Genomic and Metabolic Hallmarks of TCA-Cycle-Mutant Renal Cell Carcinomas

Memorial Sloan Kettering Cancer Center

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Background

•Succinate Dehydrogenase (SDH) and Fumarate Hydratase (FH) catalyze two consecutive and high-flux reactions in the TCA cycle, and SDH additionally encodes Complex II of the mitochondrial electron transport chain

•Mutations to the SDH or FH predispose individuals to unique subtypes of renal cell carcinoma, SDHRCC and FHRCC

•Due to their rarity, comprehensive evaluation of the genetic and metabolic features of SDHRCC and FHRCC tumors has been limited

•We assembled a multi-institutional, genomically profiled cohort of SDHRCC and FHRCC tumors to characterize and compare molecular characteristics of SDHRCC relative to FHRCC

Methods

• *MSK IMPACT targeted sequencing*: tumor and matched-normal samples were sent for targeted sequencing using our previously-validated sequencing panel (MSK-IMPACT®), of which three versions exist, targeting 341, 410, or 468 actionable cancer-associated genes, respectively. [1]

•*Whole Exome Sequencing, processing, and mutation analysis:* WES samples (from 8 SDHRCC patients; 2 with matched normals and 6 unmatched) were processed and analyzed using the TEMPO pipeline (v1.3, https://ccstempo.netlify.app/).

•*Copy Number and Mutation Analysis:* For zygosity determination, genome-wide total and allele-specific DNA copy number, purity, and ploidy were calculated via FACETS version 0.5.6 [2]. The expected number of copies for each mutation was generated based on observed variant allele fraction and local ploidy [3]. Cancer cell fractions were calculated using a binomial distribution and maximum likelihood estimation normalized to produce posterior probabilities [4].

•*Metabolomic Profiling:* Metabolomic profiling was performed using MS/MS mass spectrometry in collaboration with Metabolon Inc

References

1. Cheng et al. *Journal of Molecular Diagnostic* (2015)
2. Shen et al. *Nuclear Research Acids* (2016)
3. Dentro et al. *Cold Spring Harbor Perspec Med* (2017)
4. McGranahan et al. *Science Translational Medicine* (2015)

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Results

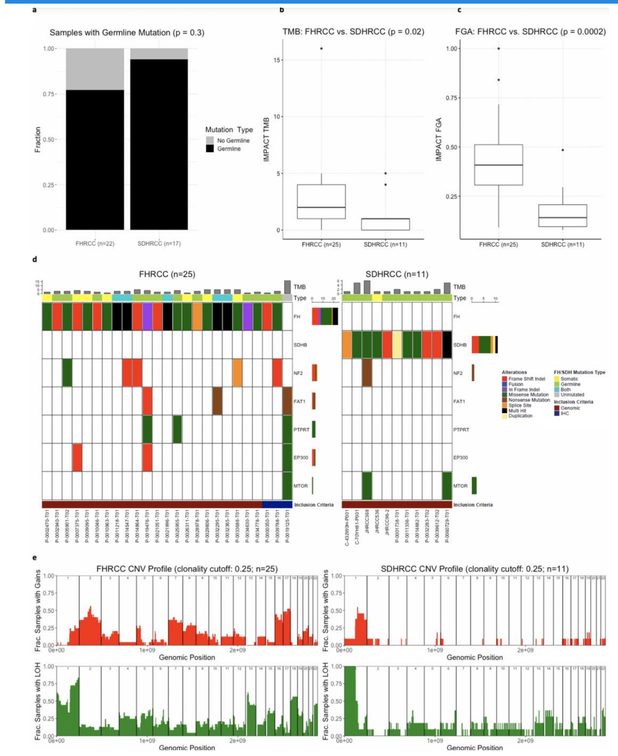

Figure 1: Genomic analysis and comparison of FHRCC and SDHRCC
(A) Comparison of incidence of germline mutations present in the FHRCC cohort vs. SDHRCC cohort. SDHRCC tumors are more likely to harbor pathogenic germline variants. Only 22 out of 25 FHRCC patients were consented for germline mutation analysis. (B) FHRCC tumors have a higher tumor mutation burden and (C) fraction of the genome altered (D) Oncoprint displaying recurrent (genes mutated in ≥3 patients) somatic mutations in FHRCC and SDHRCC tumors. Within the oncoprint, 2 out of 11 SDHRCC and 10 out of 25 FHRCC had recurrent mutations. However, 5/11 SDHRCC (45%) and 21/25 (84%) FHRCC when evaluating any occurrence of at least one somatic mutation. (E) Copy number profiles of FHRCC and SDHRCC. Top panels indicate the fraction of samples with gains. Bottom panel indicates the fraction of samples with LOH (including copy-neutral LOH). SDHRCC demonstrates universal LOH of chromosome arm 1p, whereas FHRCC often demonstrates LOH of 1q.

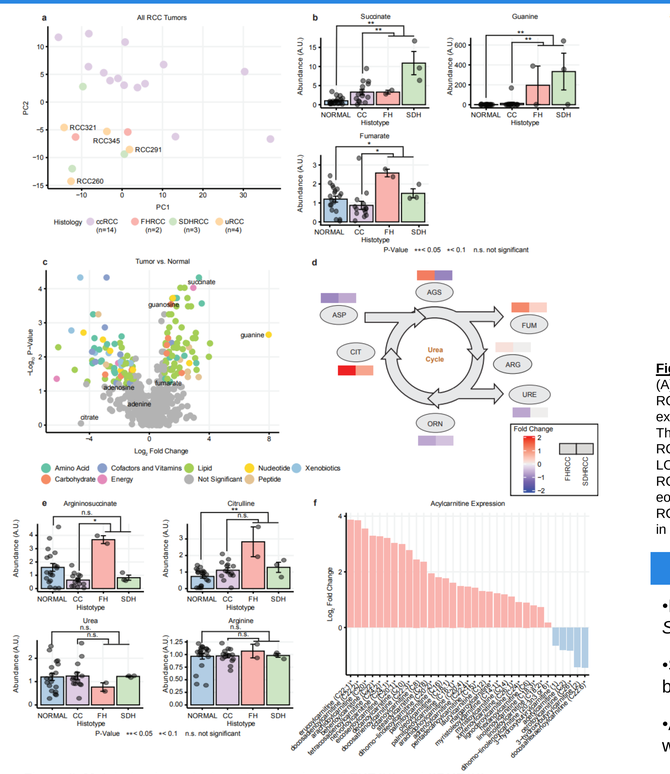

Figure 2. Metabolomic analysis and comparison of FHRCC and SDHRCC
(A): PCA plot of ccRCC (n=14), Unclassified tumors (n=4), SDHRCC (n=3) FHRCC tumors (n=2). The unclassified RCC tumors as well as the SDHRCC and FHRCC tumors cluster away from the clear cell tumors. (B) Barplot showing levels of succinate, fumarate, and guanine in normal kidney tissue, clear cell RCC, FHRCC, and SDHRCC tumors. (C) Volcano plot of metabolites that were elevated in SDHRCC/FHRCC tumors compared to normal tissue, including succinate and guanine. (D) Urea cycle metabolites (argininosuccinate and citrulline) are more elevated in FHRCC vs normal as compared to SDHRCC vs normal. AGS (argininosuccinate), Fum (fumarate), Arg (arginine), Ure (urea), Orn (ornithine), Cit (Citrulline), Asp (aspartate) (E) Barplot showing levels of argininosuccinate, urea, citrulline, and arginine in normal kidney tissue, clear cell, FHRCC, and SDHRCC tumors. Argininosuccinate and citrulline were uniquely elevated in FHRCC but urea and arginine were not. (F) Barplot depiction of various acylcarnitine expression levels in SDHRCC compared to FHRCC that demonstrate an elevation of acylcarnitine in SDHRCC tumors.

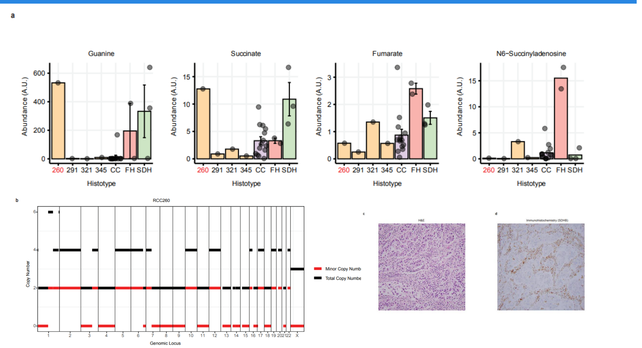

Figure 3. Metabolomic comparison of Unknown Sample with FHRCC and SDHRCC
(A) Barplot of levels of guanine, succinate, fumarate, and N6-Succinyladenosine in four unclassified RCC samples [260, 291, 321, 345], ccRCC, FHRCC, and SDHRCC tumors. Sample 260 demonstrated extreme elevation of guanine and succinate, without elevation of fumarate, and N6-succinyladenosine. This resembles the metabolic profile of other SDHRCC tumors. (B) Copy Number profile of sample RCC260. Black indicates total copy number and red indicates the minor copy number. Note copy-neutral LOH of chromosome arm 1p, the locus of *SDHB* gene (C) Representative H&E image of the tumor RCC260 showing infiltrating tubules and nests of neoplastic cells with high grade nuclear features, eosinophilic cytoplasm, and scattered cytoplasmic vacuoles. (D) SDHB immunohistochemical stain of RCC260 shows a loss of SDHB protein expression in the neoplastic cells, whereas the stain is retained in the stromal, endothelial, and inflammatory cells.

Conclusions

•In the germline analysis, 16/17 SDHRCC harbored a germline alteration in *SDHB*, whereas only 17/22 FHRCC had pathogenic germline *FH* variants.

•SDHRCC had a lower mutation burden (p = 0.02) and copy number alteration burden (p = 0.0002) than FHRCC.

•All SDHRCC presented with deletion of chromosome 1p (overlapping *SDHB*), whereas FHRCC demonstrated high but not ubiquitous loss of 1q (*FH* locus).

•Both SDHRCC and FHRCC demonstrated significant, idiopathic accumulation of the metabolite guanine.

•FHRCC tumors had elevated levels of urea cycle metabolites (argininosuccinate, citrulline, and fumarate), whereas SDHRCC had elevation of numerous acylcarnitines.

•These characteristic metabolic changes enabled the identification of a previously unrecognized SDH-deficient RCC.