-DST药敏试验。切取的标本2h内送实验室处理，余均送病理学检查确诊。所有患者均签署知情同意书。

63例高级别脑胶质瘤患者，其中男性34例，女性29例；平均年龄61.3岁，年龄≥60岁35例，<60岁28例；病理分级：胶质瘤3级22例，4级41例。

1.2 胶滴肿瘤药敏检测(CD-DST)

替莫唑胺、VP-16、顺铂、卡铂和卡莫司汀，以及联合方案长春新碱联合卡莫司汀、替莫唑胺联合顺铂、顺铂联合卡莫司汀、替莫唑胺联合VP16和VP16联合卡铂。各试验药物分别为：替莫唑胺2mg/L、VP-16 1.6mg/L、顺铂0.5mg/L、卡铂3mg/L和卡莫司汀0.6mg/L。试验步骤：先修剪标本，剪去脂肪组织和纤维组织。标本以除菌洗涤液浸洗10min共2次，切成0.5mm^3~1.0mm^{3}组织块，切碎成泥状，加入培养基移入胶原凝胶包被培养瓶，培养并制成胶原原液，滴注到6孔板制成胶原凝胶滴，每孔接种3滴。待药物处理后进行MTT (0.5mg/ml)固定染色，测定光密度值。T值为药物处理后的光密度值，C为对照的光密度值，T/C值表示相对增殖率，其数值越低表明抑制越强，试验药物越敏感。

1.3 根据药敏试验结果选择化疗方案规范治疗

(1)敏感药物≥2种时，选择药敏抑制率最高的化疗药的辅助化疗方案；(2) 只有一种敏感药物：选择包含这种药物的单药或者联合化疗方案。(3)无敏感药物：可选择替莫唑胺化疗方案。化疗2个疗程后评价疗效，治疗有效者化疗4个疗程。未按照上述进行的治疗归为未规范治疗组。

1.4 1p/19q杂合缺失检测

采用石蜡包埋切片，HE染色选取肿瘤区域，采用1p/19q荧光探针试剂盒(美国雅培公司)，在一张切片上分别标记1p36和1q25，分别标记为红色靶点探针、绿色参考探针，另一张切片上标记19q13和19p13，分别标记为红色靶点探针、绿色参考探针。DAPI标记细胞核，为蓝色探针。缺失细胞判断标准为红色信号数目小于绿色信号数目，当缺失细胞比例>30%时为1p/19q杂合缺失阳性。

1.5 统计学处理

实验结果使用SPSS21.0软件进行分析，计量资料结果以均数±标准差($\bar{x}\pm s$)表示；运用Log-rank法行单因素分析，采用Cox-Regression模型行多因素分析，同时采用Kaplan-Meier法绘制生存曲线。$P<0.05$为差异有统计学意义。

2 结 果

2.1 高级别脑胶质瘤CD-DST检测药敏结果

63例高级别脑胶质瘤患者的替莫唑胺、VP-16、卡铂、卡莫司汀和顺铂的体外增殖率T/C值分别为68.97%、75.43%、68.96%、79.59%和78.14%，联合方案长春新碱联合卡莫司汀、替莫唑胺联合顺铂、顺铂联合卡莫司汀、替莫唑胺联合VP16和VP16联合卡铂的T/C值分别为70.80%、62.23%、72.30%、63.97%和72.13%。

CD-DST指导的行规范治疗的患者共26例，未行规范治疗患者37例。规范治疗组上述单药T/C值分别为66.54%、74.72%、70.43%、82.20%和76.53%，上述联合方案的T/C值分别为77.34%、66.02%、79.00%、65.74%和75.44%。未接受规范化治疗组单药T/C值分别为70.50%、75.88%、68.03%、77.94%和79.16%，联合方案的T/C值分别为66.68%、59.84%、68.07%、62.85%和70.04%。详见Table 1。

2.2 CD-DST指导高级别胶质瘤患者的总生存

CD-DST指导化疗方案共计26例，其中以替莫唑胺单药最多，共计18例，其他方案包括卡莫司汀单药1例，卡莫司汀联合VP-16 3例、顺铂联合卡莫司汀2例和替莫唑胺联合VP-16 2例。CD-DST指导

的行规范治疗者中位总生存期为14个月，显著优于未行规范化治疗者的6个月，差异有统计学意义（P<0.001）。见Figure 1A。

2.3 1p/19q杂合性缺失

63例高级别脑胶质瘤患者中1p/19q杂合性缺失组19例，发生率30.2%，lp/19q正常组44例。1p/19q杂合性缺失组上述单药T/C值分别为59.06%、71.50%、65.31%、80.79%和76.72%，上述联合方案T/C值分别为68.69%、57.18%、70.36%、57.75%和69.70%；1p/19q正常组单药T/C值分别为76.50%、78.43%、71.74%、78.67%和79.23%，联合方案T/C值分别为72.40%、66.07%、73.77%、68.70%和73.97%，详见Table 2。

总生存亚组分析中，1p/19q杂合性缺失组中CD-DST指导的行规范治疗者中位总生存期为15个月，明显优于未行规范化治疗者的5个月，差异有统计学意义（P<0.001）。1p/19q正常组CD-DST指导的行规范治疗者中位总生存期为8个月，优于未行规范化治疗的7个月（P=0.005）。详见Figure 1B、C。

对于总生存进行多因素Cox分析，结果显示，总生存与高级别胶质瘤患者CD-DST指导规范治疗密切相关，但与年龄、性别、肿瘤级别及1p/19q杂合性缺失无相关性（P>0.05）（见Table 3）。

Table 1 Sensitivity of CD-DST of chemotherapy regimen in high-grade gliomas

T/C value (%)	All (n=63)	CD-DST pilot (n=26)	Control (n=37)	t	P
Temozolomide	68.97	66.54	70.50	−0.669	0.507
VP-16	75.43	74.72	75.88	−0.027	0.978
Cisplatin	68.96	70.43	68.03	0.473	0.639
Carboplatin	79.59	82.20	77.94	1.963	0.058
Carmustine	78.14	76.53	79.16	−0.706	0.484
Vinorelbine+carmustine	70.80	77.34	66.68	1.722	0.092
Temozolomide+cisplatin	62.23	66.02	59.84	1.295	0.202
Cisplatin+carmustine	72.30	79.00	68.07	2.464	0.018
Temozolomide+VP16	63.97	65.74	62.85	0.620	0.538
VP16+ carboplatin	72.13	75.44	70.04	1.382	0.174

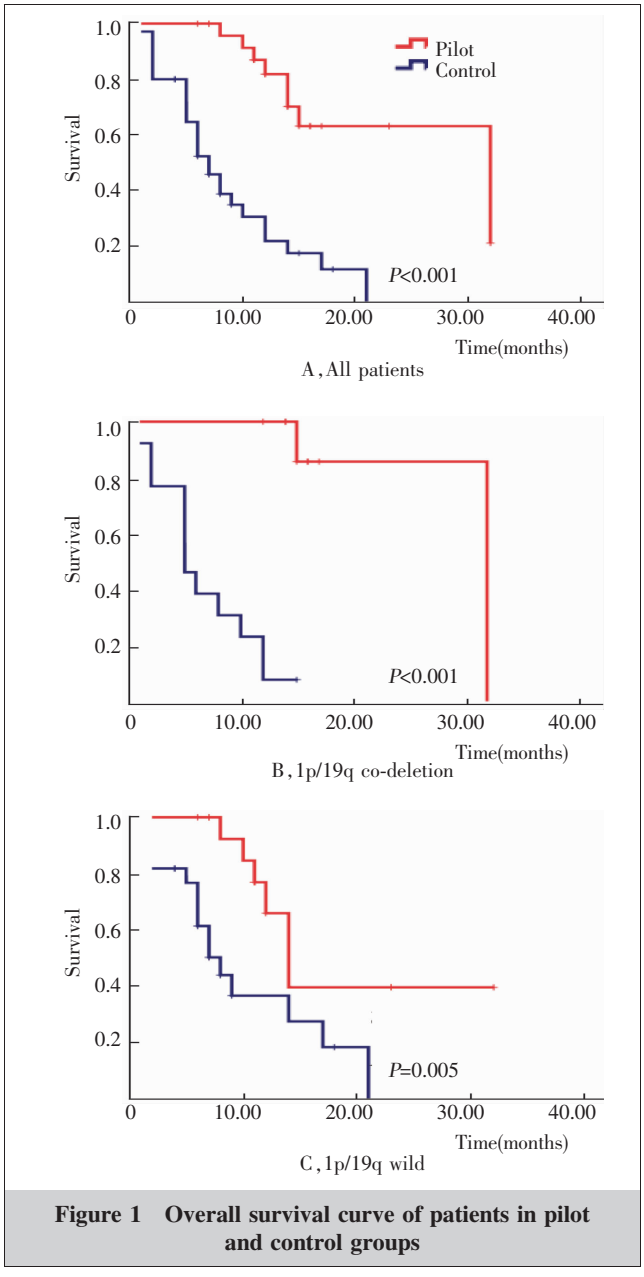

Figure 1 Overall survival curve of patients in pilot and control groups

3 讨 论

高级别胶质瘤以手术结合辅助放化疗的综合治疗其疗效得到很大改观，但总体生存获益仍不乐观。研究报道术后辅助化疗联合全脑放疗的恶性神经胶质瘤的中位生存期仅12~15个月，5年生存率不及10%[3,4]。本次体外药敏试验采用的化疗药物均为常用术后辅助化疗药物，包括替莫唑胺、VP-16、卡铂、卡莫司汀和顺铂，以及联合方案长春新碱联合卡莫司汀、替莫唑胺联合顺铂、顺铂联合卡莫司汀、替莫唑胺联合VP16和VP16联合卡铂。研究结果显示，替莫唑胺和顺铂T/C值最高，联合方案以替莫唑胺联合VP-16和顺铂联合VP-16最高，这与国外报道的临床研究结果一致，替莫唑胺是胶质瘤治

Table 2　Sensitivity of CD-DST of chemotherapy regimen in high-grade gliomas with 1p/19q co-deletion

T/C vaule (%)	All (n=63)	1p/19q co-deletion (n=19)	1p/19q normal (n=44)	*t*	*P*
Temozolomide	68.97	59.06	76.50	−4.021	<0.001
VP-16	75.43	71.50	78.43	−2.153	0.037
Cisplatin	68.96	65.31	71.43	−1745	0.088
Carboplatin	79.59	80.79	78.67	0.703	0.486
Carmustine	78.14	76.72	79.23	−1.050	0.300
Vinorelbine + carmustine	70.80	68.69	72.40	−0.656	0.515
Temozolomide + cisplatin	62.23	57.18	66.07	−1.698	0.102
Cisplatin + carmustine	72.30	70.36	73.77	−0.703	0.486
Temozolomide +VP16	63.97	57.75	68.70	−2.259	0.029
VP16+ carboplatin	72.13	69.70	73.97	−1.219	0.230

Table 3　Multivariable Cox survival analysis in patients with high-grade gliomas

Index	Exp(B)	β	SE	Wald	*P*	95%CI
Age	0.643	−0.442	0.373	1.410	0.235	0.310~1.334
Gender	0.997	−0.003	0.379	0.000	0.994	0.474~2.096
Grade(WHO)	0.712	−0.340	0.388	0.767	0.381	0.333~1.523
1p/19q co-deletion	1.059	0.057	0.383	0.022	0.882	0.499~2.244
CD-DST pilot	0.079	−2.537	0.494	26.321	0.000	0.030~0.209

疗的基石，而VP-16联合方案能显著改善胶质瘤患者的生存预后[5~7]，但在每例患者中不同药物的敏感率不一样，说明胶质瘤的肿瘤异质性存在，因此，高级别胶质瘤的治疗需要个体化治疗。

胶滴肿瘤药敏检测技术(CD-DST)是一种三维立体的化疗药物体外药敏检测技术，与传统MTT法、组织块培养终点染色计算机分析法以及ATP生物荧光肿瘤体外药敏检测技术相比，具有模拟体内微环境的生物学特征、所需细胞量少且成功率高，结果与临床用药一致性高的优点，解决了原代培养成功率低和成纤维细胞混淆的难题[8]。Naitoh等[9]报道在晚期胃癌患者根据CD-DST检测结果共33例患者表现至少一种化疗药物的高度敏感，即T/C值小于60%，而31例患者表现对三种药物完全低度敏感，即T/C大于60%，按照CD-DST结果指导治疗中位总生存从12.5个月提升至15.5个月[9]。Ⅱ期JACCRO-GC04研究在术后辅助治疗方案中5-Fu应用CD-DST方法进行药敏检测，在前瞻性研究结果发现体外药敏反应型生存预后显著优于无反应者，且与5-Fu剂量无关[10]。有研究显示CD-DST检测结果发现原发病灶和转移病灶对于体外药物敏感性不同，说明异质性的存在，也提示对于存在转移病灶者的化疗药物方案推荐应该慎重，可能充分考虑原发灶和转移灶的药物敏感性更佳[11]。Takahashi等[12]报道在结直肠癌探索新药疗效CD-DST提供了较细胞株更模拟体内状态和药物疗效的新方法。本研究结果证实按照CD-DST结果在高级别胶质瘤指导术后辅助化疗方案中，行规范治疗者中位总生存显著优于未行规范化治疗者。

本研究结果显示1p/19q杂合性缺失在高级别胶质瘤中发生率达30.2%，与国外报道数据类似，其检测手段包括FISH、核素CT和NGS等[13~15]。Meta分析结果显示在3408例患者中分析1p/19q杂合性缺失可增强化疗敏感性，其预后PFS和OS显著优于正常患者(HR=0.63，HR=0.43)，且生存获益与检测方法、胶质瘤级别无关[16]；在少突胶质细胞瘤1p/19q杂合性缺失与肿瘤干细胞特性和SOX17等有关[17~19]。本研究中CD-DST指导规范治疗结合1p/19q杂合性缺失检测，未行规范治疗的方案包括替莫唑胺、卡莫司汀等，在体外药敏检测中提示使用的药物敏感性劣于其他药物，以及未行化疗的患者。结果表明1p/19q杂合性缺失组中CD-DST指导的行规范治疗者中位总生存期为15个月，明显优于未行规范化治疗者的5个月；而1p/19q正常组两组中位总生存期相似。证明CD-DST可以推广应用于高级别胶质瘤的术后辅助治疗，具有可行性和可靠性。

总之，本研究表明CD-DST指导术后辅助化疗方案显著改善高级别脑胶质瘤患者的总生存，结合1p/19q杂合性缺失对于胶质瘤的治疗具有预后和预测价值，为建立高级别胶质瘤个体化规范化治疗体系提供依据。

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