Integrated Molecular Characterization of Fumarate Hydratase–deficient Renal Cell Carcinoma

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ABSTRACT

Purpose: Fumarate hydratase–deficient renal cell carcinoma (FH-deficient RCC) is a rare but lethal subtype of RCC. Little is known about the genomic profile of FH-deficient RCC, and the therapeutic options for advanced disease are limited. To this end, we performed a comprehensive genomics study to characterize the genomic and epigenomic features of FH-deficient RCC.

Experimental Design: Integrated genomic, epigenomic, and molecular analyses were performed on 25 untreated primary FH-deficient RCCs. Complete clinicopathologic and follow-up data of these patients were recorded.

Results: We identified that FH-deficient RCC manifested low somatic mutation burden (median 0.58 mutations per megabase), but with frequent somatic copy-number alterations. The majority of FH-deficient RCCs were characterized by a CpG islands methylator phenotype, displaying concerted hypermethylation at numerous CpG sites in genes of transcription factors, tumor suppressors, and tumor hallmark pathways. However, a few cases (20%) with low metastatic potential showed relatively low DNA methylation levels, indicating the heterogeneity of methylation pattern in FH-deficient RCC. Moreover, FH-deficient RCC is potentially highly immunogenic, characterized by increased dominant T-cell infiltration but high expression of immune checkpoint molecules in tumors. Clinical data further demonstrated that patients receiving immune checkpoint blockade–based treatment achieved improved progression-free survival over those treated with antiangiogenic monotherapy (median, 13.3 vs. 5.1 months; P = 0.03).

Conclusions: These results reveal the genomic features and provide new insight into potential therapeutic strategies for FH-deficient RCC.

Introduction

Fumarate hydratase–deficient renal cell carcinoma (FH-deficient RCC) is a rare subtype of kidney cancer characterized by pathologic germline/somatic mutation of the fumarate hydratase (FH) gene, with a lack of FH staining on IHC. FH-deficient RCC is difficult to diagnose because it frequently shows mixed pathologically architectural patterns; therefore, it is highly susceptible to be misdiagnosed as other RCC subtypes (1, 2). Clinically, most cases of FH-deficient RCC are very aggressive and frequently present with locally advanced or metastatic disease at initial diagnosis (3). Unfortunately, owing to its rarity, little is known about its genetic basis, and there are currently no standard treatment strategies for patients with advanced disease.

To date, our knowledge of the genetic basis of FH-deficient RCC mainly comes from the reports of The Cancer Genome Atlas (TCGA) database. Using comprehensive genomic analyses, TCGA identified a distinct group of papillary RCC characterized by a CpG island methylator phenotype (CIMP), of which five cases were found to harbor germline or somatic FH mutation (4). However, due to the limited cases in TCGA cohort, specific research aiming to explore the genomic landscape of FH-deficient RCC is still lacking. In this study, we performed a high-throughput genomic and epigenomic analysis of untreated primary FH-deficient RCC tumors from multinational patient cohorts to provide molecular insights into FH-deficient RCC and suggest paths for the development of biology-driven precision medicine.

Materials and Methods

Patient identification

We cooperated with nine medical centers to collect FH-deficient RCC samples. All suspicious RCC tumors were referred for pathology.
Clinicopathologic data were retrospectively collected, including age, gender, family history, metastatic sites, tumor–node–metastasis stage, histologic type, International Society of Urological Pathology grade, surgery type, and systemic treatment type. Synchronous metastasis was defined as the diagnosis of a distant metastasis at the time of the primary RCC diagnosis. Metachronous metastasis was defined as the metastasis occurred after 3 months postoperatively.

Outcomes
Patients visited the urologists for regular evaluations (physical examination, radiologic assessment, and laboratory tests) every 4–6 weeks. Response was defined by RECIST version 1.1 (5). The endpoints were objective response rate (ORR), disease control rate (DCR), disease-free survival (DFS), progression-free survival (PFS), and overall survival (OS). ORR and DCR were assessed according to RECIST 1.1. ORR was defined as the proportion of patients with complete or partial responses as the best radiological response to therapy. DCR encompassed the proportion of patients who achieved an objective response plus stable disease. DFS was defined as the time from the diagnosis to local or regional recurrence or distant metastasis or death from any cause. PFS was defined as the time from initial diagnosis to disease progression or death. First-line PFS was defined as the time from the start of first-line systemic treatment to disease progression or death. OS was defined as the time from initial diagnosis to all-cause death. Systemic treatment OS was defined as the time from the start of systemic treatment to all-cause death.

WES and methylation sequencing
For samples performing WES, high-quality genomic DNA was extracted using the GeneRead DNA FFPE Kit (180134, QIAGEN) according to the manufacturer’s specifications. Exome capture was performed using SureSelect Human All ExonV6 kit (Agilent Technologies) followed by paired-end sequencing using the Illumina Hiseq Xten platform (Illumina Inc). For panel sequencing, DNA was extracted and estimated by a targeted sequencing strategy capturing all exons of 642 tumor-related genes (Supplementary Table S1). Human Infinium MethylationEPIC BeadChip (Illumina) was used to analyze DNA methylation. Extracted DNA was treated with RNase A/T1 Mix (Thermo Fisher Scientific) and subsequently purified using the Genomic DNA Clean & Concentrator – 10 Kit (Zymo Research). Bisulfite pyrosequencing was used to validate the results of the microarray analysis. The WES data generated in this article have been deposited at the NCBI Sequence Read Archive (SRA) hosted by the NIH (SRA accession: PRJNA533711). The methylation data reported in this article were deposited in the Gene Expression Omnibus database (accession numbers GSE155207). More detailed materials and methods can be found in the Supplementary Methods.

IHC
IHC for FH, CD8, CD4, and PD-L1 were performed using an automatic staining platform, Ventana NexES (Roche). Commercially available primary anti–FH (1: 800, sc-100743, Santa Cruz Biotechnology), CD8 (1:100, clone C8/144B, Dako), CD4 (2M-0418, 1:100), and PD-L1 (740-4859, 1:40, Roche) were used in this study. PD-L1 was determined by quantification of the density of positive cells (defined as the number of positive tumor cells per mm2) and the percentage of tumor cells. PD-L1 positivity was defined as PD-L1 expression ≥1%. CD4 and CD8 were determined by quantification of the density of positive cells defined as the number of positive tumor-infiltrating lymphocytes (TILs) per mm2 and the percentage of TILs.

Multiple immunofluorescence
Multiplex staining and multispectral imaging were performed to identify the different types of immune cells. Multiplex immunofluorescence staining was obtained using PAN0 7-plex Kit (0004100100, Panovue). Different primary antibodies, including CD8 (clone C8/144B, Panovue), CD56 (clone 123C3, Panovue), HLA-DR (clone EPR3692, Panovue), CD68 (Panovue, clone BP6036), and PanCK (0057000050, Panovue) were sequentially applied, followed by horse-radish peroxidase-conjugated secondary antibody incubation and tyramide signal amplification. The slides were microwave heat treated after each Trichostatin A operation. Nuclei were stained with 4',6-diamidino-2-phenylindole (D9542, DAPI, Sigma) after all the human antigens had been labeled. The stained slides were scanned via the Mantra System to obtain multispectral images (PerkinElmer). The scans were combined to build a single stack image. All slides were
scanned at an absolute magnification of ×200 and ×100. Images of unstained and single-stained sections were used to extract the spectrum of autofluorescence of tissues and each fluorescent, respectively. The extracted images were further used to establish a spectral library required for multispectral unmixing by Inform image analysis software (PerkinElmer). The CD8, macrophage, and natural killer (NK) cells expression were quantified by the number of positive cells per mm² and by the number of positive cells per number of nucleated cells.

Sanger sequencing

The presence of somatic mutations of FH was validated by Sanger sequencing. For this analysis, primers to amplify a 220-bp fragment covering each exon (1–10) of FH gene were designed (Supplementary Table S2). PCR was performed with the following thermal cycling conditions: a 95°C for 5 minutes, 35 cycles of 95°C for 20 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a 72°C for 7 minutes. PCR amplification of 10 ng of genomic DNA was performed using the Premix Taq (RR901A, Takara) on a Veriti96 PCR Cycler (Life Technologies). PCR fragments were purified with ChargeSwitch PCR Clean-Up Kit (CS12000, Invitrogen), and the sequencing reactions were performed on an ABI 3730XL automatic sequencer (Life Technologies). All analyses were performed in duplicate. Sequences of the forward and reverse strands were analyzed using Chromas (Technelysium).

Bisulfite pyrosequencing

Bisulfite modification of genomic DNA isolated from 30 samples was performed by standard methods. PCR primers were designed with the PyroMark Assay Design V.2.0 software (QIAGEN; Supplementary Table S3). PCR products were pyrosequenced with the PyromarkTM Q24 system (QIAGEN), according to the manufacturer’s protocol.

Statistical analysis

Fisher exact test was used to compare the difference between categorical variables. Median survival was estimated using the Kaplan–Meier method and the difference was tested using the log-rank test. All tests were two sided and performed using R software v.3.2.3, SPSS v.16.0 and GraphPad V.6.0. A P < 0.05 was considered statistically significant.

Results

Patient characteristics

From 2014 to 2019, a total of 25 patients with FH-deficient RCC were identified from 429 suspicious cases (Supplementary Fig. S1A). Among them, 16 (64%) patients had germline FH mutations, 8 (32%) patients had somatic FH mutations, and one patient (FHRCC20) had a germline mutation (c.1256C>T) and a second hit of an additional frameshift mutation (c.642delE) in the tumor. Baseline characteristics of all patients are summarized in Table 1; Supplementary Table S4. The median age at initial diagnosis was 38 years (range, 13–66 years) and the male: female ratio was 1.81. Male patients tended to be older at onset of primary RCC (P = 0.026; Supplementary Fig. S1B). At the end of follow-up (median, 14.3 months; range, 3.8–137.1 months), 21 patients (84%, 21/25) presented with metastases, including 14 synchronous metastases and seven metachronous metastases. The most common metastases were lymph nodes (76%, 16/21) and bones (48%, 10/21). Five patients died by the end of follow-up. We found that those diagnosed over the age of 40 years old had a higher incidence of metastasis (P = 0.032; Supplementary Fig. S1C) and worse prognosis compared with the younger patients (Supplementary Fig. S1D and S1E).

The somatic mutational landscape of FH-deficient RCC

WES was performed on 22 untreated primary tumors and matched germline samples. A median of 31 somatic mutations per tumor (range, 1–156) was identified, corresponding to an average of 0.58 mutations per megabase (range, 0.02–2.60; Fig. 1A). A total of 27 FH mutations were identified, including 15 missense mutations, 5 frame-shift indels, 5 nonsense mutations, 1 splice site change, and one large deletion (Fig. 1B). Apart from FH, the most frequently mutated genes in FH-deficient RCC were TTN (17%, 4/24) and NF2 (13%, 3/24). Assessment of mutations within pathways demonstrated a high number of alterations involving chromatin modification (33%, 8/24), RTK-RAS-PI3K (33%, 8/24), and DNA damage repair pathway (29%, 7/24). Signature analysis identified that signature 6 contributed the most to these base
Figure 1.

Somatic alterations in FH-deficient RCC. A, Oncoplot describes somatic genomic alterations of the FH-deficient RCC (n = 24). Recurrently mutated genes, which are grouped by molecular pathways, are shown on the left. Their mutation frequencies in patients with onset of RCC < 40 years and ≥ 40 years old are shown on the right. The top histogram shows the number of somatic mutations in each individual sample. Middle, The clinicopathologic features. The bottom histogram shows the grouped by molecular pathways, are shown on the left. Their mutation frequencies in patients with onset of RCC ≥ 40 years old are shown on the right. B, The types and relative positions of somatic mutations in the predicted FH protein. C, Copy-number profile shows amplifications (red) and deletions (blue) in all chromosomes for FH-deficient RCC. Cancer-related genes located within corresponding genomic regions are indicated. For genes located in 19 chromosome, the detailed locations are: AKT2 (19p13.2), ARID3A (19p13.3), CDKN2D (19p13.2), ERCC2 (19q13.32), KDM4B (19p13.3), SMARCA4 (19p13.2), XRCC1 (19q13.31). FH, fumarate hydratase; RCC, renal cell carcinoma; IHC, immunohistochemistry.

DNA methylation alterations associated with the pathogenesis of FH-deficient RCC

EPIC array was performed on 20 FH-deficient RCC tumors with adjacent normal tissues. The study of the methylation profiles revealed that FH-deficient RCCs had a sharp global hypermethylation compared with adjacent normal tissues in all epigenomic structures, except for open sea (Supplementary Fig. S4A). In contrast to control samples, we identified a total of 199,372 differentially methylated CpG sites (CpGs) in FH-deficient RCCs, of which 66% (132,220) were hypermethylated CpGs, whereas only 34% (67,152) were hypomethylated CpGs (Fig. 2A; Supplementary Fig. S4B and S4C).

Gene ontology enrichment analysis revealed that hypermethylated CpGs were significantly enriched in genes related to DNA-binding transcription activity and sequence-specific DNA binding (Fig. 2B), indicating the transcriptional regulators might participate in the pathogenesis of FH-deficient RCC. Moreover, we found part of hypermethylated CpGs located in multiple well-established tumor suppressor genes (CDKN2A, APC, MGMT, and TP53) and small RNAs (MIR200a, MIR200b, MIR200c, and MIR429; Supplementary Fig. S4D and S4E). Bisulfite pyrosequencing was used to validate DNA methylation levels in four gene regions in three hypermethylated loci (CDKN2A, APC, and MGMT) and one hypomethylated locus (IL4R). A high correlation was observed between values obtained from microarray and pyrosequencing.
Furthermore, functional enrichment analysis identified an enrichment of genes of hallmark pathways in cancer for aberrant methylation, including WNT, mTOR, TGFβ, NOTCH, and epithelial–mesenchymal transition (EMT) pathways (Fig. 2B; Supplementary Fig. S4G). Hypermethylated CpGs also mapped to genes involving the DNA damage repair pathways (Supplementary Fig. S4H). Gene set enrichment analysis (GSEA) using genome array data further revealed that genes linked to EMT, mTORC1, DNA damage repair and glycolysis pathways were upregulated in FH-mutated RCCs versus normal tissues (Fig. 2C).

Identification of the methylation patterns of FH-deficient RCC

To illuminate the DNA methylation pattern of FH-deficient RCC, we merged data from our FH-deficient RCC and TCGA KIRC cohorts. Using unsupervised clustering analysis, we identified three DNA methylation clusters (Fig. 2D). The majority of our FH-deficient RCC cases (80%, 16/20) and all of the CIMP-RCC cases in KIRC cohort were categorized into the CIMP cluster, exhibiting a global DNA hypermethylation phenotype. This finding supports that most FH-deficient RCCs are largely characterized by a CIMP. However, a small proportion of our FH-deficient RCC cases (20%, 4/20) were classified into cluster 1, manifested a relatively low genome-wide DNA methylation, indicating that not all FH-deficient RCCs exhibited CIMP. To further validate these findings, a T-distributed stochastic neighbor embedding analysis was performed to integrated ours and all TCGA RCC subtypes data. The results confirmed that the majority of FH-deficient RCCs were distributed in the CIMP-RCC cluster, while the presence of two different DNA methylation profiles existed in our FH-deficient RCCs. The CIMP and CIMP-like subtypes were further categorized into three clusters (Fig. 2D).
RCCs (Fig. 2E). Notably, we found patients in the non-CIMP group had a lower incidence of metastasis compared with the CIMP group (25% vs. 94%, \(P = 0.013\)). Three patients in the non-CIMP group did not develop recurrences or metastases up to the end of follow-up (median, 19.7 months), with one patient (FHRC03) followed for over 6 years after nephrectomy. Morphologically, the majority of CIMP tumors (69%, 11/16) showed a predominated papillary pattern, but only one non-CIMP tumor (25%, 1/4) showed a dominated papillary pattern. Two non-CIMP tumors (FHRC03 and FHRC12) were presented with a dominated low-grade oncocytic pattern, which has been reported with a favorable outcome (2, 6, 7). Methylation profiling analysis indicated that CIMP tumors had sharp global hypermethylation compared with non-CIMP tumors in all epigenomic structures, except for open seas (Supplementary Fig. S5A and S5B). Signature analysis further identified a total of 234,563 different signatures between these two methylation groups (Supplementary Fig. SSC).

FH-deficient RCC manifests high immune infiltration and imprints of immune evasion

As mentioned previously, a fraction of CpGs were hypomethylated in FH-deficient RCC. Strikingly, these hypomethylated CpGs were enriched in genes involving the inflammation pathway and cytokine activity (Fig. 3A). GSEA analysis of the genome array further validated the increased expression of TNFα-NFκB and inflammatory response signaling in FH-mutated RCC, indicating the enhanced antitumor inflammatory signature in FH-deficient RCC (Fig. 3A). Using IHC staining, we identified that the majority of tumors (96%, 24/25) showed increased CD8+ and CD4+ T-cell infiltration. Sixty-four percent (16/25) of tumors had extensive CD8+ T-cell infiltration (CD8+ T cells \(\geq 100 \text{ mm}^2\); Supplementary Table S4; Fig. 3B). In situ multiple immunofluorescence staining further revealed the increased immune infiltrated in FH-deficient RCC, with marked activated NK (CD56+), CD8+ T cells, and macrophages (CD68+/HLA-DR+) infiltrations in tumor stroma (Fig. 3C). Importantly, GSEA analysis confirmed that IFNγ response and IFNγ-dependent cancer immunostimulation pathways (IL-6-JAK-STAT3) were significantly enriched in FH-mutated RCC, indicating the presence of activated T-cell signature. (Supplementary Fig. S6A).

Considering that the production of IFNγ can induce PD-L1 expression and subsequently leads to immune evasion (8), we next checked the expression of PD-L1 in FH-deficient RCC. Intriguingly, we found that PD-L1–associated CpGs were hypomethylated and modified in tumors (Supplementary Fig. S6B). In addition, hypomethylated CpGs also mapped to other immune inhibitory checkpoint genes (including PDCD1L2 and CTLA4; Fig. 3D; Supplementary Fig. S6B). IHC experiments further demonstrated a high PD-L1-positive rate (80%, 20/25) in tumors (Supplementary Table S4). Of note, nearly half of tumors (48%,12/25) exhibited type II tumor immune microenvironment (TIME; ref. 9), characterized by massive CD8+ T-cell infiltration but positive PD-L1 expression in tumors (Fig. 3D). Collectively, our data demonstrated that the antitumor immunity in FH-deficient RCC might be largely blocked by PD-1/PD-L1 pathway-mediated suppression mechanism, suggesting the potential of PD-1/PD-L1 inhibitors.

Patients with metastatic FH-deficient RCC benefit from immune checkpoint blockade–based immunotherapy

At the end of follow-up, 18 patients with metastatic diseases received systemic therapy. First-line therapy was tyrosine kinase inhibitor (TKI) monotherapy (67%, 12/18), anti–PD-1 inhibitor plus TKI (27%, 5/18), and anti–PD-1 inhibitor monotherapy (6%, 1/18). For those receiving first-line therapy, the DCR was higher in patients receiving immune checkpoint blockade (ICB)–based treatment (anti–PD-1 inhibitor + TKIs or anti–PD-1 inhibitor monotherapy) versus those on TKIs monotherapy (DCR: 100% vs. 58.3%; Fig. 4A). Moreover, median first-line PFS was superior in the ICB-based treatment group compared with that in TKIs group (median, 13.3 vs. 5.1 months; \(P = 0.024\); Fig. 4B). All patients (6/6, 100%) in the ICB-based first-line treatment group achieved a PFS \(\geq 6\) months, higher than those (5/12, 42%) in the TKIs group (Fig. 4C and a typical case was illustrated in Fig. 4D). The median OS of systematic treatment was also longer in the ICB-based group compared with the TKIs group (median, not reached vs. 21.4 months); however, the differences did not reach a statistical significance (\(P = 0.209\); Fig. 4B). Two patients received additional anti–PD-1 therapy plus TKIs after disease progression during first-line TKIs monotherapy; remarkably, both of them achieved favorable disease control (PFS: 13.5 and 10.3 months, respectively).

Discussion

FH-deficient RCC is a rare but lethal malignancy, and its genetic basis is poorly understood. In this study, we performed comprehensive molecular profiling to characterize the genomic foundation of FH-deficient RCC. We determined that FH-deficient RCC was a genetically simple tumor with a relatively low mutation burden, but its distinct epigenetic features could be the biological foundation for tumor pathogenesis. Moreover, FH-deficient RCC was an intrinsically immunogenic tumor that is likely to benefit from ICB-based treatment strategies. Our study provides novel insights into the molecular landscape of FH-deficient RCC and identifies promising therapeutic targets for potential exploitation.

Although with low somatic mutation burden, we identified increased SCNAs in FH-deficient RCC, with significant loss of 1p, 8q, 10p, and 15q and gain of 4p, 7q, 11q, and 19p. Previous studies identified that a subgroup of papillary type 2 RCCs were characterized by a high degree of aneuploidy and frequent loss of 9p (4), which was not significantly altered in our FH-deficient RCCs. The different SCNAs patterns between FH-deficient RCC and papillary type 2 RCC indicates the distinct genomic alterations during tumorigenesis, although the majority of FH-deficient RCC was predominantly of type 2 histology. Recent studies also showed an increased chromosome 22 loss in CIMP-RCC (10); however, it was not a significant event in our cohort. This discrepancy may reflect the heterogeneity of CIMP-RCC and warrant further investigation.

Loss-of-function mutations in FH resulting in the accumulation of fumarate impairs the function of \(\alpha\)-KG–dependent dioxygenases, such as histone and DNA demethylases, which disrupts the normal epigenetic genes regulation (11). Consistent with this, we identified FH-deficient RCC-specific DNA methylation changes within a broad panel of transcription factors, as well as genes significantly associated with tumor progression and patient outcomes, including CDKN2A, MGMT, APC, and TP53. The loss of CDKN2A by either mutation, deletion, or promoter hypermethylation occurred in 15.8% RCCs and correlated with decreased survival in all RCC histologic subtypes (10). Of note, we observed that CDKN2A promoter hypermethylation is a common event in FH-deficient RCCs. Loss of CDKN2A can be targeted by inhibitors of cyclin-dependent kinase 4/6 (CDK4/6; ref. 12), suggesting the potential of CDK4/6 inhibitors for FH-deficient RCCs. Also, it should be pointed out that it remains unclear if the loss of CDKN2A could be a predictor for response to CDK4/6 inhibitors, and still warrant further investigation (13). Currently, epigenetic agents...
FH-deficient RCCs exhibit intrinsic immunogenic phenotype. A, Bubble chart (top) shows the significantly hypomethylated CpGs in FH-deficient RCCs. Enrichment q values (−log_{10} P) are calculated by hypergeometric test. GSEA enrichment scores (bottom) of TNFα-NFκB and inflammation response signaling in HLRCC versus normal tissues. See also Supplementary Fig. S6. B, Representative IHC demonstrating the typical TIME type II in CIMP (FHRCC13) and non-CIMP tumors (FHRCC12) in our cohort, is characterized by PD-L1−positive expression and massive CD8+ and CD4+ T-cell infiltrations. TIME type II is defined as PD-L1 positive in tumors and massive CD8+ T-cell infiltration (>100 cells/mm²). Magnification ×200. Scale bar = 100 μm. C, Representative immunofluorescence demonstrating the presence of overall CD8+ T cell, tumor-associated macrophage (CD68 and HLA-DR) and NK cell (CD56) infiltration in two selected samples (FHRCC16 and FHRCC19) in our cohort. Magnification ×400. Scale bar = 100 μm. D, Heatmap shows the immune features among FH-deficient RCCs (n = 25). Top, The number of CD8+ and CD4+ T-cell infiltrations, PD-L1 expression, and TIME type. Middle, The methylation status of a CpGs in the promoter region of immune modulation-associated genes. The bottom histogram shows the FH mutation type, FH protein expression, and CIMP status. FH, fumarate hydratase; RCC, renal cell carcinoma; HLRCC, hereditary leiomyomatosis and renal cell carcinoma; GSEA, gene set enrichment analyses; TIME, tumor immune microenvironment; CIMP, CpG island methylator phenotype.
have been approved for the treatment of hematologic malignancies (14) and are now undergoing testing in hereditary leiomyomatosis and renal cell carcinoma (ClinicalTrials.gov identifier NCT03165721). Our data imply that these aberrant DNA methylations could be the biological foundation of FH-deficient RCC, highlighting the potential of demethylation agents for treating this disease.

Another important finding of our study is that FH-deficient RCC exhibits a distinct immunophenotype. Albeit with low mutation burden, most FH-deficient RCCs exhibited typical types II TIME, characterized by increased levels of tumor infiltrating lymphocytes and activated chemotaxis signaling, but with high expression level of PD-L1. Therefore, it is reasonable to assume that anti–PD-1/PD-L1 therapy can be a potential treatment option for patients with FH-deficient RCC (9, 15). Indeed, we observed a favorable PFS for patients receiving ICB-based treatment compared with those receiving antiangiogenic monotherapy. Mechanistically, this immunogenicity may be mediated by epigenomic and metabolic alterations. Using TCGA data, Singh and colleagues found that hypomethylation of genes was associated with immune function in papillary RCC (16). Recent studies also show that tricarboxylic acid cycle metabolites, including fumarate, can regulate methylation status in immune cells and facilitate transcription and expression of proinflammatory factors (17–20). Similarly, we observed epigenetically enhanced genes involved in proinflammatory and immune suppression pathways in FH-deficient RCC. Taken together, our data suggest that FH-deficient RCC is intrinsically immunogenic and could be targeted by ICB-based immunotherapy. Given the global hypermethylation phenotype, coadministration of epigenomic agents could be a promising strategy to improve the efficacy of PD-1/PD-L1 inhibitors.

In addition to the treatments mentioned previously, there are other potential therapeutic targets according to our findings. Mutation of the Hippo pathway tumor suppressor, NF2, was detected in several tumors. Thus, targeting NF2 (e.g., dasatinib; ref. 21) may be effective in a subset of patients. Moreover, preclinical studies suggested that elevated fumarate could suppress the homologous recombination DNA repair pathway through inhibition of demethylases (22, 23). We observed mutation and frequently aberrant methylation of genes involved in DNA damage repair pathways in FH-deficient RCC, which supports the poly (ADP)-ribose polymerase inhibitors for targeted therapy. In addition, both ours and previous studies showed the
activation of glycolysis (4, 24, 25) and mTOR signaling in FH-deficient RCCs. Results from a single-center phase II trial (ClinicalTrials.gov Identifier: NCT01130519) and retrospective studies (26, 27) reported the combination of bevacizumab plus erlotinib could achieve fair objective response for patients with FH-deficient RCC, indicating the potential therapeutic value of this combination therapy.

In summary, this study expands our knowledge of the FH-deficient RCC somatic alteration landscape, emphasizes the importance of DNA methylation profiles for tumor pathogenesis and molecular classification, and reveals its immunogenic phenotype. Our study represents a step forward in understanding the biology of FH-deficient RCC and provides a genetic basis for biomarker classification and potential therapeutic strategies.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

G. Sun: Conceptualization, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. X. Zhang: Software, formal analysis, investigation, visualization, methodology, writing-review and editing. J. Liang: Data curation, software, formal analysis, investigation, methodology, writing-original draft, writing-review and editing. X. Pan: Resources, data curation, validation, methodology, writing-review and editing. S. Zhao: Conceptualization, data curation, software, formal analysis, methodology, writing-original draft, writing-review and editing. Z. Liu: Conceptualization, resources, data curation, methodology, writing-review and editing. C.M. Armstrong: Methodology, writing-review and editing. J. Chen: Resources, data curation. W. Lin: Resources, data curation. B. Liao: Resources, data curation, software, methodology. T. Lin: Resources, data curation, investigation, methodology. R. Huang: Resources, data curation, software, visualization. M. Zhang: Investigation, visualization, methodology. L. Zheng: Data curation, visualization. X. Yin: Validation, methodology. L. Nie: Methodology. P. Shen: Methodology, writing-review and editing. J. Zhao: Methodology. H. Zhang: Resources, data curation. J. Dai: Resources, data curation. Y. Shen: Resources, data curation. Z. Li: Resources, data curation. J. Liu: Resources, data curation, J. Chen: Methodology, writing-review and editing. J. Liu: Resources, data curation. Z. Wang: Data curation. X. Zhu: Data curation. Y. Ni: Data curation. D. Qin: Software, formal analysis, methodology. L. Yang: Data curation. Y. Chen: Data curation. Q. Wei: Supervision. X. Li: Supervision. Q. Zhou: Supervision. H. Huang: Conceptualization, supervision, methodology, writing-review and editing. J. Yao: Conceptualization, resources, supervision, investigation, visualization, methodology, project administration. N. Chen: Conceptualization, resources, data curation, supervision, funding acquisition, investigation, visualization, methodology, project administration, writing-review and editing. H. Zeng: Conceptualization, resources, supervision, funding acquisition, methodology, project administration, writing-review and editing.

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