DUSP4 is an Oncogenic Gene for the Carcinogenesis of Clear cell Renal Cell Carcinoma

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Short Report

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Abstract

DUSP4 is considered as an oncogenic gene. However, the effect of DUSP4 on the oncogenesis of Clear cell Renal cell carcinoma (CCRCC) is still unclear. In this study, we explored the expression pattern of DUSP4 in CCRCC cancer tissues and CCRCC cell lines by qRT-PCR. Furthermore, we investigated the roles of DUSP4 in CCRCC using gain-of-function and loss-of-function assays. Here, DUSP4 mRNA levels were significantly increased in CCRCC tissues and cell lines. DUSP4 overexpression promotes the proliferation, migration and tumorigenicity of CCRCC cells while DUSP4 silencing showed the opposite effects. DUSP4 serves as an oncogenic gene in CCRCC carcinogenesis, indicating the potential value of DUSP4 in the diagnosis and treatment of CCRCC.

Introduction

Here, we focused on DUSP4, whose expression is responsible for the carcinogenesis of various malignancies [9, 13–17]. With the further study on the role of DUSP4 gene in tumors, it was found that DUSP4 is closely related to CCRCC. Remarkably, high DUSP4 levels in CCRCC cancer tissues are displayed in the GEPIA of TCGA database. However, the relationship between CCRCC carcinogenesis and DUSP4 is still unknown. In our study, DUSP4 expression in CCRCC tissues and cell lines was higher than that in non-cancer tissues and normal renal tubular epithelial cell line, respectively. Importantly, with the invasion or metastasis of CCRCC, DUSP4 levels in cancer tissues was further increased. The analyses related to the correlation between DUSP4 levels and the clinicopathological features showed that high DUSP4 levels indicated the poor prognosis of CCRCC patients. Accordingly, DUSP4 may be a promising biomarker for the prognosis of CCRCC.

In addition, it was found that DUSP4-overexpressed CCRCC cells showed higher proliferative and migratory levels. In contrast, CD142 knockdown was harmful to the proliferation and migration of CCRCC cells. Xenograft assays also showed that DUSP4-overexpressed CCRCC cells had the stronger tumorigenicity *in vivo*, while DUSP4-silenced CCRCC cells showed the reverse effect. Taken together, DUSP4 is likely to be an oncogenic gene and treatment target for CCRCC according to the evidence *in vitro* and *in vivo*. However, the intrinsic mechanism underlying DUSP4-regulated CCRCC carcinogenesis requires the further exploration.

Through this study, we revealed a novel biomarker for CCRCC, which may act as an oncogenic gene in the carcinogenesis of CCRCC. The detection of DUSP4 levels may be helpful to judge the prognosis of CCRCC. Moreover, targeting DUSP4 may be a potential treatment protocol for CCRCC. Based on current experimental data, the prognosis and treatment of CCRCC may be further improved in future.

Results

DUSP4 gene was overexpressed in the tissues and cell lines of CCRCC

Firstly, the GEPIA of TCGA database showed that the mRNA level of DUSP4 in CCRCC cancer tissues was significantly higher than that of paracancerous tissues (Fig. 1A). As shown in Fig. 1B, DUSP4 was significantly overexpressed in CCRCC tissue samples compared with paracancerous tissues. The correlation analyses related to clinical data showed that the increased DUSP4 levels was positively correlated with the growth, aggressiveness and metastasis of CCRCC cancer tissues (**Table 1**). Besides, 20 CCRCC samples with metastatic characteristic showed higher DUSP4 levels than 26 non-metastatic CCRCC samples (Fig. 1C). Moreover, compared with 21 CCRCC samples with a diameter less than 3 cm, 25 CCRCC samples with a diameter greater than 3 cm had higher DUSP4 expression (Fig. 1D). The detection of DUSP4 mRNA levels in normal renal tubular epithelial cell line HK-2 and CCRCC cell lines OS-RC-2, 786-O and Caki-1 showed that DUSP4 was significantly overexpressed in CCRCC cell lines (Fig. 1E).

DUSP4 overexpression promoted the proliferation and migration of CCRCC cells while DUSP4 silencing showed the opposite effect

To investigate the roles of DUSP4 in CCRCC, we overexpressed or silenced DUSP4 by transducing lentiviruses encoding DUSP4 (LV-DUSP4) or siRNAs against DUSP4 (si-DUSP4) into OS-RC-2 and 786-O cells (Fig. 2.**A,E**). CCK-8 assays displayed that DUSP4 overexpression promoted the proliferative levels of OS-RC-2 and 786-O cells (Fig. 2.**B,F**). In addition, Transwell assays showed that DUSP4 overexpression increased the number of migratory cells (Fig. 2.**C,D,G,H**). By contrast, DUSP4 knockdown showed the opposite effects in the proliferative and migratory abilities of OS-RC-2 and 786-O cells (Fig. 2.**B-D, F-H**).

DUSP4 overexpression promoted the tumorigenicity of CCRCC cells in vivo **while DUSP4 silencing showed the opposite effect**

To investigate the roles of DUSP4 in CCRCC *in vivo*, we injected DUSP4-overexpressed or DUSP4-silenced 786-O cells subcutaneously into nude mice. The import efficiency of DUSP4-overexpressed or DUSP4-silenced 786-O cells in xenograft tumors *in vivo* was verified by the results in Fig. 3A. As shown in Fig. 3.**B-D**, DUSP4 overexpression significantly enhanced the sizes, growth curve and weights of CCRCC xenograft tumors, while DUSP4 knockdown showed the opposite results, which supports the promotional effect of DUSP4 gene on CCRCC tumorigenicity.

Declarations

Author contributions Xianxin Zhu and Xianyou Zeng conceived and designed experiments; Xianyou Zeng and Changyan Zhu performed experiments, analyzed data, and prepared figures; Xianxin Zhu wrote the manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest No potential conflict of interest was disclosed by all authors.

Ethical approval All protocols of animal experiments were approved by the Institutional Animal Care and Use Committee of 900th Hospital of Joint Logistics Support Force. The Institutional Review Board of 900th Hospital of Joint Logistics Support Force reviewed and approved the collection and study of tumor samples.

Ethical approval Not applicable.

Consent to participate and publish This manuscript has been approved for submission and future publication by all authors.

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Figures

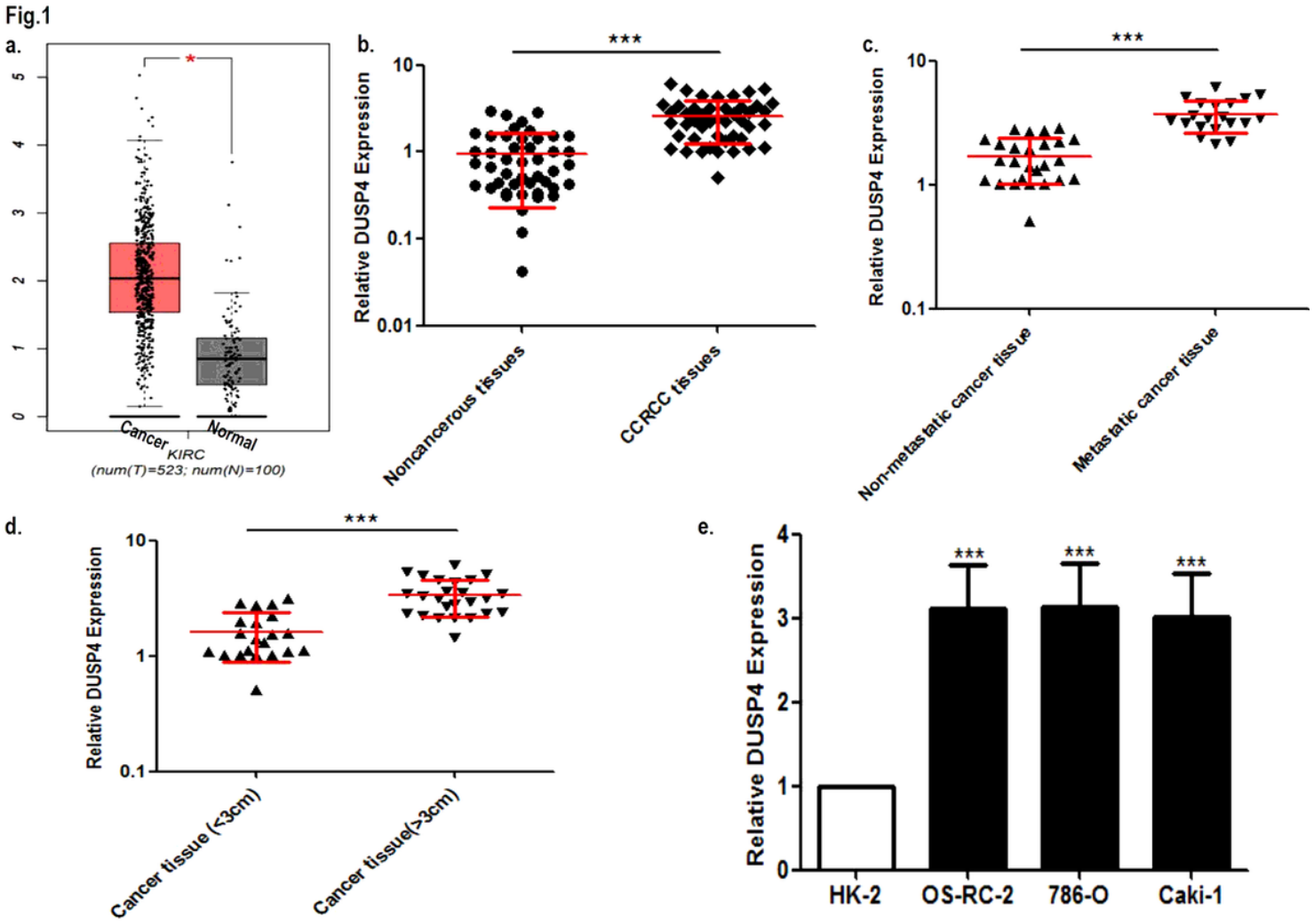

Figure 1

DUSP4 gene was overexpressed in the tissues and cell lines of CCRCC. (a) mRNA abundance analysis of DUSP4 gene in GEPIA database (http://gepia.cancer-pku.cn/index.html). (b) DUSP4 expression levels in 46 pairs of CCRCC tissues and adjacent paracancerous tissues. Results are presented as median with interquartile range. ***P<0.001 by Wilcoxon signed-rank test. (B) DUSP4 expression levels in 20 CCRCC samples with metastasis and 26 nonmetastatic CCRCC samples. Results are presented as median with interquartile range. ***P<0.001 by Wilcoxon rank sum test. (c) DUSP4 expression levels in 25 CCRCC samples with diameter more than 3 cm and 21 CCRCC samples with diameter less than 3 cm. Results are presented as median with interquartile range. ***P<0.001 by Wilcoxon rank sum test. (d) DUSP4 expression levels in normal renal tubular epithelial cell line HK-2 and CCRCC cell lines OS-RC-2, 786-O and Caki-1. Results are presented as mean±SEM from three independent experiments. ***P<0.001 by one-way ANOVA test.

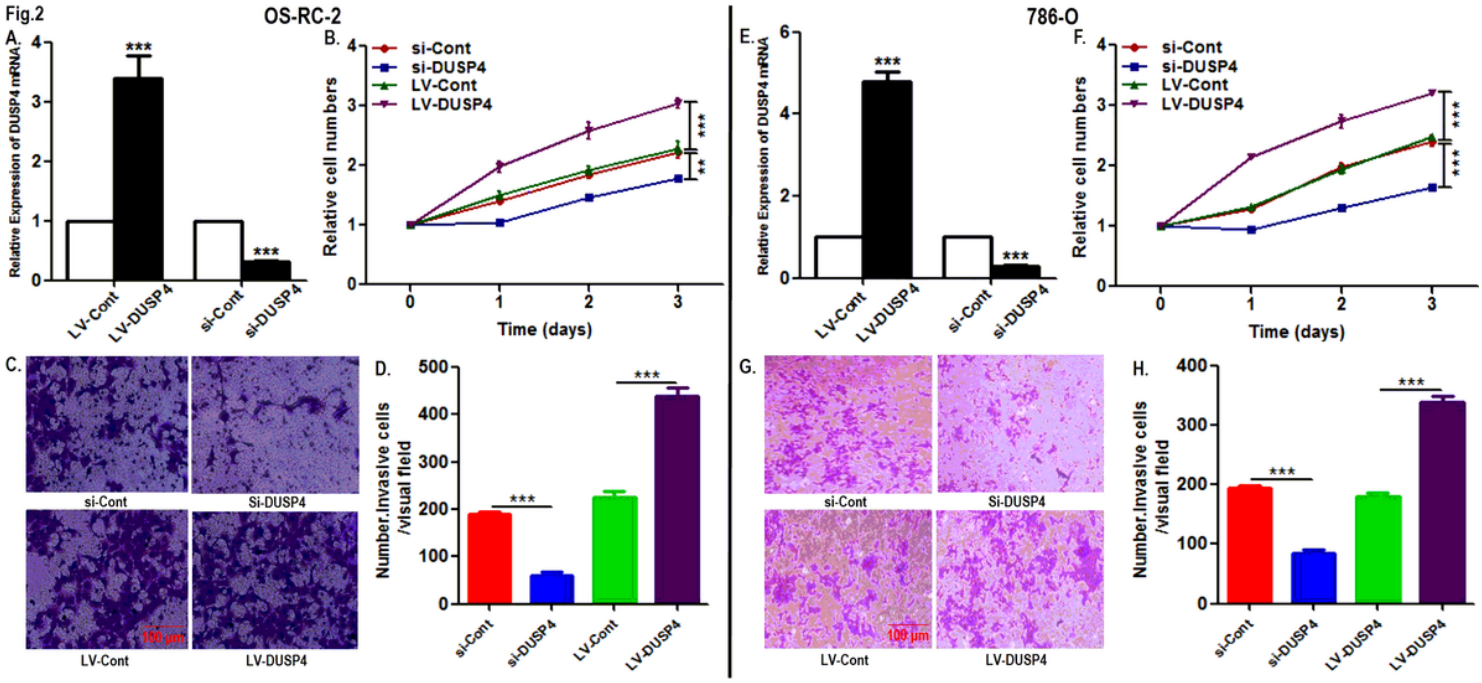

Figure 2

DUSP4 overexpression promoted the proliferation and migration of CCRCC cells while DUSP4 silencing showed the opposite effect. (a) DUSP4 expression levels in OS-RC-2 cells transduced with DUSP4-siRNA (si-DUSP4), lentivirus encoding DUSP4 (LV-DUSP4) and the corresponding control-vectors (si-Cont, LV-Cont). (b) Cell proliferation of the treated OS-RC-2 cells was assessed using CCK-8 assays. (c-d) The migration of the treated OS-RC-2 cells was assessed using Transwell assays. Scale bar: 100 μm. (e-h) Repetition of operations as described for (A-D) on 786-O cells. Results are presented as mean±SEM from three independent experiments. ***P<0.001 by Student's t-test.

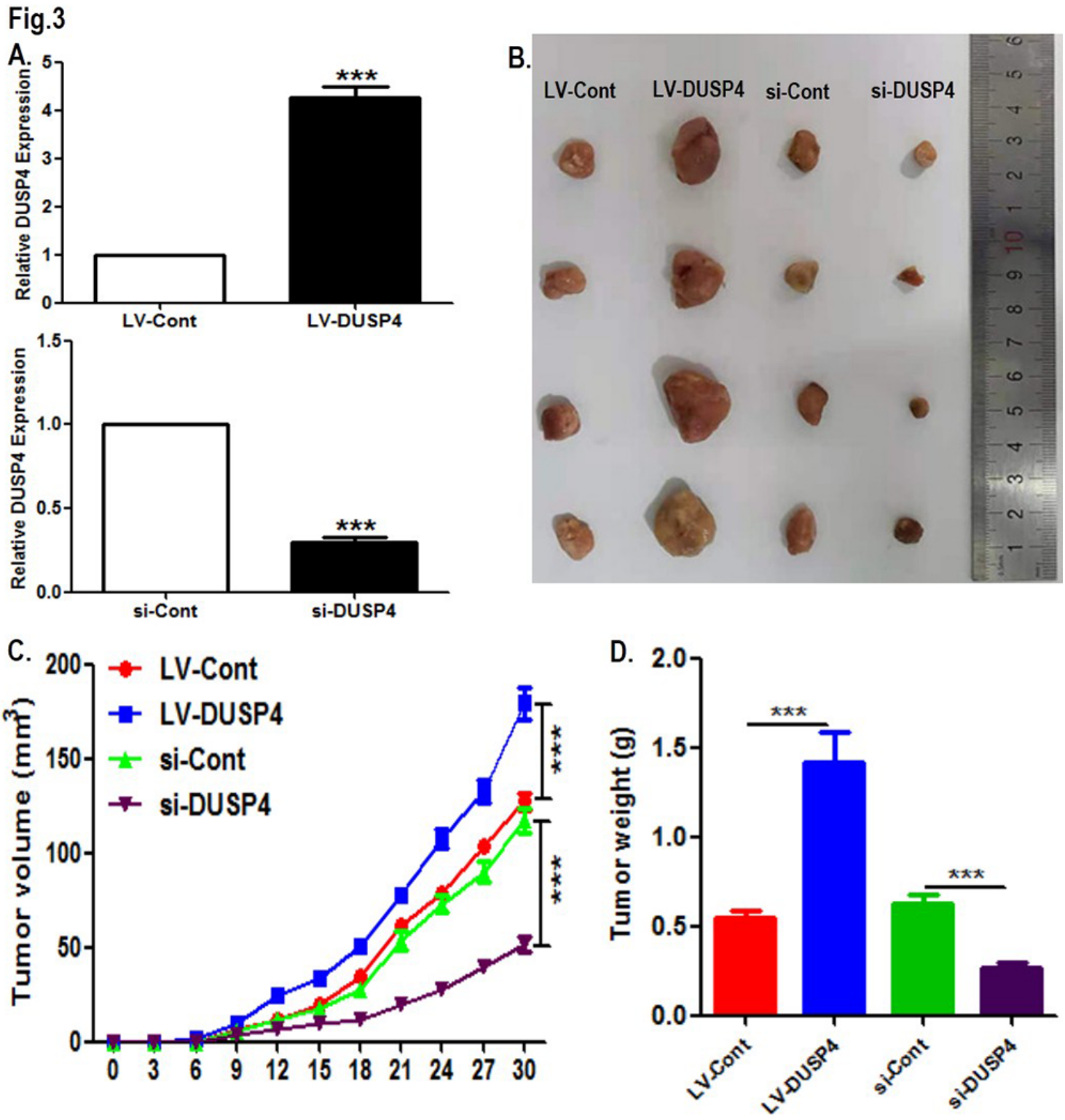

Figure 3

DUSP4 overexpression promoted the tumorigenicity of CCRCC cells in vivo while DUSP4 silencing showed the opposite effect. DUSP4-overexpressing, DUSP4-silencing or the corresponding control 786-O cells was inoculated into nude mice. 30 days later, all mice were killed, and tumors were removed and weighted. (a) mRNA levels of DUSP4 in xenograft tumor tissues were measured by qPCR assays (N=8). (b) Representative images of the removed tumors. (B) The scatter plot indicates the growth curves of

tumor volumes in four groups (N=8). (C) The statistical graph indicates the quantitative results of the weights of the removed tumors in four groups (N=8). Results are presented as mean±SEM. ***P<0.001 by Student's t-test.

Supplementary Files

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