EJMO

DOI: 10.14744/ejmo.2024.43877
EJMO 2024;8(2):216–224

Research Article

FHL2 Determines Poor Outcomes and Responsiveness of Immunotherapy Plus Tyrosine Kinase Inhibition in Metastatic Renal Cell Carcinoma

Xianglai Xu,[1,2] Jinglai Lin,[1,3] Yanjun Zhu,[2] Jiajun Wang,[2] Jianming Guo[1,2]

[1]Department of Urology, Zhongshan Hospital (Xiamen), Fudan University, Xiamen, China
[2]Department of Urology, Zhongshan Hospital, Fudan University, Shanghai, China
[3]Xiamen Clinical Research Center for Cancer Therapy, Zhongshan Hospital (Xiamen), Fudan University, Xiamen, China

**These authors contributed equally to this work.*

Abstract

Objectives: Immunotherapy plus a tyrosine kinase inhibitor (IO+TKI) is now the first line therapy for metastatic renal cell carcinoma (mRCC). Nevertheless, IO+TKI treatment's moderate response rate limited the treatment selection for mRCC patients without a useful biomarker in clinical practice.

Methods: Cohorts from our institution and from a clinical study were included (ZS-MRCC and JAVELIN Renal 101). RNA expressions were identified by sequencing. The immune infiltrate and tumor microenvironment were evaluated by flow cytometry and immunohistochemistry.

Results: Participants with high-FHL2 had a lower rate of objective response and a higher non-response rate. Longer PFS was identified in ZS-MRCC and JAVELIN Renal 101 cohorts. In the group with high FHL2, the quantity of tumor-infiltrating lymphocytes was enhanced; however, CD8+ T cells demonstrated an exhaustion phenotype. Incorporating FHL2 expression and TME markers, a machine learning model was constructed using random forest.

Conclusion: There was a strong connection between a high level of FHL2 and immunosuppression, in addition to a response to IO+TKI treatment. Additionally, there was a connection between T-cell malfunction. The expression of FHL2 was a prognostic factor in mRCC therapy, and the FHL2-based RFscore may serve as a potential biomarker to distinguish an ideal strategy between TKI monotherapy and IO+TKI.

Keywords: Renal cell carcinoma, FHL2, immune checkpoint inhibition plus tyrosine kinase inhibition, t cell dysfunction

Cite This Article: *Xu X, Lin J, Zhu Y, Wang J, Guo J. FHL2 Determines Poor Outcomes and Responsiveness of Immunotherapy Plus Tyrosine Kinase Inhibition in Metastatic Renal Cell Carcinoma. EJMO 2024;8(2):216–224.*

Recent significant breakthroughs have been conducted in the therapy of metastatic renal cell carcinoma (mRCC).[1] Prior to a decade ago, despite the poor responses, therapies that depended on cytokine were the cornerstone of RCC management since RCC was resistant to radiation and chemotherapy. Later, multitarget tyrosine-kinase inhibitors (TKIs) that target VEGF receptors and other growth receptors offered notable advancements and established the first benchmark for the management of later-stage RCC.[2] Subsequent to the immune

Address for correspondence: Jianming Guo; Jiajun Wang; Yanjun Zhu, MD. Department of Urology, Zhongshan Hospital, Fudan University, No.180 Fenglin Road, Shanghai 200032, China; Department of Urology, Zhongshan Hospital, Fudan University, No.180 Fenglin Road, Shanghai 200032, China; Department of Urology, Zhongshan Hospital, Fudan University, No.180 Fenglin Road, Shanghai 200032, China
Phone: +86-021-64041990 **E-mail:** guo.jianming@zs-hospital.sh.cn; address: w.jiajun@hotmail.com; zhu.yanjun@zs-hospital.sh.cn

Submitted Date: April 25, 2024 **Accepted Date:** May 30, 2024 **Available Online Date:** July 10, 2024

checkpoint inhibitors (ICIs) progress, clinical trials quickly demonstrated that immunotherapy (IO) and TKI combinations had better prognosis than TKI monotherapy.[3-5] In medical practice, individuals with mRCC are often categorized in accordance with the International Metastatic RCC Database Consortium (IMDC) criteria. These criteria divide patients into favorable, intermediate, or poor risk groups.[6] It has been utilized in clinical trials for subgroup analysis and is a validated predictive model for individuals with progressive RCC. Nevertheless, a sizable portion of patients succumb to the progression of mRCC while still displaying developing acquired resistance or intrinsic resistance to IO+TKI. It is vital that novel biomarkers be developed for translational research or medical decision-making for prediction and enhancement of the mRCC prognosis.

The Four-and-a-half LIM (FHL)-only protein subfamily is a part of the LIM-only protein family. FHL2 is a member of the subfamily of FHL proteins that has been the subject of most research. In light of its distinctive structure of LIM motifs, it has been discovered that FHL2 interacts with a wide variety of proteins, including IER3 MDM2 and EGFR.[7,8] Consequently, FHL2 is engaged in biological mechanisms like gene expression, cell proliferation, and motility,[9] playing vital functions in tumorigenesis and tumor advancement in hepatocellular carcinoma,[10] breast cancer,[11] ovarian cancer,[12] esophageal squamous cell carcinoma,[13] colorectal cancer,[14] non-small cell lung cancer.[15] FHL2 was informed to be involved in the Wnt/β-catenin signaling pathway by working as a co-activator of β-catenin, causing improvement of transactivation activity of β-catenin.[9,16,17] Furthermore, the FHL family is able to modulate VEGF expression in cancer cells, which may regulate the angiogenesis and TKIs therapy effectiveness.

Herein, we present real-world results from patients with mRCC who received IO+TKI therapy. We identified higher FHL2 expression as a possible marker for the IO+TKI therapy response and progression-free survival (PFS), and this finding was validated in a different external cohort.

We also discovered the correlation between enhanced FHL2 expression and macrophages, CD8+ T cells dysfunction and exhaustion. Moreover, we established a model of predictive random-forest depended on FHL2 and TME biomarkers in mRCC to anticipate the effectiveness of IO+TKI therapy in drug selection. Our outcomes exhibited the potential prognostic role and immunologic connections of FHL2 and suggested that suppressing FHL2 may improve the outcome of IO+TKI in individuals with mRCC.

Methods

Study Cohorts and Data Collection

The cohort of ZS-MRCC consisted of fifty-one participants with mRCC who underwent mixed therapies with IOIO between January 2017 and December 2020. The criteria for inclusion and exclusion had already been explained.[18,19] Six participants were eliminated from the study because tissue specimens were not available or follow-up was discontinued. The RECIST 1.1 standards were employed to determine the response to treatment and disease advancement.[20]

The JAVELIN Renal 101 group consisted of 726 people with metastatic advanced RCC who were engaged in a clinical study, as documented by Robert J. Motzer et al. before.[4,21]

The TCGA (The Cancer Genome Atlas) investigation recruited 530 individuals with clear cell RCC in the cohort of TCGA-KIRC. More information may be found at xena.ucsc.edu.[22]

Between January 2020 and December 2021 at Zhongshan Hospital, Fudan University, a total of 43 subjects diagnosed with high-risk localized RCC received radical nephrectomy were enrolled in our study. The criteria of inclusion and exclusion have been stated before.[18] Three individuals were eliminated owing to the absence of tissue specimens or the inability to fulfill sample quality control requirements based on clinical criteria.

RNA-seq and Data Processing

The RNA from primary RCC tissues that were resected was isolated. The procedure of preparing and organizing the specimen, as well as constructing the library, was explained in detail previously.[18] The read count and values of FPKM was obtained utilizing additional standardization of the sequencing data.

Immunohistochemistry Staining

The immunohistochemistry (IHC) processes and use of antibodies were conducted according to the methods previously reported.[18,23] PANNORAMIC® 250 Flash III DX scanner manufactured by 3DHISTECH Ltd was employed to scan the sides. The targeted cell densities were determined by measuring the average number of cells/mm2. T-cell exhaustion indicators were standardized to tumor-infiltrating lymphocytes. Three investigators were tasked with quantifying the number of positive cells in six fields chosen at random.

Flow Cytometry

The flow cytometry methodology and antibodies have already been identified.[18] In summary, following the Fc receptors blockage, individual cells and white blood cells

underwent a 30-minute staining process at 4°C employing membrane biomarker antibodies that were labeled with fluorescent. Intracellular staining of proteins was performed employing antibodies and the Inside Cells Fixation & Permeabilization Buffer from Thermo Fisher Scientific.

Statistical Analysis

Continuous variables among different groups were compared utilizing the Kruskal-Wallis H test. The chi-square test employed categorical variables. Quantitative correlation analysis was performed employing Spearman's correlation analysis. Survival analysis was conducted utilizing log-rank regression of Kaplan-Meier analysis. The cutoff was optimized as 36% by package 'survminer'. A predictive study was conducted employing Cox proportional hazard models.

Results

FHL2 Expression Connected with IO+TKI Therapy Response and Prognosis

RNA sequencing was utilized to assess the FHL2 expression in the cohort of TCGA. FHL2 expression was higher in ISUP grade G3 plus G4 RCC tissues in contrast to tissues of G1 plus G2 ($p<0.01$, Fig. 1a), and it was significantly raised in later TNM T stage and clinical stage ($p<0.001$, Fig. 1b, c). No significant variation in OS was detected between TxNxM1 of low and high FHL2 in individuals with mRCC in the TCGA cohort (Fig. 1d). Based on the EAU RCC direction, the recommended initial therapy for mRCC is a combined treatment of IO+TKI. Nevertheless, this treatment approach might

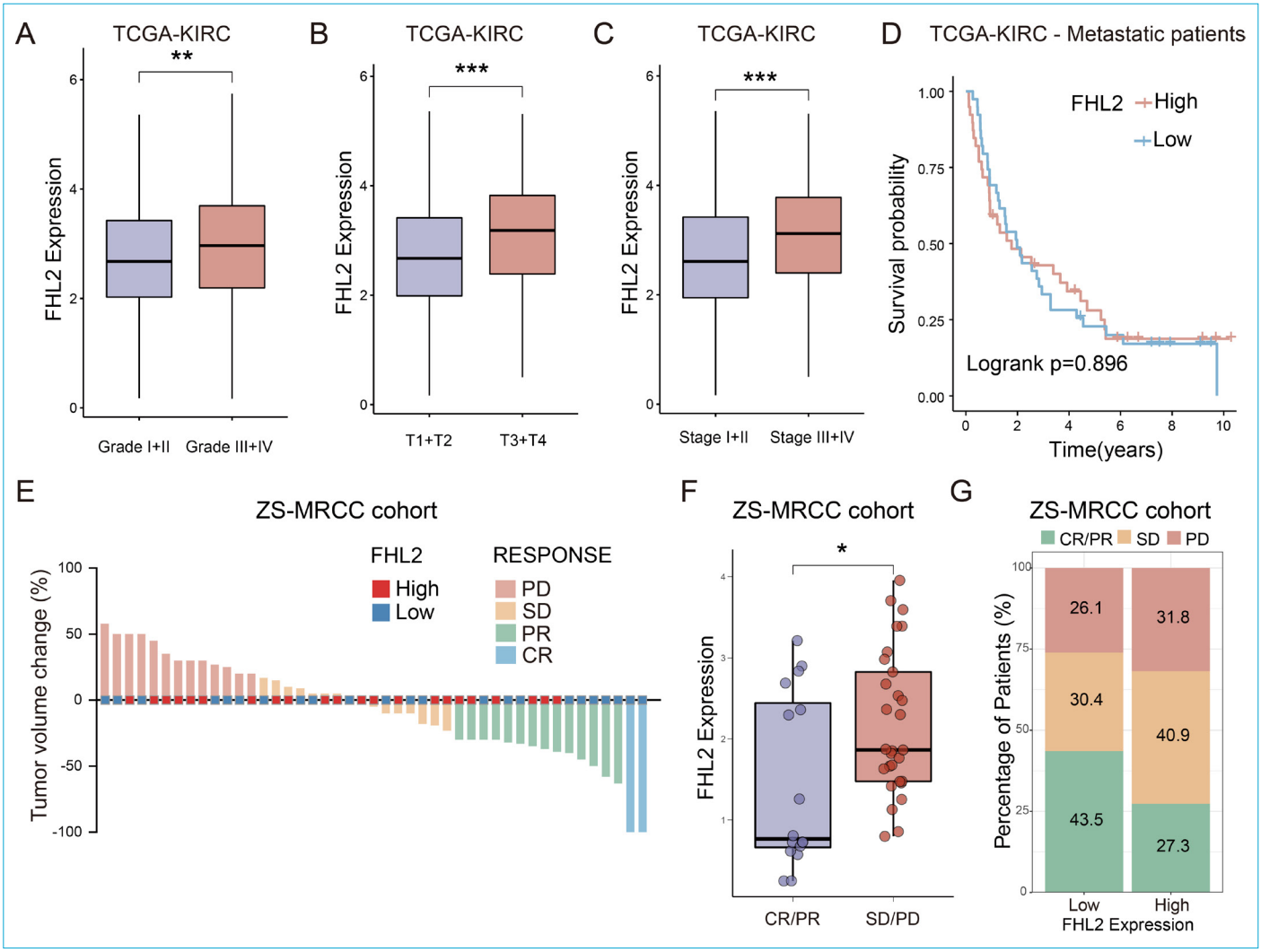


Figure 1. FHL2 is connected with resistance to combination therapy of IO+TKI in renal cell carcinoma.

(a-c) Relationship between FHL2 expression and ISUO grade **(a)**/T stage **(b)**/clinical stage **(c)** in RCC. P values, Kruskal-Wallis H test. **(d)** PFS following treatment of IO+TKI in the TxNxM1 samples of the cohort of TCGA. **(e)** Tumor best proportion alteration from baseline. **(f)** FHL2 expression between non-responders and responders of combined IO+TKI in the cohort of ZS-MRCC. P values, Kruskal-Wallis H test. **(g)** Response rate based on FHL2 expression in the cohort of ZS-MRCC under TKI+IO combined treatment. ***, P<0.001; *, P<0.05; **, P<0.01; ns, not significant.

result in different therapeutic outcomes (Fig. 1e). Further, we wonder whether it could serve as a prognostic factor in IO+TKI treatment, although we found out that FHL2 was not a prognostic gene in TxNxM1 cases of the TCGA cohort (Fig. 1d). Responders to IO+TKI revealed a substantial reduction in FHL2 expression ($p<0.05$, Fig. 1f). In the meantime, there were more responders (PR/CR) in the subgroup with low expression of FHL2 compared with the high FHL2 group (43.5% versus 27.3%, Fig. 1g).

Consequently, clinical and pathological factors, such as grade, IMDC group, and FHL2 expression, were combined into the model of Cox regression in the cohort of ZS-MRCC. FHL2 expression independently showed a poor PFS prognosis (multivariate COX: HR = 2.342, 95% CI = 1.015–5.402, p=0.046; univariate COX: hazard ratio (HR) = 2.397, 95% confidence interval (CI) = 1.081-5.315, p=0.031; Fig. 2a). In our cohort of ZS-MRCC, participants exhibiting reduced FHL2 expression had a prolonged PFS (p=0.026, Fig. 2b), and this finding was further validated in the cohort of JAVELIN Renal 101 (p=0.031, Fig. 2c). This may be related to the fact that the TCGA cohort consists of a substantial group of

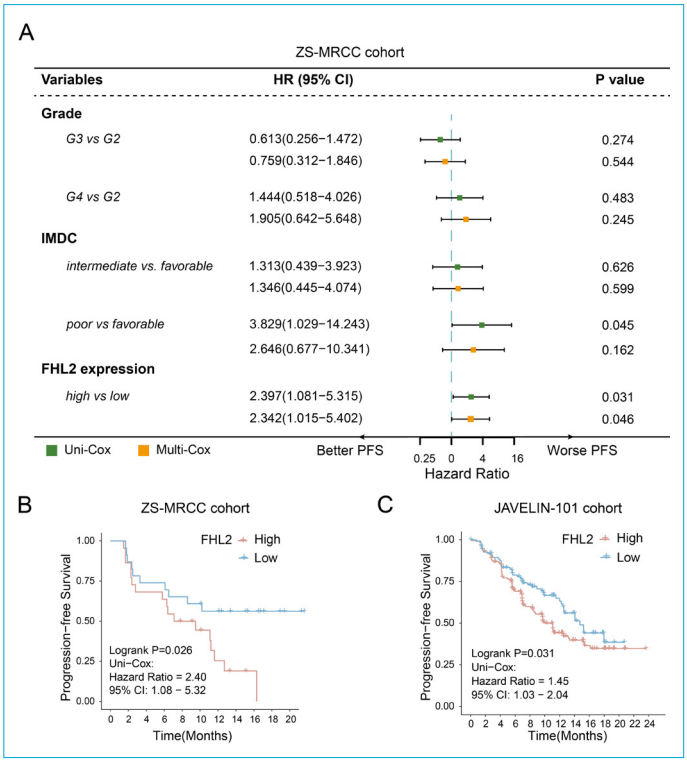


Figure 2. FHL2 linked with treatment of combined IO+TKI prognosis in renal cell carcinoma.

(a) Multivariate and Univariate Cox regression were employed to reckon HR and 95% CI. HR < 1 suggests better survival. (B&C) PFS following treatment of IO+TKI in the ZS-MRCC **(b)** and Javelin Renal 101 cohorts **(c)** of treatment of combined IO+TKI. P value, Kaplan-Meier analysis, and log-rank test.

subjects who were in early-stage RCC, and for those who were diagnosed with advanced RCC at a later stage, therapy was entirely altered at that moment. Our outcomes indicated that FHL2 expression could act as an independent negative predictor for subjects with mRCC who underwent treatment with IO+TKI.

FHL2 was Connected with T-Cell Infiltration

As noted earlier, FHL2 may be associated with the IO+TKI response. We evaluated the TME components of mRCC specimens employing H&E and IHC, ranked by FHL2 expression (Fig. 3a). As anticipated, Tumor-infiltrating lymphocytes (TILs) increased in samples with high FHL2 levels ($p<0.05$, Fig. 3b). Nevertheless, neither CD8+ T nor CD4+ T cells (Figs. 3c, d) exhibited a distinguishing pattern. To confirm the results of IHC, we utilized flow cytometry to analyze the amount of T cells in resected nephrectomy samples of high-risk localized RCC at our center (Fig. 3e). The expression of FHL2 was measured by RNA-seq as well. Consistent with IHC data, CD8+ T and CD4+ T cells amount did not suggest a significant trend between low- and high-FHL2 expression groups (Fig. 3g-h), while TILs increased significantly in high-FHL2 tissues ($p<0.05$, Fig. 3f). These outcomes exhibited that the amount of CD8+ and CD4+ T cells increased in the high FHL2 samples.

FHL2 Linked with T Cells Dysfunction and Suppressive TME

Antitumor immunity is not only related to the number of TILs, but also the function. TILs possess the capacity to identify and react to antigens that are particular to the tumor. The connection between the amount of TILs and CD8+ T cells and the effectiveness and resistance to immunotherapy or IO+TKI treatment is often insignificant. This might be attributed to T-cell exhaustion or malfunction. Utilizing flow cytometry, the function of TILs was further evaluated. Rather than CD4+ T cells (Fig. S3F), the amount of GZMB+ CD8+ T cells were observed to be downregulated in high FHL2 level samples ($p< 0.05$; Fig. 4a), while PD1+ CD8+ T cells were upregulated in high FHL2 specimens ($p<0.05$; Fig. 4b). A significant positive association was determined between TIM3+ (Fig. 4g, $p<0.05$), TBX21+ cells (Fig. 4h, $p<0.05$) and FHL2, respectively, and TOX p-value was not statistically significant; however, it was close (p=0.065, data not shown). IHC biomarkers positive cells were standardized to the TILs (Fig. 4f). The outcomes exhibited a connection between FHL2 expression and T-cell function. T cells malfunction might result from the microenvironment of the immune suppressive tumor. Thus, in the present investigation, IHC and flow cytometry were performed. Macrophages

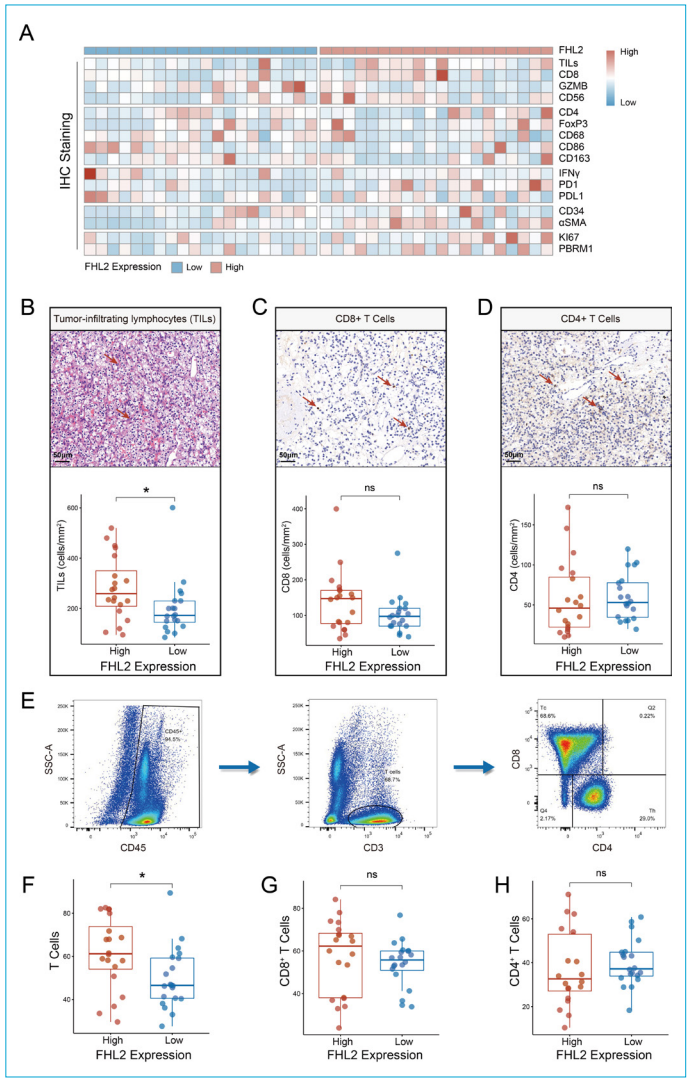


Figure 3. The connection between FHL2 and T cell infiltration in RCC.

(a) Heatmap exhibiting tumor microenvironment components ordered by FHL2 in the cohort of high-risk localized RCC. **(b-d)** Illustrative images and measurement of TILs **(b)**, tumor-infiltrating CD8+ T **(c)**, and CD4+ T cells **(d)** arranged by level of FHL2. P values, Kruskal-Wallis H test. **(e-g)** Demonstrative images of flow cytometry and the connection between TILs **(f)**, CD8+ T **(g)** or CD4+ T cells **(h)**, and FHL2 expression.

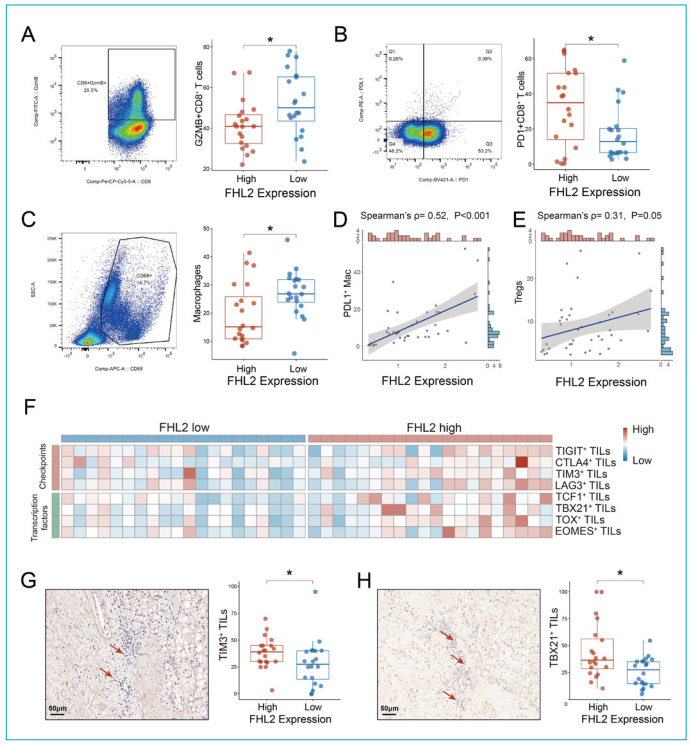


Figure 4. Suppressive microenvironment in RCC with raised FHL2 expression.

(a-d) Illustrative images of flow cytometry and the relationship between GZMB+ CD8+ T cells **(a)**, PD1+ CD8+ T cells **(b)**, macrophages **(c)**, PDL1+ macrophages **(d)**, or Tregs **(e)**, and FHL2 expression. P values, Kruskal-Wallis H test; ρ and P values, Spearman's rank-order connection. **(f)** Heatmap exhibiting checkpoints and key transcription factors of T cell exhaustion ordered by FHL2 in the cohort of high-risk localized RCC. **(g-h)** Demonstrative images and quantification of TIM3+ **(g)**, TBX21+ **(h)**, and their connection with FHL2 in a cohort of high-risk localized RCC by IHC. P values, Kruskal-Wallis H test.

were proved to be reduced in the high FHL2 group ($p<0.05$; Fig. 4c), confirmed by IHC ($p<0.05$, data not presented). However, in high FHL2 patients, PDL1+ macrophages were significantly elevated (Spearman's $\rho=0.52$, $p<0.001$, Fig. 4d), which may indicate a greater immune suppressive pattern of macrophages in those samples. Tregs were discovered to be positively connected with the expression of FHL2 ($p=0.05$; Fig. 4E). The connection between FHL2 expression and suppressive TME was suggested by these findings.

Association between FHL2 and Somatic Mutations in mRCC

An analysis was conducted to summarize the tumorigenesis mechanisms mutations based on the expression of FHL2 in the cohort of JAVELIN Renal 101. BAP1 (16%) mutations, SETD2 (25%) mutations, PBRM1 (32%) mutations, and VHL (55%) mutations were recognized as frequent in mRCC. Only SETD2, KDM5C, and PTEN mutation demonstrated a significant relationship with FHL2 expression (Fig. S1A & S1B). The results indicated that the poor IO+TKI outcome in individuals with elevated FHL2 might not be due to these well-known mutations.

Risk Model Construction and Contribution of Components

IO+TKI's treatment efficacy varies from patient to patient. Although TKI monotherapy remains an alternative option for individuals with mRCC as a first-line treatment, clinical

trials demonstrated a better outcome for IO+TKI combinations. Consequently, it is essential to construct a model that can ascertain the differential response of a subset to the treatment of IO+TKI. A randomized forest, a well-recognized machine learning method, was employed to develop a prognostic model. The model parameters consisted of the expression levels of FHL2 and TME biomarkers, which include PD1, CD8A, GZMB, GZMK, CD4, CTLA4, and PDL1. The significance of random forest parameters to the riskscore (RFscore) was illustrated (Fig. 5a). The prognostic effect of RFscore was confirmed by Kaplan-Meier analysis (Fig. 5f) in patients with avelumab+axitinib in the JAVELIN Renal 101 cohort (p<0.001, Fig. 5b), but not in patients in the sunitinib arm (Fig. 5c). Nevertheless, the combination of IO+TKI demonstrated a superior PFS in contrast to TKI alone only in subgroups with low RFscore (p<0.001, Figs. 5d, e). This random forest based model was applied to analyzed the PFS data from JAVELIN Renal 101, it was found that in the low RFscore arm, the combined treatment of IO+TKI revealed a tendency towards longer PFS (p<0.001, P value for interaction <0.001; HR 0.42; 95% CI 0.31-0.58; Fig. 5g). However, in the high RFscore arm, there was no variation in PFS between TKI+IO and TKI alone (HR 0.96; 95% CI 0.72-1.27, p=0.763). The model of random forest indicated promise for determining IO+TKI prognosis as opposed to TKI alone for individuals with mRCC.

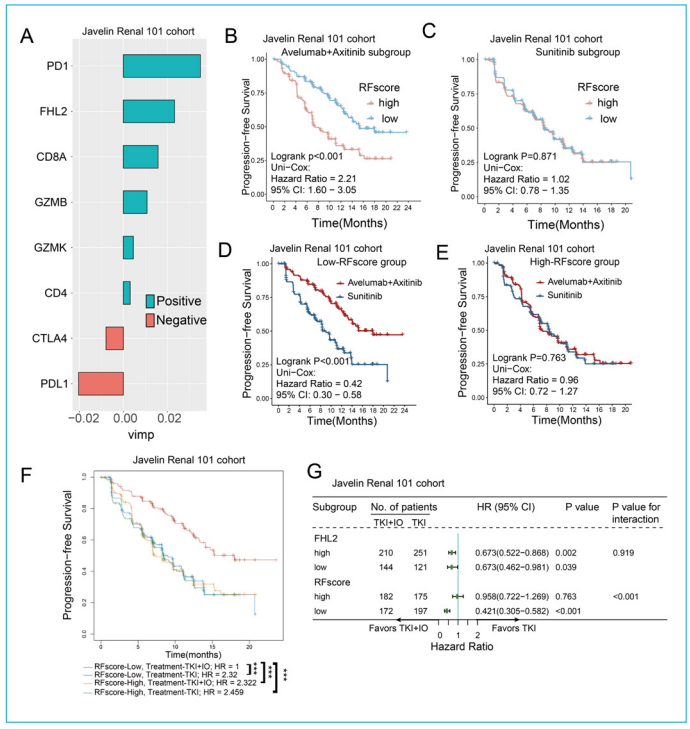


Figure 5. An integrated random forest risk score (RFscore) for IO+TKI benefit prediction

(a) Variables' significance of RFscore connected with the expression of FHL2 and some other TME biomarkers, like PD1, CD8A, GZMB, GZMK, CD4, CTLA4, and PDL1. **(b, c)** PFS based on high- and low-RFscore in RCC managed by IO+TKI **(b)** or TKI monotherapy **(c)**. **(d, e)** PFS of low-RFscore subgroup **(d)** or high-RFscore subgroup **(e)** in subjects with combined treatment of IO+TKI or only TKI. **(f)** PFS of RFscore in subjects who underwent combined IO+TKI treatment or TKI monotherapy. **(g)** HR and 95% CI of random forest model parameters detection was conducted utilizing the Cox regression model. HR < 1 suggests better survival with the treatment of IO+TKI. HR >1 shows better survival with the treatment of TKI alone. ***, p<0.001.

Discussion

The LIM homeodomains of FHL2 are composed of four and a half highly preserved cysteine-rich regions. FHL2's distinctive construction enables it to interact with a variation of proteins.[9] In cancer, FHL2 may act as an oncogenic protein or an inhibitor of tumor growth. Although FHL2 has been associated with tumor progression, it has also been linked to inhibiting neuroblastoma and myeloid disorders.[24,25] The primary objective of our investigation was to examine the systematic therapy for progressing RCC. Individuals who had low FHL2 expression weren't showing a statistically significant increase in OS in contrast to those with high FHL2 expression. This might be attributed to the fact that the common patients in the TCGA cohort were in the early stages and were not undergoing efficient therapy at that moment. However, it is worth noting that the expression of FHL2 had a connection to the stage and grade at that time (Fig. 1b, c). In ZS-MRCC and Javelin Renal 101 cohorts being treated with IO+TKI combination therapy, FHL2 has been observed to be associated with poor prognosis and poor therapeutic outcomes (Fig. 2b, c).

The specific pathways behind the oncogenic impacts of FHL2 are yet not well understood. It has been shown that FHL2 is connected to the Wnt/β-catenin signaling pathway,[9] which not only directly promotes carcinogenesis but also is involved in tumor immune evasion.[26-28] While APC mutations have been linked with CD8+ T-cell infiltration in colon malignancy in some studies, TCGA data provided a more comprehensive relationship between T-cell infiltration and Wnt/β-catenin signaling.[29] High amounts of β-catenin were connected with low CD8+ T cells infiltration levels, as demonstrated by the study's findings. Generally, T cells infiltration is the cornerstone for tumor self-elimination and immunotherapy. According to the findings of the current study, the expression of FHL2 was also associated with TILs (Fig. 3b-f). Interestingly, the ratio between CD8+ (Fig. 3c, g) and CD4+ T cells (Fig. 3d, h) did not differ significantly, which revealed that the amount of CD8+ T cells elevated in the high FHL2 specimens.

WNT/β-catenin pathway upregulation may result in tumor immune escape, tumor recurrence, and immunotherapy re-

sistance.[30,31] CD8+ T cells have a critical function as triggers in the immune-tumor cycle. Due to extended tumor antigen exposure, tumor immunosuppressive factors, and suppressive tumor microenvironment, tumor-infiltrating CD8+ T cells gradually lose their effector capabilities. In these processes, the WNT/β-catenin pathway has a vital function in T cell differentiation, migration, and activation.[32] In line with our present data, we exhibited that FHL2 expression was connected with GZMB+ CD8+ and PD1+ CD8+ T cells by flow cytometry (Fig. 4a, b), which suggested that CD8+ T cell function was impaired in higher FHL2 samples.

The use of VEGFR-TKIs in the treatment of mRCC has made significant advancements over the last decade until the introduction of immunotherapy. First-line therapy recommendations were revised by the EAU to the combination therapy of IO plus TKI. While IO+TKI treatment has achieved great success, the rate of response varies considerably from patient to patient (Fig. 1e). Through our investigation, IO+TKI therapy prognosis may be predictable by the expression of FHL2 (Fig. 2b, c). Further, we demonstrated that the RFscore, constructed with FHL2 and other immune-related factors, may be used to help the clinical selection between IO+TKI treatment versus monotherapy (Fig. 5a, g). Targeting the pathways via which FHL2-related function affects immune cells and immunological-mediated anticancer responses could help with the development of novel therapeutic approaches. There are a number of limitations to this study. The small sample size and retrospective approach may produce an enrollment bias. In the future, a prospective validation study with a larger sample size will be conducted.

Conclusion

FHL2 may function as a marker for unfavorable survival and the effectiveness of IO+TKI. FHL2 expression was associated with exhaustion and malfunction of CD8+ T cell, as well as an increase in Tregs and a reduction in macrophages. In mRCC patients, the FHL2 and PD1-based RFscore may be useful in determining the selection of therapy strategies.

Disclosures

Acknowledgments: We express our thankfulness to the authors who made their datasets available and shared them in the TCGA databases and JAVELIN Renal 101 clinical trial.

Declaration of Interest: The authors affirm that they do not have any conflicting interests.

Ethics Committee Approval: The investigation adhered to the directions set out in the Declaration of Helsinki and received authorization from the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University (B2021-119). Each participant provided informed consent.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Funding: This investigation received a fund from National Natural Science Foundation of Fujian Province, China(2023J05297), National Natural Science Foundation of China (81700660, 81902898, 82272776, 81974393), Shanghai Sailing Program (19YF1407900), Medical Project of Xiamen (3502Z20214ZD1080), Fudan University (2020ZSXMYS17), Zhongshan Clinical Research Project (ZSLCYJ202338), and Science Foundation of Zhongshan Hospital (Xiamen). None of these investigation funders are contributed to the study's design, data collection, analysis, or data interpretation.

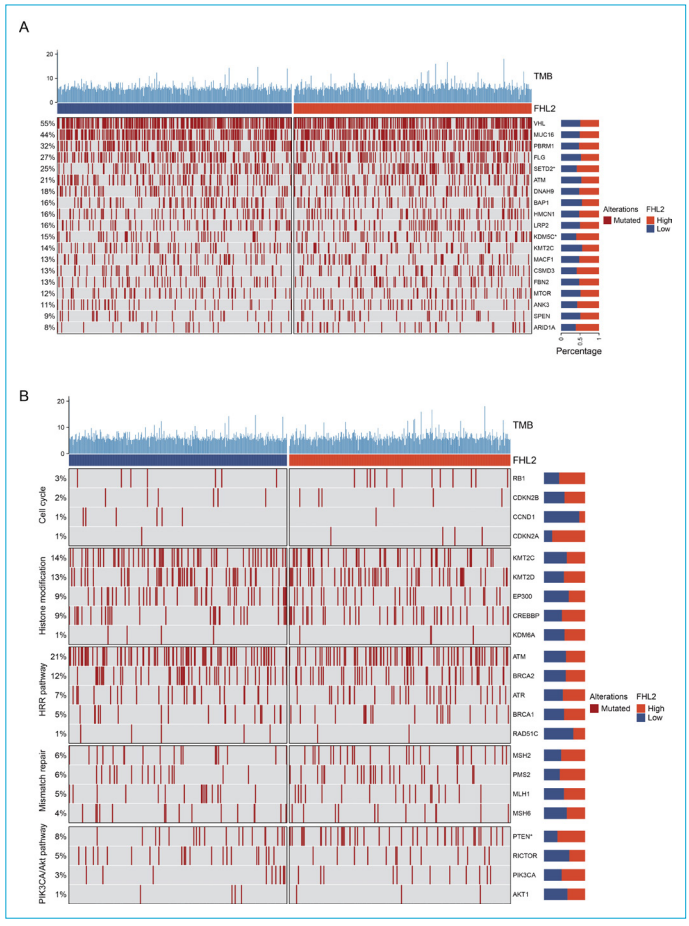
Availability of Data and Materials: In the present investigation, the used datasets are publicly accessible via the TCGA and JAVELIN Renal 101 clinical trials. The data from our cohorts, which validate the findings of this research, may be acquired from the corresponding author upon a fair demand.

Authorship Contributions: Concept – X.X.; Design – J.W.; Supervision – J.G.; Materials – J.L.; Data collection &/or processing – J.L.; Analysis and/or interpretation – X.X.; Literature search – J.W.; Writing – X.X.; Critical review – Y.Z.

References

1. Geynisman DM, Maranchie JK, Ball MW, Bratslavsky G, Singer EA. A 25 year perspective on the evolution and advances in an understanding of the biology, evaluation and treatment of kidney cancer. Urol Oncol 2021;39(9):548–60.
2. Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, et al. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): A randomised phase 3 trial. Lancet 2011;378(9807):1931–9.
3. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380(12):1116–27.
4. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L, Campbell MT, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380(12):1103–15.
5. Motzer R, Alekseev B, Rha SY, Porta C, Eto M, Powles T, et al. Lenvatinib plus pembrolizumab or everolimus for advanced renal cell carcinoma. N Engl J Med 2021;384(14):1289–300.
6. Heng DY, Xie W, Regan MM, Harshman LC, Bjarnason GA, Vaishampayan UN, et al. External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: A population-based study. Lancet Oncol 2013;14(2):141–8.
7. Jin H, Lee K, Kim YH, Oh HK, Maeng YI, Kim TH, et al. Scaffold protein FHL2 facilitates MDM2-mediated degradation of IER3 to regulate proliferation of cervical cancer cells. Oncogene 2016;35(39):5106–18.
8. Sun L, Yu S, Xu H, Zheng Y, Lin J, Wu M, et al. FHL2 interacts with EGFR to promote glioblastoma growth. Oncogene 2018;37(10):1386–98.

9. Johannessen M, Moller S, Hansen T, Moens U, Van Ghelue M. The multifunctional roles of the four-and-a-half-LIM only protein FHL2. Cell Mol Life Sci 2006;63(3):268–84.
10. Dahan J, Nouët Y, Jouvion G, Levillayer F, Adib-Conquy M, Cassard-Doulcier AM, et al. LIM-only protein FHL2 activates NF-kappaB signaling in the control of liver regeneration and hepatocarcinogenesis. Mol Cell Biol 2013;33(16):3299–308.
11. Boateng LR, Bennin D, De Oliveira S, Huttenlocher A. Mammalian actin-binding protein-1/Hip-55 interacts with FHL2 and negatively regulates cell invasion. J Biol Chem 2016;291(27):13987–98.
12. Huang Z, Li Q, Luo K, Zhang Q, Geng J, Zhou X, et al. miR-340-FHL2 axis inhibits cell growth and metastasis in ovarian cancer. Cell Death Dis 2019;10(5):372.
13. Gao A, Su Z, Shang Z, He C, Miao D, Li X, et al. TAB182 aggravates progression of esophageal squamous cell carcinoma by enhancing beta-catenin nuclear translocation through FHL2 dependent manner. Cell Death Dis 2022;13(10):900.
14. Verset L, Tommelein J, Moles Lopez X, Decaestecker C, Mareel M, Bracke M, et al. Epithelial expression of FHL2 is negatively associated with metastasis-free and overall survival in colorectal cancer. Br J Cancer 2013;109(1):114–20.
15. Li N, Xu L, Zhang J, Liu Y. High level of FHL2 exacerbates the outcome of non-small cell lung cancer (NSCLC) patients and the malignant phenotype in NSCLC cells. Int J Exp Pathol 2022;103(3):90–101.
16. Brun J, Dieudonné FX, Marty C, Müller J, Schüle R, Patiño-García A, et al. FHL2 silencing reduces Wnt signaling and osteosarcoma tumorigenesis in vitro and in vivo. PLoS One 2013;8(1):e55034.
17. Zhang W, Jiang B, Guo Z, Sardet C, Zou B, Lam CS, et al. Four-and-a-half LIM protein 2 promotes invasive potential and epithelial-mesenchymal transition in colon cancer. Carcinogenesis 2010;31(7):1220–9.
18. Wang J, Zhang S, Wang Y, Zhu Y, Xu X, Guo J. Alternative complement pathway signature determines immunosuppression and resistance to immunotherapy plus tyrosine kinase inhibitor combinations in renal cell carcinoma. Urol Oncol 2023;41(1):51.e13-51.e23.
19. Xu X, Wang Y, Chen Z, Zhu Y, Wang J, Guo J. Unfavorable immunotherapy plus tyrosine kinase inhibition outcome of metastatic renal cell carcinoma after radical nephrectomy with increased ADAM9 expression. Immunogenetics 2023;75(2):133–43.
20. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Journal Article; Practice Guideline. Eur J Cancer 2009;45(2):228–47.
21. Motzer RJ, Robbins PB, Powles T, Albiges L, Haanen JB, Larkin J, et al. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: Biomarker analysis of the phase 3 JAVELIN Renal 101 trial. Nat Med 2020;26(11):1733–41.
22. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol 2020;38(6):675–8.
23. Wang J, Liu L, Bai Q, Ou C, Xiong Y, Qu Y, et al. Tumor-infiltrating neutrophils predict therapeutic benefit of tyrosine kinase inhibitors in metastatic renal cell carcinoma. Oncoimmunology 2019;8(1):e1515611.
24. Han W, Wu Z, Zhao Y, Meng Y, Si Y, Yang J, et al. FHL2 interacts with and acts as a functional repressor of Id2 in human neuroblastoma cells. Nucleic Acids Res 2009;37(12):3996–4009.
25. Hou Y, Wang X, Li L, Fan R, Chen J, Zhu T, et al. FHL2 regulates hematopoietic stem cell functions under stress conditions. Leukemia 2015;29(3):615–24.
26. Fu C, Liang X, Cui W, Ober-Blöbaum JL, Vazzana J, Shrikant PA, et al. β-Catenin in dendritic cells exerts opposite functions in cross-priming and maintenance of CD8+ T cells through regulation of IL-10. Proc Natl Acad Sci U S A 2015;112(9):2823–8.
27. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 2015;523(7559):231–5.
28. Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, et al. Wnt/beta-catenin pathway: Modulating anticancer immune response. J Hematol Oncol 2017;10(1):101.
29. Zhou Y, Xu J, Luo H, Meng X, Chen M, Zhu D. Wnt signaling pathway in cancer immunotherapy. Cancer Lett 2022;525:84–96.
30. Wang B, Tian T, Kalland KH, Ke X, Qu Y. Targeting wnt/beta-catenin signaling for cancer immunotherapy. Trends Pharmacol Sci 2018;39(7):648–58.
31. Galluzzi L, Spranger S, Fuchs E, Lopez-Soto A. WNT signaling in cancer immunosurveillance. Trends Cell Biol 2019;29(1):44–65.
32. Gattinoni L, Ji Y, Restifo NP. Wnt/beta-catenin signaling in T-cell immunity and cancer immunotherapy. Clin Cancer Res 2010;16(19):4695–701.



Supplement 1. Somatic mutations are sorted depending on the expression of FHL2. P values, Chi-square test. *, p<0.05.

(a, b) Waterfall plot showing genomic mutations ordered by FHL2 expression in the cohort of JAVELIN-101. P values, Chi-square test. *, p<0.05.