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单羧酸转运蛋白MCT1和MCT4在肾透明细胞癌中的表达水平作为靶向治疗预后指标的研究

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摘　要

目的：靶向治疗晚期肾透明细胞癌远未达到临床预想的效果。如何能寻找一种能够预测其治疗效果的生物标志物成为迫切需要解决的问题。本课题主要研究单羧酸转运蛋白MCT1和MCT4的表达水平与晚期肾癌靶向治疗疗效之间的关系，以期望找到准确的靶向治疗肾癌的预后因子。方法：采用组织芯片法检测2009~2017年75例接受索拉非尼或舒尼替尼治疗的肾透明细胞癌(clear cell renal cell carcinoma, ccRCC)组织中MCT1、MCT4的表达，探讨其与临床及病理指标以及预后之间的关系。结果：MCT1、MCT4的表达与年龄、肿瘤直径、肿瘤分期、Furmann分级、MSKCC无显著性差异($P > 0.05$)。生存分析结果显示MCT1高表达与总生存期OS ($P = 0.023$, HR = 0.14, 95%CI = 0.03~0.50)以及肿瘤无进展期PFS ($P = 0.026$, HR = 0.19, HR = 0.19, 95%CI = 0.07~0.54)显著相关。MCT4高表达与OS ($P = 0.015$, HR = 0.16, 95%CI = 0.04~0.56)和PFS ($P = 0.03$, HR = 0.29 95%CI = 0.12~0.73)显著有关。COX回归分析显示MCT4为独立预后因素，MCT4高表达与OS ($P = 0.03$, HR = 0.09, 95%CI = 0.12~0.82)和PFS ($P = 0.047$, HR = 0.35, 95%CI = 0.12~0.99)显著有关。MCT1表达水平与PFS相关($P = 0.014$, HR = 0.26, 95%CI = 0.09~0.76)而与OS无显著相关($P = 0.79$)。结论：MCT1和MCT4的表达水平与OS和PFS明显相关。肿瘤MCT1、MCT4表达水平是预测ccRCC或靶向治疗疗效预后的独立因素，因此MCT1和MCT4表达水平可能成为预测靶向治疗效果的新生物标记物。

关键词

单羧酸转运蛋白，透明细胞肾癌，总生存时间，无进展期，靶向治疗

The Study of the Expression of MCT1 and MCT4 as a Prognostic Indicator of Targeted Therapy in Renal Clear Cell Carcinoma

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Abstract

Purpose: Targeted therapy for advanced renal clear cell carcinoma is far from achieving the expected clinical effect. How to find a biomarker that can predict its therapeutic effect has become an urgent problem to be solved. This project is to study the relationship between the expression levels of MCT1 and MCT4 and the efficacy of targeted therapy in advanced renal cell carcinoma, in order to find the accurate prognostic factors of targeted therapy for renal cell carcinoma. Methods: Tissue microarray was used to detect the expression of MCT1 and MCT4 in 75 cases of clear cell renal cell carcinoma (ccRCC) treated with sorafenib or sunitinib from 2009 to 2017. Results: The expression of MCT1 and MCT4 had no significant difference with age, tumor diameter, tumor stage, Furmann grade and MSKCC ($P > 0.05$). Survival analysis showed that high expression of MCT1 was significantly associated with OS ($P = 0.023$, HR = 0.14, 95%CI = 0.03~0.50) and PFS ($P = 0.026$, HR = 0.19, HR = 0.19, 95%CI = 0.07~0.54). High expression of MCT4 was significantly associated with OS ($P = 0.015$, HR = 0.16, 95%CI = 0.04~0.56) and PFS ($P = 0.03$, HR = 0.29, 95%CI = 0.12~0.73). Cox regression analysis showed that MCT4 was an independent prognostic factor. High expression of MCT4 was significantly associated with OS ($P = 0.03$, HR = 0.09, 95%CI = 0.12~0.82) and PFS ($P = 0.047$, HR = 0.35, 95%CI = 0.12~0.99). MCT1 expression was correlated with PFS ($P = 0.014$, HR = 0.26, 95%CI = 0.09~0.76), but not with OS ($P = 0.79$). Conclusion: The expression levels of MCT1 and MCT4 were significantly correlated with OS and PFS. The expression levels of MCT1 and MCT4 are independent factors for predicting the prognosis of ccRCC or targeted therapy. Therefore, the expression levels of MCT1 and MCT4 may become new biomarkers for predicting the effect of targeted therapy.

Keywords

Monocarboxylate Transporter, Clear Cell Renal Cell Carcinoma, Overall Survival, Progression Free Survival, Targeting Therapy

1. 引言

肾透明细胞癌是一种高度血管化的肿瘤。近十年抗血管生成药物如舒尼替尼、索拉非尼等在晚期肾透明细胞癌治疗中取得了一定的临床效果[1]。然而，就无进展生存期(PFS)和总生存率(OS)来评估治疗效果时，大多数治疗药物提供的姑息效益低于我们的预期，PFS 和 OS 的反应率分别为 70.6%和 46.3% [2]。自从“Warburg”和“Reverse Warburg 效应”被提出[1] [2] [3]以来，肿瘤的糖酵解供能方式越来越被重视。单羧酸转运蛋白(MCTs)主要负责乳酸的转运，在调节糖酵解和维持细胞微环境中的酸碱平衡起重要作用[4]。因此，我们推测 MCTs 可通过促进糖酵解，从而削弱抗血管生成药物的作用。本研究旨在探讨单羧酸转运蛋白(MCT1，MCT4)的表达水平是否可以作为靶向治疗肾透明细胞癌的预后指标。

2. 材料与方法

2.1. 一般材料

回顾性研究 2010 年 6 月至 2015 年 6 月无法手术切除的肾透明细胞癌(ccRCC)患者 75 例。所有患者均采用口服索拉非尼或舒尼替尼作为一线治疗方案，持续至病情进展或出现不能耐受的不良反应。排除标准：接受肿瘤手术治疗病人；年龄大于 70 岁，合并有其他类型肿瘤；由于经济或个人主观原因自动放弃治疗；不规律服用药物；转移器官 ≥ 两处。临床参数包括年龄、性别、肿瘤大小(直径)、肿瘤分期、Furmann 分级 I/II/III)、MSKCC 评分、PFS、OS (表 1)。伦理符合“赫尔辛基宣言”和“青岛附属医院伦理委员会宣言”准则。

Table 1. Clinical data and expression levels of MCT1 and MCT4 in patient receiving targeted therapy (n = 75)
表 1. 接受靶向治疗患者的临床资料及 MCT1、MCT4 的表达水平(n = 75)

临床资料	例数(n)	MCT1 (低)	MCT1 (高)	P	MCT4 (低)	MCT4 (高)	P
性别							
男	41 (54.7%)	25 (60.9%)	16 (39.1%)	0.38	27 (65.9%)	14 (34.1%)	0.22
女	34 (45.3%)	22 (64.7%)	12 (35.3%)		23 (67.6%)	11 (32.4%)	
年龄(年)	56.5 ± 10	55.7 ± 9.7	57.7 ± 10.6	0.46	56.1 ± 8.6	56.9 ± 11.5	0.76
肿瘤直径(cm)	10.4 ± 3.3	9.6 ± 2.8	11.8 ± 3.7	0.22	9.7 ± 2.7	11.2 ± 3.7	0.11
T							
T1	0	0	0		0	0	
T2	0	0	0	0.35	0	0	0.95
T3	8 (10.7%)	6 (75.0%)	2 (25.0%)		4 (50.0%)	4 (50.0%)	
T4	67 (89.3%)	45 (67.1%)	22 (32.9%)		34 (50.7%)	33 (49.3%)	
N							
N0	18 (24%)	12 (66.7%)	6 (33.3%)	0.89	10 (55.6%)	8 (44.4)	0.47
N (1~2)	57 (76%)	33 (57.9%)	24 (42.1%)		4	4	
M							
M0	49 (65%)	31 (63.2%)	18 (36.8%)	0.24	25 (51.0%)	24 (49.0%)	0.70
M1	26 (35%)	12 (46.2%	14 (53.8%)		12 (46.2%)	14 (53.8%)	
Furmann 分级							
1	3 (4%)	2 (66.7)	1 (33.3%)	0.69	1 (33.3%)	2 (66.7)	0.17
2	17 (23%)	12 (70.6%)	5 (29.4%)		7 (41.2%)	10 (58.8%)	
3	55 (73%)	31 (56.3%)	24 (43.6%)		31 (56.4%)	24 (43.6%)	
MSKCC 评分							
1	5 (7%)	3 (60.0%)	2 (40.0%)	0.54	3 (60.0%)	2 (40.0%)	0.91
2	15 (20%)	7 (46.7%)	8 (53.3%)		8 (53.3%)	7 (46.7%)	
3	55 (73%)	14 (25.5%)	41 (74.5%)		28 (50.9%)	27 (49.1%)	

2.2. 组织芯片构建和免疫组织化学分析

肾癌标本在 4%多聚甲醛溶液中固定过夜，然后石蜡包埋。采用 Envision 二步法，按照试剂盒说明书进行操作。抗 MCT1、抗 MCT4 工作液浓度分别为 1:100、1:200。

2.3. 统计学分析

显微镜下观察染色结果，进行统计学分析。三位病理科医生分别对同一视野进行了观察。MCT1、4的表达以半定量的方式解释。染色分级为 0 (<5%染色细胞)、1 (<25%染色细胞)、2 (25%~50%染色细胞)和 3 (>50%染色细胞)。MCT1 和 MCT4 染色阳性细胞数<25%或≥25%者，染色评分为低或高[5]。所有统计分析均使用 SPSSWindowsVersion19.0 (IBM 公司，纽约州阿蒙克)进行。定量数据用单因素方差分析、T 检验进行分析。用 Spearman 检验、Mann-Whitney 检验和 Kruskal Wallis 检验对分布中具有非正态分布的范畴变量和秩变量进行了比较。用 Kaplan-Meier 方法估计 PFS 和 OS，Logrank 进行统计学分析。COX 回归模型用于评估各变量的预后意义。危险比(HRs)用 95%置信区间表示。*P* 值小于 0.05 的双尾试验被认为是有意义的。

3. 结果

3.1. 免疫组化结果

本组患者中男性 41 例(54.7%)，女性 34 例(45.3%)，年龄(55.49 ± 11.53)岁，随访时间(33.01 ± 14.01)个月。采用组织芯片免疫组织化学方法检测 MCT1、MCT4 在肿瘤组织中的表达。MCT1 在肿瘤细胞胞浆中表达。MCT4 呈膜性染色(图 1，图 2)。表 1 是接受靶向治疗患者的临床资料及 MCT1、MCT4 表达水平(n = 75)的结果。结果显示：在全部 ccRCC 患者(n = 150)和 TT 患者(n = 28)中，与 MCT1 或 MCT4 低表达或高表达相比，年龄、肿瘤直径、肿瘤分期、Furmann 病理分级和 MSKCC 均无显著性差异($P > 0.05$)。MCT1 和 MCT4 在 ccRCC 细胞中的表达趋势一致($R = 0.55$, $P < 0.01$)。

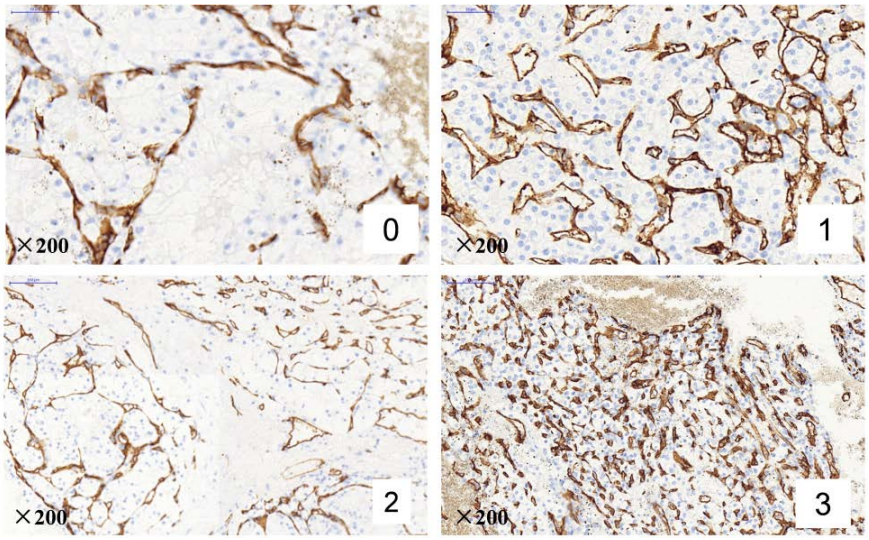
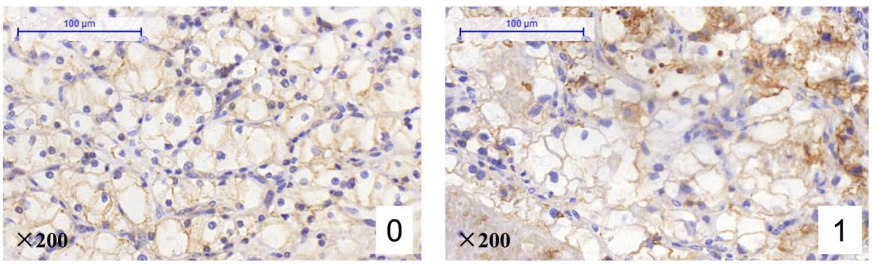


Figure 1. Results of MCT1 semi quantitative immunohistochemistry. The staining score was 0, 1, 2, 3
图 1. MCT1 免疫组化半定量检测结果。染色评分为 0，1，2，3



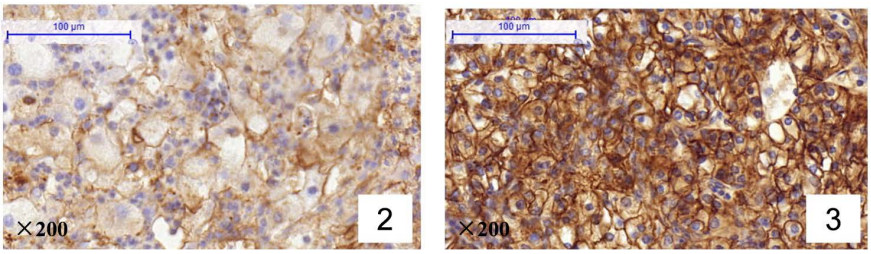


Figure 2. Results of MCT4 semi quantitative immunohistochemistry. The staining score was 0, 1, 2, 3
图 2. MCT4 免疫组化半定量检测结果。染色评分为 0，1，2，3

3.2. ccRCC 患者的生存分析

全部 ccRCC 患者的生存分析($n = 75$)：对免疫组织芯片结果进行评估后，进行 Kaplan-Meier 分析(图 3)。

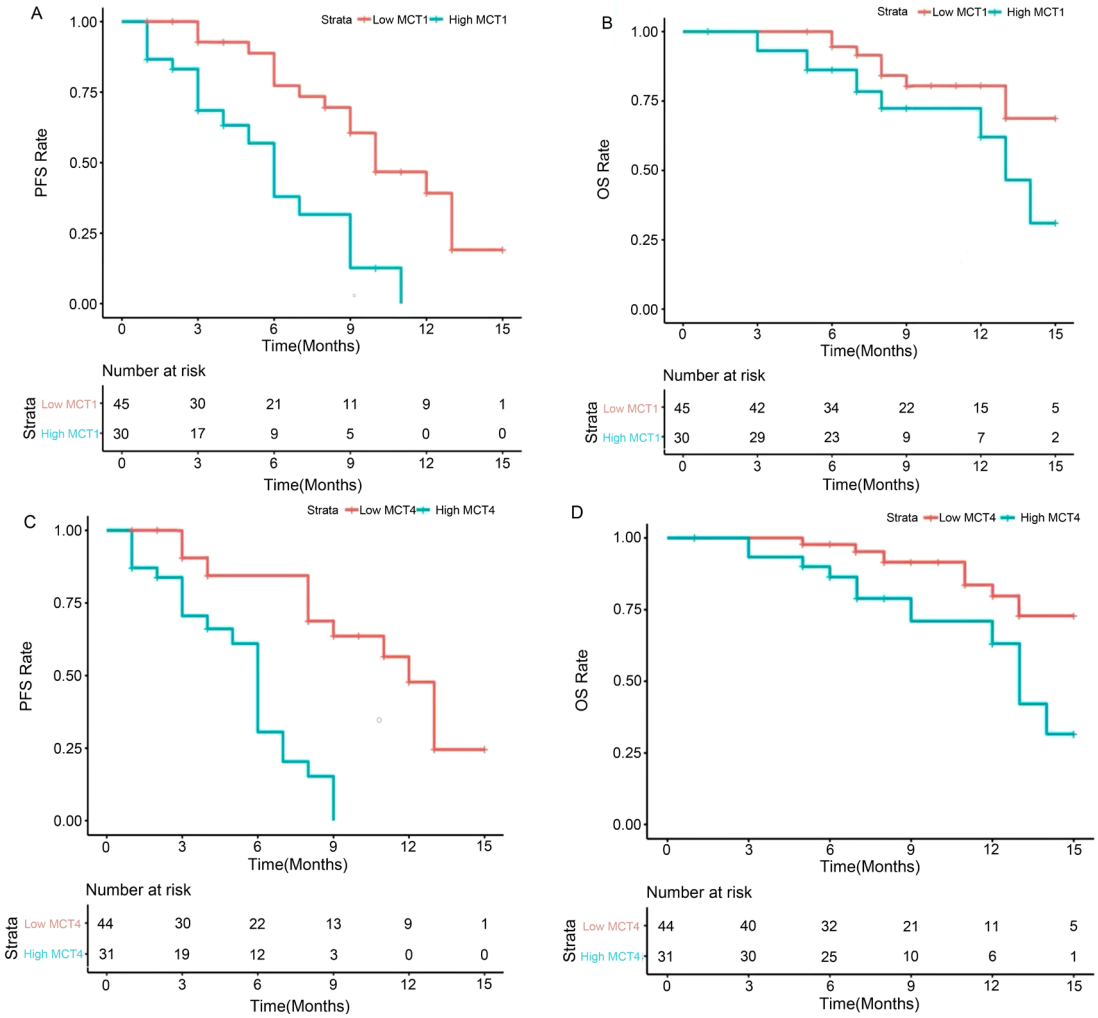


Figure 3. (A) The expression of MCT1 was significantly correlated with PFS ($P = 0.026$, HR = 0.19, 95%CI = 0.07~0.54); (B) The expression of MCT1 was significantly correlated with OS ($P = 0.023$, HR = 0.14, 95%CI = 0.03~0.50); (C) The expression of MCT4 was significantly correlated with PFS ($P = 0.03$, HR = 0.29, 95%CI = 0.12~0.74); (D) The expression of MCT4 was significantly correlated with OS ($P = 0.015$, HR = 0.16, 95%CI = 0.05~0.56)
图 3. (A) MCT1 表达水平与 PFS ($P = 0.026$, HR = 0.19, 95%CI = 0.07~0.54)显著相关；(B) MCT1 表达水平与 OS ($P = 0.023$, HR = 0.14, 95%CI = 0.03~0.50)显著相关；(C) MCT4 表达水平与 PFS ($P = 0.03$, HR = 0.29, 95%CI = 0.12~0.74)显著相关；(D) MCT4 表达水平与 OS ($P = 0.015$, HR = 0.16, 95%CI = 0.05~0.56)显著相关

MCT1 低表达 45 例(60.0%)，高表达 30 例(40.0%)，总生存率 MCT1 低表达患者为 100%，而高表达患者为 54.6% (P<0.001)。MCT1 表达增加与 OS (P = 0.023, HR=0.14, 95%CI = 0.03~0.50)以及肿瘤无进展期 PFS (P = 0.026, HR = 0.19, 95%CI = 0.07~0.54)显著相关。MCT1 高表达患者的 OS 中位生存期为 48 个月，PFS 中位期为 47 个月。COX 回归分析显示 MCT1 表达水平与 PFS 相关(P = 0.014, HR = 0.26, 95%CI, 0.09~0.76)而与 OS 无显著相关(P = 0.79)。

MCT4 低表达 44 例(59%)，高表达 31 例(41%)，总生存率 MCT4 低表达患者为 96.4%，而高表达患者为 66.7% (P = 0.005)。MCT4 表达水平与 OS (P = 0.015, HR = 0.16, 95%CI = 0.05~0.56)以及 PFS (P = 0.03, HR = 0.29, 95%CI = 0.12~0.74)显著相关。MCT4 高表达的中位 OS 为 53 个月，PSF 中位期为 48 个月。COX 回归分析显示，MCT4 表达水平与 OS (P = 0.031, HR = 0.10, 95%CI =0.13~0.81)和 PFS (P = 0.047, HR = 0.35, 95%CI 为 0.12~0.99)显著相关。

4. 讨论

肾肿瘤靶向药物如舒尼替尼和索拉非尼可以阻断酪氨酸激酶或与血管内皮生长因子(VEGF)配体结合，从而抑制肿瘤血管生成。在 105 例转移性透明细胞癌患者的舒尼替尼 II 期试验中，客观缓解率(ORR)仅为 34% [4]。从一项随机的舒尼替尼 III 期研究中，PFS 仅为 11 个月，OS 为 26.4 个月。PFS 和 OS 的反应率分别为 70.6%和 46.3% [3] [6]。从一线药物到第三线药物的所有靶向治疗都显示了相似的结果，这意味着只有一部分晚期肾癌患者面临着一系列副作用的风险而中从中获益[7] [8]。然而，到目前为止，在大多数肾癌临床试验和研究中，患者的选择完全是基于资格标准，包括患者状态、肿瘤概况，副作用耐受等[9]。根据我们目前的数据，医生们很难为患者提供一些预后信息。

单羧酸转运蛋白(MCT)是单羧酸转运蛋白家族，由 SLC16A 家族编码的 14 种跨膜蛋白组成。MCTs 的两个成员 MCT1 和 MCT4 是重要的单羧酸转运蛋白，负责丙酮酸、L-乳酸和酮体细胞膜间转运。MCT 高表达与肿瘤的侵袭性和预后有关[9] [10]，MCT1 和 MCT4 在胃癌、口腔癌、大肠癌、宫颈癌、前列腺癌、乳腺癌和胶质母细胞瘤中均呈高表达，并与肿瘤预后有关[2] [9] [11] [12]。MCT 作为肾癌的生物标记物和预后因子，已引起人们的广泛关注和研究。我们采用组织芯片法对晚期肾癌瘤组织中 MCT1 和 MCT4 的表达进行分析，并将其表达水平与靶向药物治疗后的临床效果进行分析，结果发现 MCT1 表达水平与 PFS 显著相关，MCT4 表达水平与 OS 和 PFS 均显著相关。虽然我们在 COX 回归分析总并未发现 MCT1 与 OS 的相关性，但从 Kaplan 生存分析结果存在明显统计学差异，这可能是影响整体生存的因素复杂，也可能是样本量过小所致。但无论如何，MCT1 和 MCT4 显示对靶向治疗的肾癌患者的预后具有明显的预测作用。显然转移对于靶向治疗的预后至关重要，我们下一步应重点放在对于转移患者 MCT 表达与靶向药物治疗预后之间的关系研究上。

MCT1 和 MCT4 抑制剂可能具有抗代谢、抗血管生成和抗迁移作用，从而抑制肿瘤生长，并有可能使肿瘤进展到转移状态[13]。Ar-C155858 为高效 MCT1/2 抑制剂[14]，AZD3965 为第二代 MCT1/2 抑制剂[15]。7ACC2 是主要的化合物，最近被发现能选择性地抑制表达 MCT1 和 MCT4 的癌细胞对乳酸的摄取。因此，明确 MCT1、MCT4 和 ccRCC 的相关性，可能为 ccRCC 的靶向治疗开辟一条新的途径。

5. 结论

MCT1 和 MCT4 的表达水平与肾癌靶向治疗的生存率、总生存期和无进展生存期明显相关。肿瘤 MCT1、MCT4 表达水平是预测 ccRCC 或靶向治疗疗效预后的独立因素，因此 MCT1 和 MCT4 表达水平可能成为预测靶向治疗效果的新生物标记物。

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