研究论文

dUTPase在胃癌中的表达及其对癌细胞增殖、迁移的影响

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摘要 脱氧尿苷三磷酸核苷酸水解酶(deoxyuridine 5'-triphosphate nucleotidohydrolase, dUTPase)是由*DUT*基因编码的DNA合成过程中的关键酶。研究显示其在维护基因组稳定性过程中充当基因组管家的作用, 而其在胃癌中的表达及作用尚不清楚。该研究首先利用iTRAQ标记的定量蛋白组学从胃癌组织样本中鉴定出dUTPase高表达, 经TIMER数据库分析得出dUTPase在胃癌中高表达, 并经实时荧光定量PCR(quantitative real-time PCR, qPCR)及免疫组化(immunohistochemistry, IHC)等方法检测显示, dUTPase在胃癌组织中显著高表达, 且其表达与组织学分级及TNM分期等临床病理因素相关。预后分析结果显示: dUTPase高表达与胃癌患者的首次进展生存期(first progression survival, FP)、总生存期(overall survival, OS)、复发后生存期(post progression survival, PPS)及HER2阳性状态显著相关。基因富集分析(gene set enrichments analysis, GSEA)结果显示: *DUT*高表达胃癌患者中有185条信号通路显著被激活, 涉及DNA合成、DNA修复及基因组稳定性等多个方面。一系列体外功能实验结果显示, 体外抑制*DUT*基因表达可显著抑制胃癌细胞的增殖及体外迁移。总之, 该研究证实了dUTPase在胃癌中显著高表达, 其表达可能与多条参与DNA合成、修复及基因组稳定性的肿瘤信号通路相关, 靶向dUTPase可显著抑制胃癌细胞的增殖及迁移。

关键词 dUTPase; GSEA; 增殖; 迁移; 胃癌

Expression of dUTPase in Gastric Cancer and Its Effect on Proliferation and Migration of Cancer Cells

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Abstract dUTPase (deoxyuridine 5'-triphosphate nucleotidohydrolase) encoded by *DUT* gene is a key enzyme in the DNA synthesis process. Recent studies have shown that it acts as a "House keeper" in the protection of

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genome stability, but its expression and roles in gastric cancer are still unclear. In this study, iTRAQ-labeled quantitative proteomics was used to identify the high expression of dUTPase in gastric cancer tissue samples. TIMER database analysis confirmed that dUTPase was highly expressed in gastric cancer. The results of qPCR (quantitative real-time PCR) and IHC (immunohistochemistry) showed that the expression of dUTPase was significantly higher in gastric cancer tissues, its expression was correlated with clinicopathological factors such as histological grade and TNM stages. Prognostic analysis showed that high expression of dUTPase was associated with FP (first progression survival), OS (overall survival), PPS (post progression survival) and HER2 positive status. GSEA (gene set enrichment analysis) showed that 185 signaling pathways were significantly activated in gastric cancer patients with high expression of *DUT*, involving DNA synthesis, DNA repair and genome stability. A series of *in vitro* functional experiments showed that inhibition of *DUT* gene expression *in vitro* could significantly inhibit the proliferation and the migration of gastric cancer cells. In conclusion, this study confirmed that dUTPase was significantly overexpressed in gastric cancer, and its expression might activate multiple tumor signaling pathways involved in DNA synthesis, DNA repair, and genomic stability. Targeting dUTPase can significantly inhibit the proliferation and migration of gastric cancer cells.

Keywords dUTPase; GSEA; proliferation; migration; gastric cancer

胃癌是我国最常见的恶性肿瘤之一, 是导致消化道恶性肿瘤死亡的主要原因。由于缺乏有效的诊断和治疗方法, 大多数胃癌患者表现为进展期胃癌及预后不良[1-2]。虽然CA72-4、CA19-9已被用作胃癌诊断和预后的监测标志物, 但其检测的灵敏度较低[3-4]。因此, 亟需开发新的生物标志物, 以用于胃癌的早期诊断和治疗。

近年来, iTRAQ标记与液相色谱和串联质谱(LC-MS/MS)相结合的方法已被广泛用于肿瘤标志物研究[5-6]。通过标记生物样品的稳定同位素, iTRAQ标记方法能够通过不同报告基团离子强度的差异来确定它所标记的多肽的相对丰度[7-9]。通过体外合成经iTRAQ标记的多肽标准品做内标, 可对蛋白质进行绝对定量。

在本研究中, 我们旨在通过iTRAQ标记的定量蛋白质组学来鉴定用于胃癌早期检测的生物标志物。此外, 我们进行了体外实验以验证我们的检测结果。本研究为胃癌的早期诊断和预后监测提供了新的生物标志物。

1 材料与方法

1.1 胃癌样本及细胞培养

胃癌组织和癌旁组织取自川北医学院附属医院手术切除的98例胃癌患者, 良性病变组织取自良性胃炎病变患者, 所有患者知情同意并签署知情同意书。实验所用胃癌HGC及AGS细胞为实验室保存。利用DMEM或RPMI-1640培养基加1%双抗(青霉素+链霉素)和10% FBS, 在37 °C、5% CO_2细胞培养箱内常规培养细胞。川北医学院伦理委员会批准了本研究方案(批准号: NSMC2021077)。

1.2 数据库分析

TIMER(Tumor Immune Estimation Resource, http://timer.cistrome.org)数据库是一个将TCGA(the Cancer Genome Atlas)癌症大数据整合分析的数据库。本研究中我们利用TIMER分析了*DUT*在一些临床常见恶性肿瘤及其相应正常组织中的表达情况, 并进一步运用Kaplan-Meier Plotter数据库分析了其表达与预后的关系(http://kmplot.com/analysis/)。

1.3 蛋白提取与iTRAQ标记

胃癌组织样品用含有蛋白酶抑制剂混合物的PBS匀浆, 4 °C、1 000 ×*g*离心10 min。样品用RIPA处理, 超声波破碎, 以12 000 r/mim离心15 min。用0.22 μm滤膜过滤, 收集滤液。通过使用Bradford蛋白质测定试剂盒(Bio-Rad, Hercules, CA, USA)测定蛋白质的浓度并在–80 °C保存。用胰蛋白酶消化来自胃癌组织的蛋白质, 用iTRAQ试剂标记肽2 h。使用缓冲液A(25% CAN, 25 mmol/L NaH_2PO_4, pH2.7)重悬标记的蛋白质, 并加到Ultremex SCX柱(Phenomenex)上。用100%缓冲液A(25% ACN, 25 mmol/L NaH_2PO_4, pH2.7), 以1 mL/min的速率洗脱蛋白质, 然后用5%~35%缓冲液B(25 mmol/L NaH_2PO_4, 1 mmol/L KCl, 25% ACN, pH2.7)洗脱11 min, 35%~80%缓冲液B洗脱11 min。将样品合并成20份, 脱盐并冷冻干燥。

1.4 液相色谱–串联质谱分析与生物信息学鉴定

缓冲液A(2% CAN, 0.1% FA)重悬样品, 浓度为0.25 μg/μL。对于LTQ Orbitraq Velos(Thermo), 每次注射体积为10 μL。在350~2 000 m/z范围内采集数据。使用Mascot软件(版本2.2)在IPI数据库中搜索所获得的数据, 允许的修饰包括半胱氨酸的脲甲基。我们使用1.2倍变化的阈值来鉴定上述两组之间的差异表达蛋白。

1.5 免疫组织化学(immunohistochemistry, IHC)分析

石蜡包埋的组织切片脱蜡, 以3~4 μm切片, 60 °C烘烤2 h, 4 °C冰箱保存备用。用1:100稀释的抗脱氧尿苷三磷酸酶(dUTPase)抗体4 °C孵育过夜。PBS代替一抗作阴性对照。DAB辣根过氧化物酶显色试剂盒购自上海生工生物技术有限公司。用苏木素溶液复染切片30 s, 用100%乙醇和二甲苯脱水。染色强度按以下标准记录: 无染色(0)、淡染色(1)、中度染色(2)和强染色(3)。染色细胞百分比记录如下: ≤5%(0)、6%~25%(1)、26%~50%(2)、51%~75%(3)和76%~100%(4)。将染色强度和着色比例分值相乘, 大于3分为高表达, 3分及以下为低表达。

1.6 基因集富集分析(gene expression enrichment analysis, GSEA)

基因集富集分析筛选在dUTPase高表达样品中富集的基因集和信号通路。从基因表达综合数据库(gene expression omnibus, GEO)中下载GSE84437数据集, 这些数据包含来自韩国的433名原发性胃癌患者的全基因组mRNA表达谱, 分析了*DUT*表达与相关通路的关系。NES>1, P<0.05且FDR<0.25的通路被判定为显著富集者。

1.7 细胞转染

将胃癌细胞培养于RPMI-1640培养基中, 并向其中加入10% FBS和1%青霉素–链霉素双抗, 再将其置于37 °C、5% CO_2培养箱中培养。根据转染质粒的不同, 将其分为空白对照组、阴性对照组siRNA-NC、siRNA-*DUT1*、siRNA-*DUT2*、siRNA-*DUT3*和siRNA-*DUT4*, 转染用量为5 μg, 按照Lipofectamine 2000转染说明书进行, 分别于转染24 h、48 h、72 h和96 h后收集细胞。

1.8 CCK-8分析细胞活力

胃癌细胞以4×10^{4}个/孔的密度接种于96孔板中, 每组设6个复孔, 分别于24 h、48 h、72 h和96 h后加入CCK-8试剂, 4 h后检测各孔在波长为450 nm处的吸光度(D)值, 以空白胃癌细胞组做对照, 以GraphPad Prism 8分析实验结果。

1.9 Transwell实验

将Transwell小室聚碳酸微孔膜加入适量无血清培养基水化聚碳酸微孔膜。将胰酶消化转染后的各组胃癌细胞制成单细胞悬液(1×10^{5}个/mL), 弃去Transwell小室内的无血清培养基, 每孔内上室加入200 μL细胞悬液, 再在下室内加入300 μL含20% FBS的完全培养基。将Transwell小室放入37 °C、5% CO_2培养箱中, 于48 h后取出, PBS清洗, 4%多聚甲醛室温固定30 min, 结晶紫染色后镜下观察计数。

1.10 统计学分析

使用SPSS 22.0进行分析, 采用卡方检验和Fisher's检验进行统计学分析。采用t检验检测胃癌细胞增殖及迁移率, 以P<0.05为有统计学意义。

2 结果

2.1 dUTPase蛋白在胃癌组织中表达增高

课题组前期结果显示, 在胃癌组织与癌旁组织蛋白质谱鉴定结果中, 胃癌组织中dUTPase的表达比癌旁组织增高1.584倍[10], 在胃癌中鉴定的dUTPase的MS/MS肽谱如图1所示。同时, 通过TIMER数据库分析发现, dUTPase在多种肿瘤中也上调表达, 在胃癌中表达水平显著上升(图2)。

2.2 免疫组织化学方法验证dUTPase在胃癌中的表达

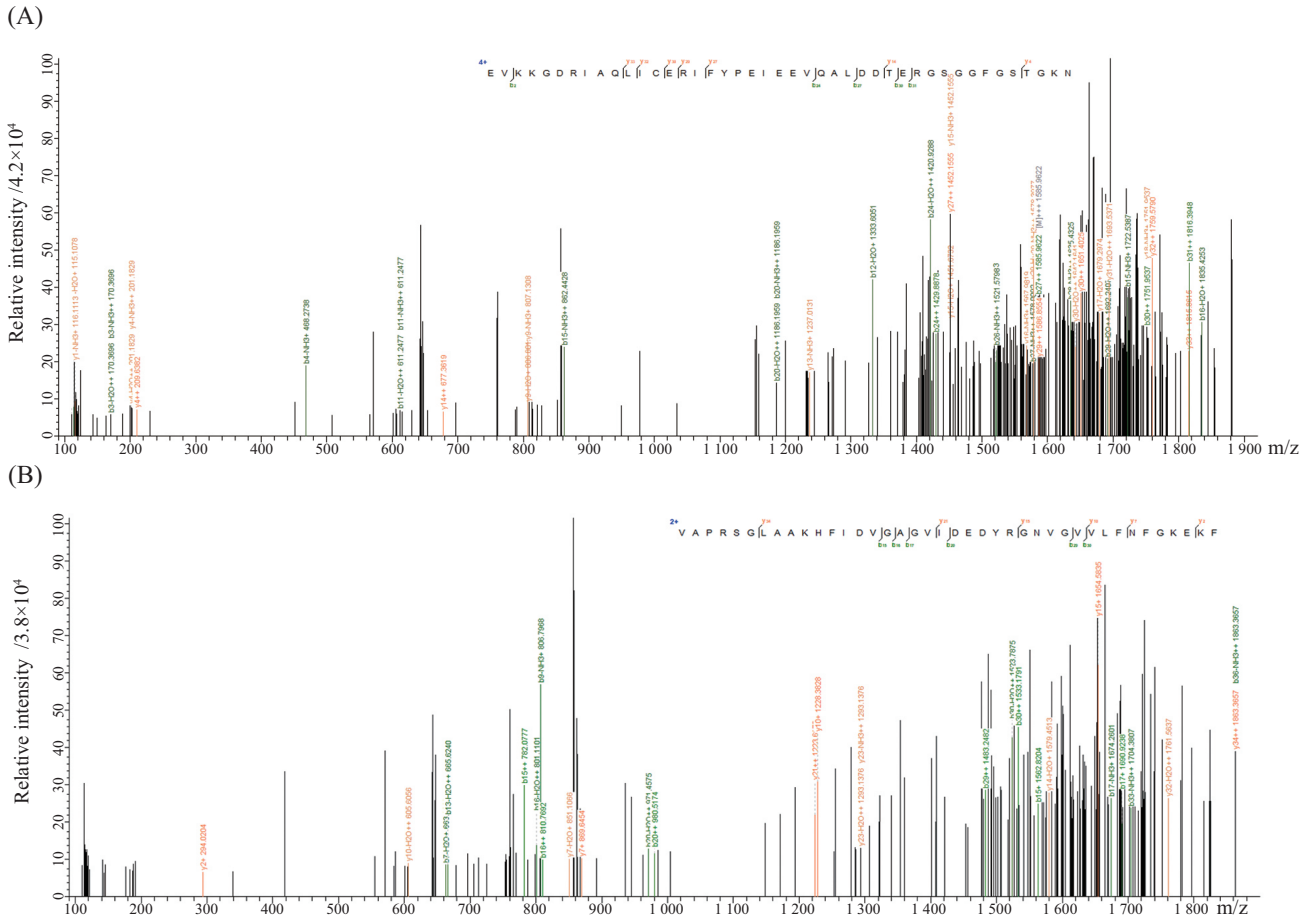
选择胃癌组织、癌旁组织和良性胃炎组织进行免疫组织化学检测, 图3显示与癌旁组织和良性病变相比, dUTPase在胃癌组织中表达上调, 与iTRAQ结果一致。在胃癌组织中, 74例(76%)呈dUTPase强阳性, 仅36例(37%)在癌旁组织中显示阳性。在胃癌中dUTPase的灵敏度为76%, 特异度为63%。

2.3 dUTPase表达与临床病理因素的关系

采用卡方检验和Fisher精确检验分析胃癌组织中dUTPase的表达与临床病理因素的关系。表1显示组织学分级II和III级患者的dUTPase表达水平显著高于组织学分级I级患者(P=0.01)。TNM分期II~IV期患者的dUTPase表达水平明显高于TNM分期I期患者(P<0.001)。

2.4 胃癌组织中dUTPase表达与临床预后的关系

通过预后分析显示, 在无淋巴结转移的胃癌患者中, dUTPase高表达在总体生存期(overall survival,

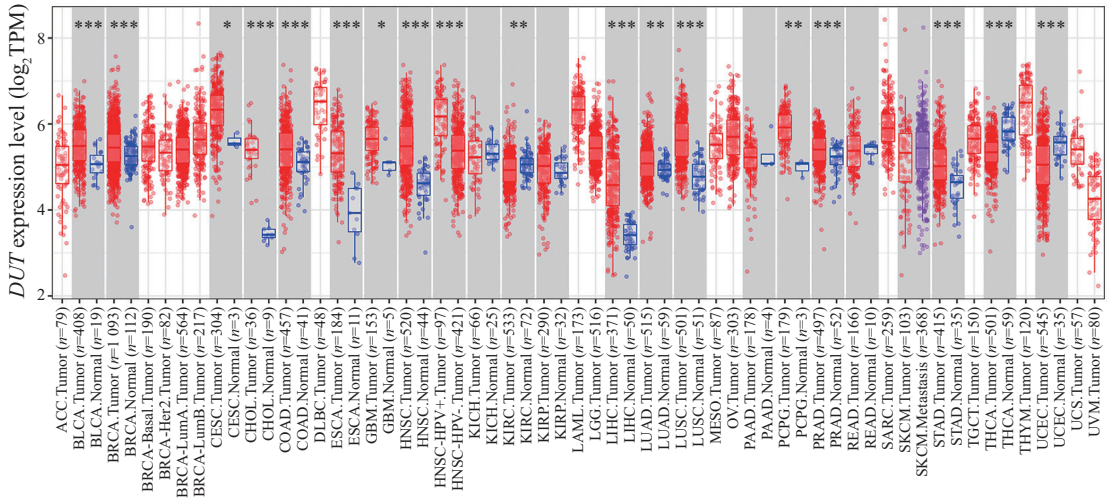


A: 胃癌组织样本中dUTPase的MS/MS肽谱; B: 癌旁组织样本中dUTPase的MS/MS肽谱。

A: the MS/MS spectrum of dUTPase in gastric cancer tissue samples; B: the MS/MS spectrum of dUTPase in paracancerous tissue samples.

图1 dUTPase质谱鉴定图谱

Fig.1 The MS/MS spectrum of dUTPase

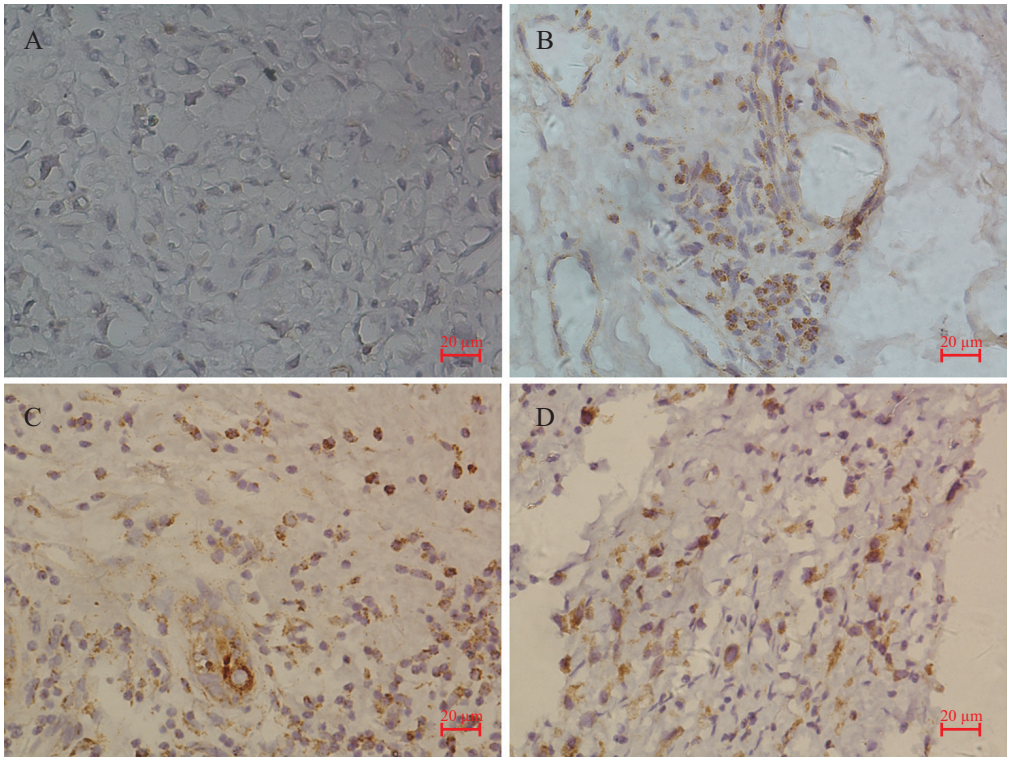


STAD: 胃腺癌; Normal: 正常组织; *P<0.05, **P<0.01, ***P<0.001, 与正常组织组比较。

STAD: stomach adenocarcinoma; Normal: normal tissues; *P<0.05, **P<0.01, ***P<0.001 compared with normal group.

图2 TIMER数据库分析*DUT*基因表达情况

Fig.2 Analysis of *DUT* expression by TIMER



A: 正常胃组织; B: 癌旁组织; C、D: 胃癌组织。

A: normal gastric tissues; B: adjacent gastric tissues; C,D: gastric cancer tissues.

图3 免疫组织化学检测dUTPase在胃癌中的表达情况

Fig.3 Analysis of dUTPase expression in gastric cancer by immunohistochemistry

表1 dUTPase表达与胃癌临床病理因素的关系

Table 1 dUTPase expression correlated with clinicopathological factors in gastric cancer

病理因素 Clinicopathological factors	dUTPase低表达 dUTPase low expression	dUTPase高表达 dUTPase high expression	病例数 Cases	*P*值 *P*-values
Age (year)				
≥60	13	53	66	0.113
≤59	11	21	32	
Sex				
Male	15	53	68	0.399
Female	9	21	30	
Histology classification				
Adenocarcinoma	20	69	89	0.144
Signet-ring cell carcinoma	4	5	9	
Histological grade				
I	11	11	22	0.002
II and III	13	63	76	
Lymph node metastasis				
Positive	10	47	57	0.059
Negative	14	27	41	
TNM staging				
I	15	14	29	<0.001
II-IV	9	60	69	

OS)和进展后生存期(post-progression survival, PPS)中没有差异(图4A和图4B)。在淋巴结转移的患者中, dUTPase的高表达预示低OS和PPS(图4C和图4D)。此外, dUTPase高表达与TNM IV期胃癌患者的低OS相关(图4E), 而I、II、III期患者的OS无显著差异(图4F~图4H)。

2.5 dUTPase高表达与不同HER2亚型的预后分析

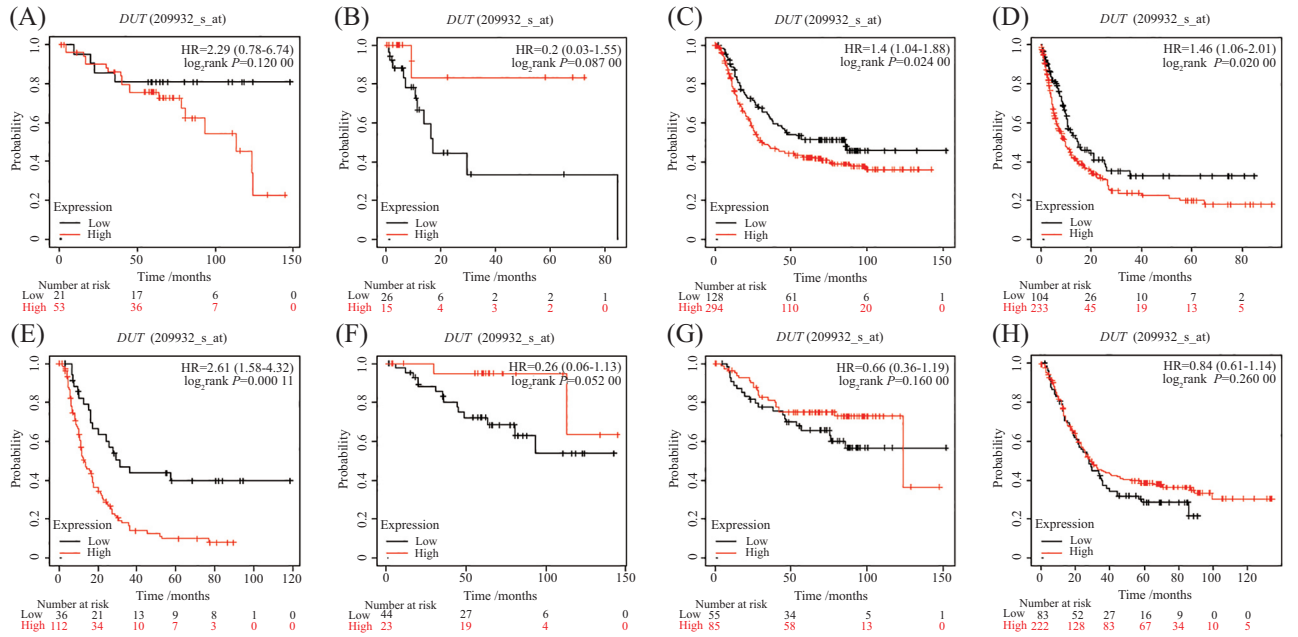
在HER2阳性的胃癌患者中, dUTPase高表达预示低OS、FP和PPS(图5A~图5C)。在淋巴结转移且HER2阳性的患者中, dUTPase高表达患者与低表达患者OS无显著差异(P>0.05, 图5D), 而在淋巴结转移且HER2阴性的患者中dUTPase高表达预示低OS(图5E)。在接受5-FU治疗和HER2阳性的胃癌患者中, dUTPase高表达与低OS相关(图5F), 尤其在男性受试者中密切相关(图5G)。但在接受5-FU治疗和HER2阴性患者中, dUTPase高表达病人与低表达病人的OS没有显著差异(图5H)。

2.6 GSEA分析胃癌中信号通路

基于*DUT*表达量, 样本分为*DUT*高表达组(前50%)和*DUT*低表达组(后50%)。在*DUT*高表达组中, 185个信号通路在*DUT*高表达组中显著富集, 而2个基因集在*DUT*低表达组中富集。在*DUT*高表达组中富集到的前10个信号通路如图6A~图6J显示。在*DUT*高表达组中, 前5个富集到的通路是Gnf2 DEK(ES=0.78, FDR<0.25), Gnf2 APEX1(ES=0.68, FDR<0.25), Fischer Dream Targets(ES=0.64, FDR<0.25), Kauffmann修复基因(ES=0.63, FDR<0.25), HSD17b8靶基因(ES=0.55, FDR<0.25)。在*DUT*低表达组中富集的前2条通路(图6K和图6L)是CL14成熟平滑肌细胞(ES=–0.46, FDR<0.25)和Hoshida肝癌亚型S1(ES=–0.33, FDR<0.25)。

2.7 干扰*DUT*基因对胃癌细胞增殖及迁移的影响

为了进一步明确*DUT*在胃癌细胞增殖、迁移中的生物学功能, 我们运用siRNA技术, 分别设计针对*DUT*基因的敲减siRNA序列和对照序列, 运用脂质体转染方法转染胃癌细胞AGS、HGC, 分别构建了干扰*DUT*的胃癌细胞株并将其命名为AGS-si*DUT1*、AGS-si*DUT2*、AGS-si*DUT3*、AGS-si*DUT4*、HGC-si*DUT1*、HGC-si*DUT2*、HGC-si*DUT3*、HGC-si*DUT4*, 其对应的对照细胞株被命名为AGS-si*RNA*-

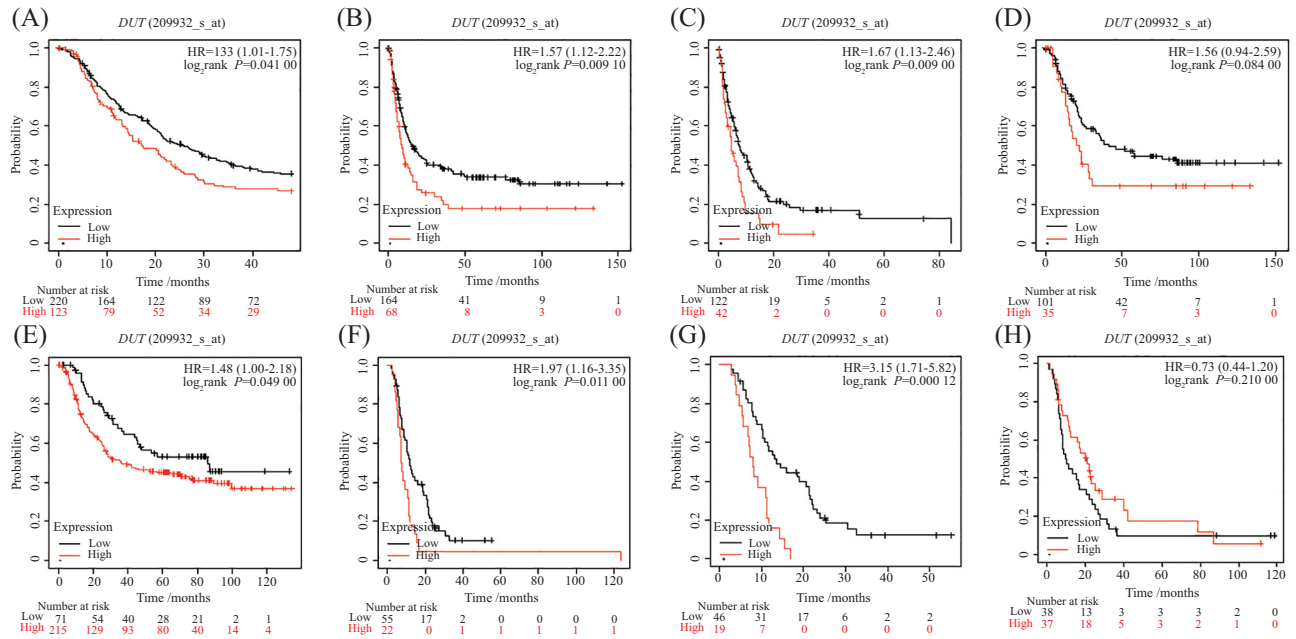


A、B: 无淋巴结转移胃癌患者中, *DUT*高表达与OS和PPS无显著关系。C、D: 淋巴结转移患者中, *DUT*高表达预测差的OS和PPS。E: dUTPase高表达与TNM IV期胃癌患者低OS显著相关。F~H: 在I、II、III期患者中, dUTPase高表达与OS无关。

A,B: no significant association of the high expression of *DUT* with OS and PPS in gastric cancer patients without lymph node metastasis. C,D: high expression of *DUT* predicted poor OS and PPS in patients with lymph node metastasis. E: high expression of dUTPase was significantly associated with poor OS in TNM stage IV patients. F-H: high expression of dUTPase was not associated with OS in TNM stage I, II, III patients.

图4 dUTPase高表达与胃癌患者预后关系

Fig.4 The relationship between dUTPase high expression and prognostic survival of gastric cancer



A~C: HER2阳性患者中, dUTPase高表达与差OS、FP和PPS显著相关。D、E: 淋巴结转移且HER2阳性患者dUTPase高表达与OS无相关(D), 而与HER2阴性患者OS显著相关(E)。F~H: 接受5-FU治疗和HER2阳性患者中, dUTPase高表达与差OS相关(F), 尤其在男性中显著相关(G), 而与HER2阴性患者的OS无关(H)。

A-C: in HER2 positive patients, high dUTPase expression was significantly associated with poor OS, FP, and PPS. D,E: in patients with lymph node metastasis and HER2 positive, the high expression of dUTPase was not significantly associated with OS (D), but was significantly associated with OS in HER2 negative patients (E). F-H: in 5-FU-treated and HER2-positive patients, high expression of dUTPase was associated with poor OS (F), especially in male subjects (G), but not in HER2-negative patients (H).

图5 dUTPase高表达与HER2阳性患者的预后关系

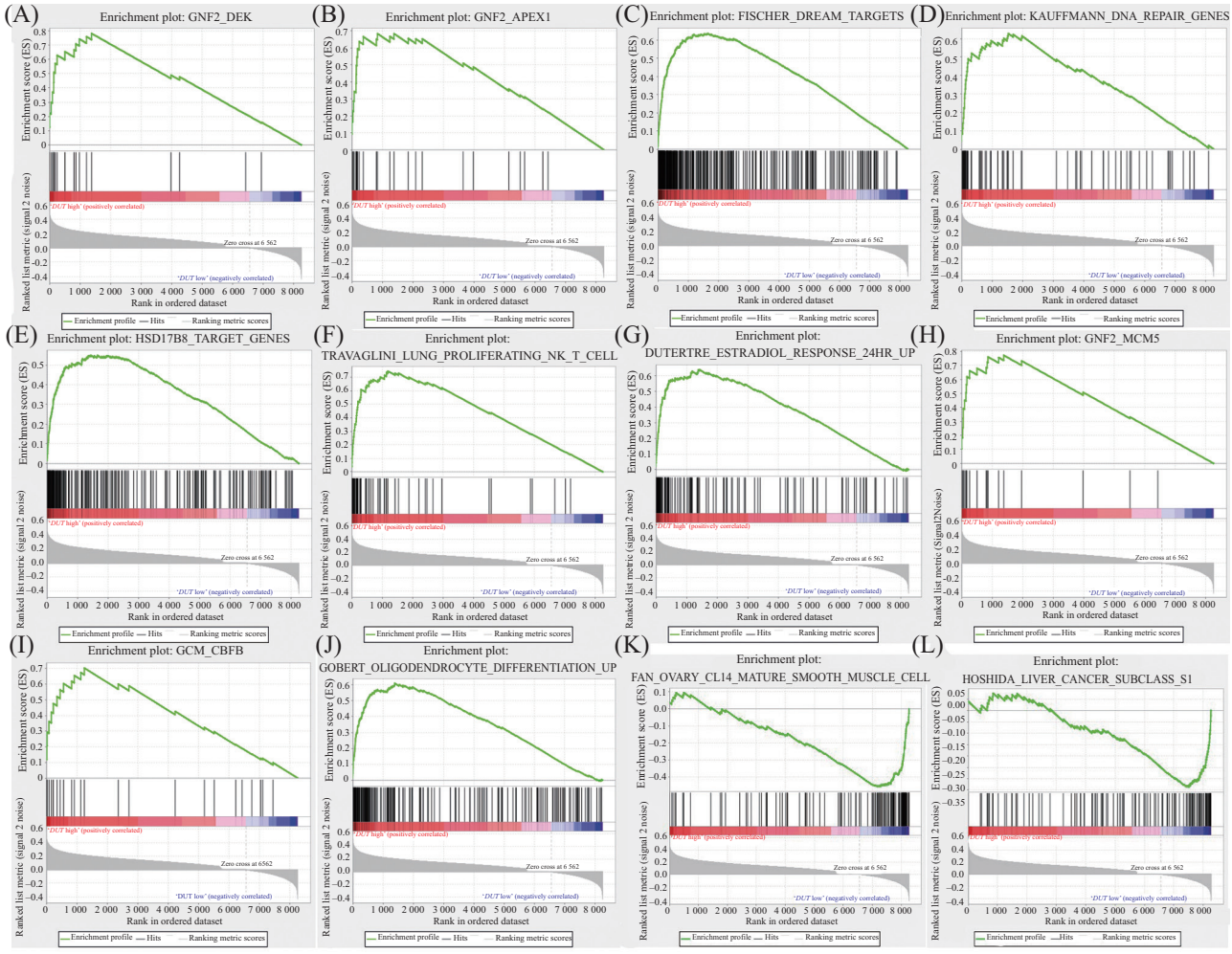
Fig.5 The relationship between the high expression of dUTPase and the prognosis of HER2 positive gastric cancer patients

NC、HGC-siRNA-NC。然后分别利用以上细胞检测细胞的增殖能力和迁移能力。CCK-8实验结果显示, 干扰*DUT*基因表达后, AGS及HGC的体外增殖能力相对于对照组细胞显著降低(图7E和图7F)。同时, Transwell实验结果显示, 下调*DUT*基因表达后的胃癌细胞迁移能力相对于对照组细胞的迁移能力显著降低(图7C和图7D)。上述体外实验结果表明, 下调*DUT*基因的表达, 可显著降低胃癌细胞的增殖能力和迁移能力。

3 讨论

脱氧尿苷三磷酸核苷酸水解酶, 由人类*DUT*基因编码, 在所有生物体中对细胞生存能力至关重要[11-12]。dUTPase催化脱氧尿苷三磷酸(dUTP)水解为dUMP和焦磷酸, 从而去除DNA合成过程中的dUTP[13-15]。在DNA合成过程中, DNA聚合酶能以相同的效率将dTTP和dUTP掺入DNA中, 高水平的dUTP导致尿嘧啶错误掺入[16-18]。因此, dUTPase是将细胞内dUTP清除的关键酶, 充当着基因组稳定性的"House keeper", 最大限度地减少尿嘧啶错误掺入[19-20]。研究证明, 当癌细胞在dUTPase强效抑制剂(TAS-114)存在的情况下, 用2-脱氧-5-氟尿苷(FDURD)处理时, FDUTP和dUTP水平增加, 5-FU和尿嘧啶在DNA中的错误掺入也增加, 癌细胞死亡率增加。因此, dUTPase抑制可增强氟嘧啶的抗肿瘤活性[21-23]。

在本研究中, 我们发现dUTPase在胃癌组织中高表达, 其阳性率为76%, 特异性为63%。此外, 我们发现dUTPase在组织学分级为II~III级和TNM分期为II~IV期的胃癌患者中的表达明显高于组织学分级为I级和TNM分期为I期的患者, 提示dUTPase的高表达与胃癌的进展有关(表1)。KAWAHARA等[24]的研究显示, dUTPase在有转移的原发性结直肠癌中的表达为54%, 他们提示dUTPase是结直肠癌转移相关标志物。此外, YE等[25]的研究表明, *DUT*基因rs3784619的GG纯合等位基因和rs11637235的TT等位基因显著增加了宫颈上皮内瘤变III(cervical intraepithelial neoplasia III, CINIII)和宫颈鳞状细胞癌



A~J: *DUT*-high胃癌患者中显著高表达的信号通路; K、L: *DUT*-low胃癌患者中显著高表达的信号通路(P<0.05, FDR<0.25)。

A-J: signaling pathways with significantly higher expression in *DUT*-high gastric cancer patients; K,L: signaling pathways with significantly high expression in *DUT*-low gastric cancer patients (P<0.05, FDR<0.25).

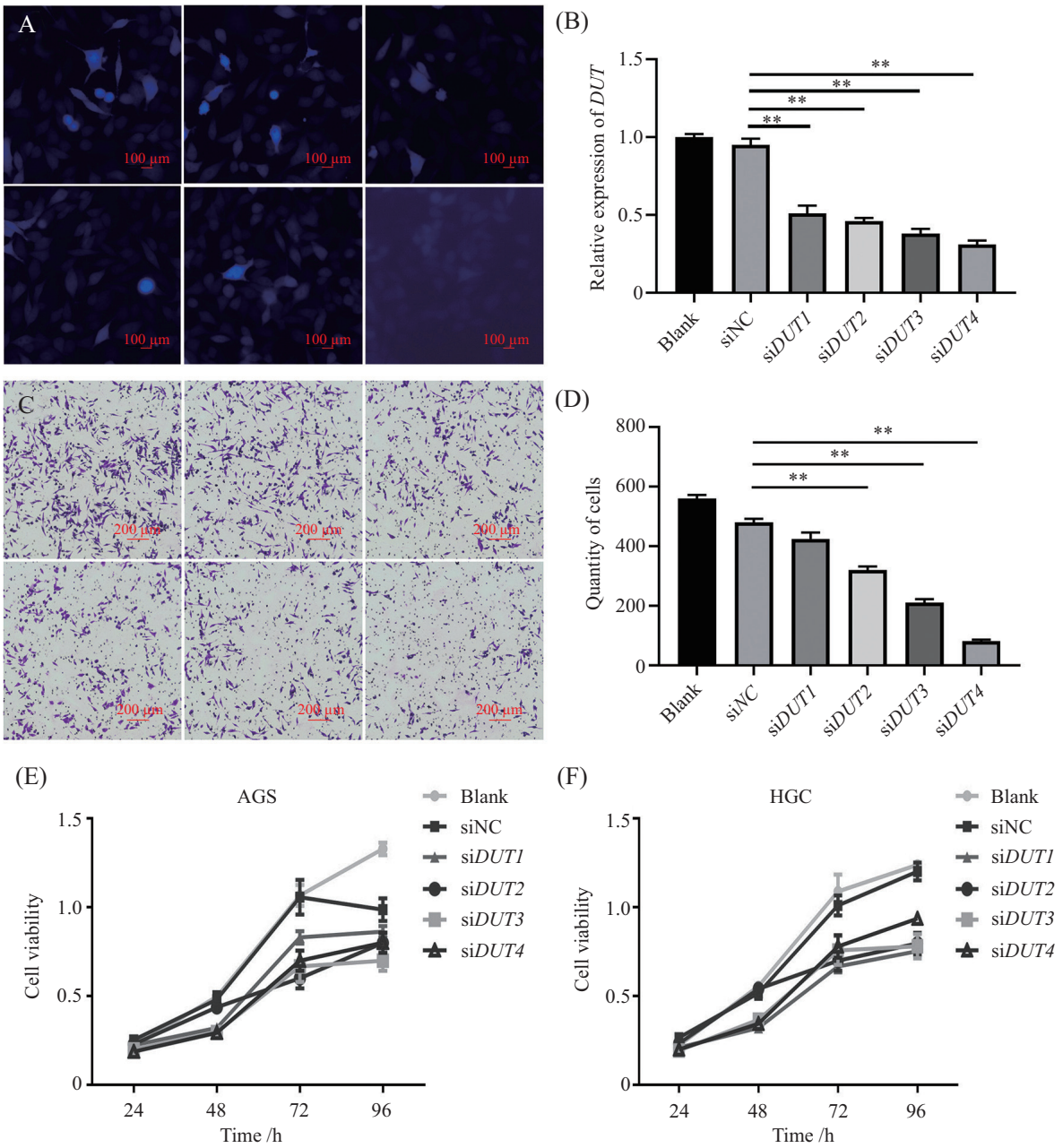
图6 基因富集分析*DUT*相关信号通路

Fig.6 Gene set enrichment analysis of *DUT*-related pathways

(cervical squamous-cell carcinoma cancer, CSCC)的风险, dUTPase可能作为CIN和CSCC的早期生物标志物。REQUENA等[26]表明, 下调dUTPase增强了地西他滨的细胞毒性作用, 引起含有尿嘧啶的核苷酸三磷酸的堆积和基因组DNA中的双链断裂。WILSON等[27]观察到了相同的结果, 即抑制dUTPase增强了胸苷酸合酶靶向化疗药物的疗效。

为了进一步研究dUTPase高表达对胃癌患者生存率的影响, 我们发现dUTPase的高表达与淋巴结转移患者差的OS和PPS显著相关(图4C和图4D), 而无淋巴结转移的患者中未观察到上述结果(图4A和图4B), 提示dUTPase可能参与了胃癌淋巴结转移的过程。此外, 我们还发现dUTPase的高表达与IV期患者的低OS显著相关(图4E)。在胃癌患者的不同HER2亚型中, 高表达dUTPase的HER2阳性胃癌患者的OS、FP和PPS预后较差(图5A~图5C)。然而, CHEN等[28]发现结直肠癌中低表达的dUTPase与高表达的核糖核苷酸还原酶R2亚单位可预测结直肠癌患者的不良生存预后。上述结果表明, dUTPase对癌症患者生存的影响需要进一步探索。

通过使用GSEA分析, 我们发现DEK基因集在dUTPase高表达组中显著富集(图6A)。DEK是一种被广泛报道的癌基因, 在快速生长的细胞和许多癌症中异常高表达[29]。最初发现DEK与核孔复合体214(nuclear pore complexes 214, NUP214)结合, 在急性髓系白血病(acute myeloid leukemia, AML)亚群中形成DEK-NUP214融合基因。随后的研究表明, DEK是一种DNA调节蛋白, 参与DNA复制、DNA修



A、B: 敲减*DUT*胃癌细胞株的建立; C、D: Transwell实验检测干扰*DUT*表达对胃癌细胞体外迁移能力的影响; E、F: CCK-8实验检测干扰*DUT*表达对胃癌细胞体外增殖能力的影响。**P<0.01。

A,B: construction of *DUT* knockdown gastric cancer cell line; C,D: Transwell assay was used to detect the effect of *DUT* knockdown on the migration of gastric cancer cells; E,F: CCK-8 assay was used to detect the effect of *DUT* knockdown on the proliferation of gastric cancer cells. **P<0.01.

图7 干扰*DUT*表达抑制胃癌细胞体外增殖和迁移

Fig.7 Downregulation of *DUT* expression inhibits the proliferation and migration of gastric cancer cells

复、RNA转录和转录调控以及表观遗传调控。本研究首次发现DEK在*DUT*高表达组中显著富集, 表明dUTPase通过DEK信号通路介导胃癌的发生发展。另外, 在*DUT*低表达组中富集的第一个信号是CL14平滑肌细胞(图6K)。FAN等[30]证明CL14中差异表达的基因如*ACTA2*、*PLN*、*ADIRF*和*MYH11*与平滑肌细胞成熟相关, 提示dUTPase可能与该生物学功能相关。

为了进一步探索*DUT*在胃癌细胞增殖、迁移中的生物学作用, 本文分别设计了针对*DUT*基因的敲减siRNA序列和对照序列, 通过脂质体转染方法分别构建干扰*DUT*的胃癌细胞株AGS-si*DUT*和HGC-

si*DUT*。CCK-8检测结果显示, 干扰*DUT*基因表达后, AGS及HGC的体外增殖能力相对于对照组细胞显著降低(图7E和图7F)。这提示下调*DUT*基因的表达, 减弱了胃癌细胞DNA损伤后修复能力, 并影响了胃癌细胞的体外增殖能力。下调*DUT*的表达导致胃癌细胞的体外迁移降低, 说明下调*DUT*基因的表达也显著抑制了胃癌细胞的体外迁移(图7C和图7D)。上述结果表明, dUTPase是一个强有力的抑制胃癌增殖和迁移过程的作用靶点。

综上所述, 本研究证实了dUTPase在胃癌中表达上调, 并与胃癌的恶性程度与预后不良密切相关, 从而可能促进了胃癌细胞的增殖和迁移。该研究为胃癌的临床诊断和治疗靶点筛选提供了一定的理论基础。

参考文献 (References)

[1] SMYTH E C, NILSSON M, GRABSCH H I, et al. Gastric cancer [J]. Lancet, 2020, 396(10251): 635-48.

[2] BLAIR V R, MCLEOD M, CARNEIRO F, et al. Hereditary diffuse gastric cancer: updated clinical practice guidelines [J]. Lancet Oncol, 2020, 21(8): e386-97.

[3] SHIMADA H, NOIE T, OHASHI M, et al. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association [J]. Gastric Cancer, 2014, 17(1): 26-33.

[4] TOH J, HOPPE M M, THAKUR T, et al. Profiling of gastric cancer cell-surface markers to achieve tumour-normal discrimination [J]. BMJ Open Gastroenterol, 2020, 7(1): e0004521-5.

[5] MURAOKA S, KUME H, WATANABE S, et al. Strategy for SRM-based verification of biomarker candidates discovered by iTRAQ method in limited breast cancer tissue samples [J]. J Proteome Res, 2012, 11(8): 4201-10.

[6] LI Y, WANG X, AO M, et al. Aberrant mucin5B expression in lung adenocarcinomas detected by iTRAQ labeling quantitative proteomics and immunohistochemistry [J]. Clin Proteomics, 2013, 10(1): 15-24.

[7] WANG Q, ZHI Y, REN W, et al. Suppression of OSCC malignancy by oral glands derived-PIP identified by iTRAQ combined with 2D LC-MS/MS [J]. J Cell Physiol, 2019, 234(9): 15330-41.

[8] WANG L, CHEN S, ZHANG M, et al. Legumain: a biomarker for diagnosis and prognosis of human ovarian cancer [J]. J Cell Biochem, 2012, 113(8): 2679-86.

[9] WIPPEL H H, SANTOS M D M, CLASEN M A, et al. Comparing intestinal versus diffuse gastric cancer using a PEFF-oriented proteomic pipeline [J]. J Proteomics 2018, 171: 63-72.

[10] JIANG Z, SHEN H, TANG B, et al. Quantitative proteomic analysis reveals that proteins required for fatty acid metabolism may serve as diagnostic markers for gastric cancer [J]. Clin Chim Acta, 2017, 464: 148-54.

[11] NYÍRI K, MERTENS H D T, TIHANYI B, et al. Structural model of human dUTPase in complex with a novel proteinaceous inhibitor [J]. Sci Rep, 2018, 8(4326): 1-15.

[12] KUMAR H, KEHRER J, SINGER M, et al. Functional genetic evaluation of DNA house-cleaning enzymes in the malaria parasite: dUTPase and Ap4AH are essential in plasmodium berghei but ITPase and NDH are dispensable [J]. Expert Opin Ther Targets, 2019, 23(3): 251-61.

[13] PÁLINKÁS H L, RÁCZ G A, GÁL Z, et al. CRISPR/Cas9-mediated knock-out of dutpase in mice leads to early embryonic lethality [J]. Biomolecules, 2019, 9(4): 136,1-14.

[14] WILLIAMS M, ARIZA M E. EBV positive diffuse large b cell: lymphoma and chronic lymphocytic leukemia patients exhibit increased anti-dutpase antibodies [J]. Cancers, 2018, 10(5): 1-15.

[15] GROGAN B C, PARKER J B, GUMINSKI A F, et al. Effect of the thymidylate synthase inhibitors on dUTP and TTP pool levels and the activities of DNA repair glycosylases on uracil and 5-fluorouracil in DNA [J]. Biochemistry, 2011, 50(5): 618-27.

[16] VODENKOVA S, BUCHLER T, CERVENA K, et al. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: past, present and future [J]. Pharmacol Ther, 2020, 206: 107447.

[17] SETHY C, KUNDU C N. 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: implication of DNA repair inhibition [J]. Biomed Pharmacother, 2021, 137: 111285: 1-15.

[18] YANO W, YOKOGAWA T, WAKASA T, et al. TAS-114, a first-in-class dual dutpase/dpd inhibitor, demonstrates potential to improve therapeutic efficacy of fluoropyrimidine-based chemotherapy [J]. Mol Cancer Ther, 2018, 17(8): 1683-93.

[19] MIYAHARA S, MIYAKOSHI H, YOKOGAWA T, et al. Discovery of a novel class of potent human deoxyuridine triphosphatase inhibitors remarkably enhancing the antitumor activity of thymidylate synthase inhibitors [J]. J Med Chem, 2012, 55(7): 2970-80.

[20] DAVISON C, MORELLI R, KNOWLSON C, et al. Targeting nucleotide metabolism enhances the efficacy of anthracyclines and anti-metabolites in triple-negative breast cancer [J]. NPJ Breast Cancer, 2021, 7(38): 1-13.

[21] YOKOGAWA T, YANO W, TSUKIOKA S, et al. dUTPase inhibition confers susceptibility to a thymidylate synthase inhibitor in DNA-repair-defective human cancer cells [J]. Cancer Sci, 2021, 112(1): 422-32.

[22] YAMAMOTO N, HAYASHI H, PLANCHARD D, et al. A randomized, phase 2 study of deoxyuridine triphosphatase inhibitor, TAS-114, in combination with S-1 versus S-1 alone in patients with advanced non-small-cell lung cancer [J]. Invest New Drugs, 2020, 38(5): 1588-97.

[23] DOI T, YOH K, SHITARA K, et al. First-in-human phase 1 study of novel dUTPase inhibitor TAS-114 in combination with S-1 in Japanese patients with advanced solid tumors [J]. Invest New Drugs, 2019, 37(3): 507-18.

[24] KAWAHARA A, AKAGI Y, HATTORI S, et al. Higher expression of deoxyuridine triphosphatase (dUTPase) may predict the metastasis potential of colorectal cancer [J]. J Clin Pathol, 2009, 62(4): 364-9.

[25] YE F, WANG H, LIU J, et al. Genetic variants of the dUTPase-encoding gene DUT increase HR-HPV infection rate and cervical squamous cell carcinoma risk [J]. Sci Rep, 2019, 9(513): 1-9.

[26] REQUENA C E, PEREZ-MORENO G, HORVATH A, et al. The nucleotidohydrolases DCTPP1 and dUTPase are involved in the cellular response to decitabine [J]. Biochem J, 2016, 473(17): 2635-43.

[27] WILSON P M, LABONTE M J, LENZ H J, et al. Inhibition of dUTPase induces synthetic lethality with thymidylate synthase-targeted therapies in non-small cell lung cancer [J]. Mol Cancer Ther, 2012, 11(3): 616-28.

[28] CHEN C W, TSAO N, HUANG L Y, et al. The impact of dutpase on ribonucleotide reductase-induced genome instability in cancer cells [J]. Cell Rep, 2016, 16(5): 1287-99.

[29] BODINE D M. All hands on DEK [J]. J Clin Invest, 2019, 129(6): 2205-6.

[30] FAN X, BIALECKA M, MOUSTAKAS I, et al. Single-cell reconstruction of follicular remodeling in the human adult ovary [J]. Nat Commun, 2019, 10(3164): 1-13.