SMARCB1 regulates the hypoxic stress response in sickle cell trait during the pathogenesis of renal medullary carcinoma.

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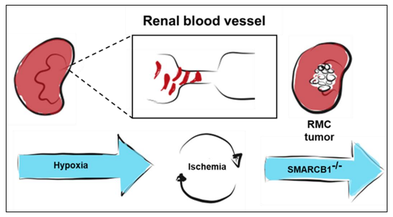
Introduction

- *SMARCB1* is one of the core subunits of the SWI/SNF complex, an ATP-dependent chromatin remodeling complex.
- Malignancies are characterized by simple genomes and lack of somatic events, suggesting that the dysregulation of the SWI/SNF machinery is sufficient to induce highly malignant states [1].
- Although SWI/SNF dysregulations are related to approximately 20% of human malignancies [2], lack of conditional genetic models of SMARCB1-deficient tumors has made it difficult to investigate the molecular bases and dependencies associated with *SMARCB1* loss due to the embryonic lethality associated with SWI/SNF disruption.
- Renal medullary carcinoma (RMC), is an aggressive renal tumor that afflicts primarily young individuals of African descent with **sickle cell trait** [3]. RMC is also characterized by the **complete loss of the SMARCB1 tumor suppressor** [4], positioning it as an ideal tumor model for studying the role of SWI/SNF dysregulation in tumorigenesis.
- **We hypothesize that sickling red blood cells are promoting a hypoxic microenvironment in the renal medulla that is selecting for the loss of the tumor suppressor SMARCB1.**



Main hypothesis: Renal medullary hypoxia promotes chronic hypoxia, creating a selective pressure for tumorigenic SMARCB1-deficient cells.

Technique/ Methods

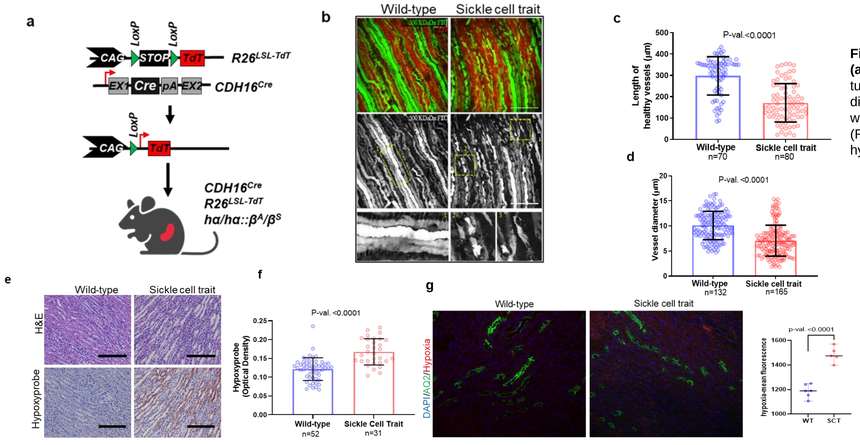


Figure 1. Renal ischemia is associated with chronic hypoxia in sickle cell trait mouse model. (a) Schematic of genetically engineered mouse model (GEMM) of SCT. **(b)** 3D image reconstruction of renal epithelia (RFP) and FITC-dextran (GFP) in adult mice (n=4-5) with kidney-specific *CDH16*^{Cre} and conditional *R26*$^{LSL\text{-}Tom}$ **(c, d)** Quantification of the diameter **(c)** and length **(d)** of the renal blood vessels (10 vessels/image, 3 locations/vessel). **(e)** IHC of mouse kidneys after injection with Hypoxyprobe. **(f)** Quantification of the optical density of horseradish peroxidase (HRP) staining for 20x images was done using ImageJ. Data are expressed as mean value ± SD, with *P* value calculated by student's *t* test. **(g)** Immunofluorescence (IF) analysis of hypoxyprobe (red) levels in wild-type mice (n=5) compared to sickle cell trait mice (n=5). Aquaporin 2 (AQ2, green) was used to localize renal medullary region.

Results

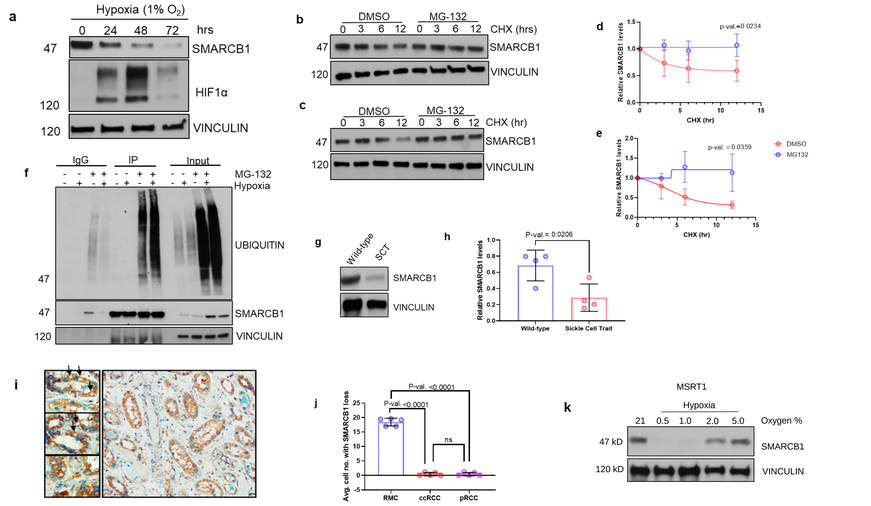


Figure 2. SMARCB1 is degraded via ubiquitin-proteasome mediated degradation.(a) Immunoblotting analysis of IMCD-3 cells after increasing exposure to hypoxia (1% oxygen). (b) Cycloheximide chase assay of IMCD-3 cells in 24 hours of normoxia and (c) 24 hours of hypoxia. (d-e) Quantification of cycloheximide (CHX) chase experiment with MG-132 protein rescue after 24 hours of growth in normoxia and hypoxia. (f) Immunoprecipitation (IP) analysis of SMARCB1 ubiquitination after 6 hours of hypoxia treatment. (g) Representative image of immunoblotting analysis of kidney lysates from wild-type mice and mice with SCT. (h) Quantification of immunoblotting analysis of SMARCB1 protein in mouse kidney lysates. (i) RMC adjacent kidneys with SMARCB1 loss characterized using IHC analysis. SMARCB1 is indicated in blue (Alkaline phosphatase) staining and eIF2-alpha is indicated in HRP 3,3'-Diaminobenzidine (DAB) staining. The arrows indicate SMARCB1 loss. (j) Quantification of SMARCB1 protein in IHC experiment comparing normal adjacent kidney tissue in different renal tumors. Five 40X images were taken per patient and quantified. The average of five images is shown per patient and represented as a single dot in the plot. P-values were calculated using Student's t-test in GraphPad. (k) SMARCB1 degradation after exposure to 0.5%, 1.0%, 2.0%, 5.0%, and 21% oxygen for 24 hours in MSRT1 tumor cells with ectopic overexpression of SMARCB1^{WT}.

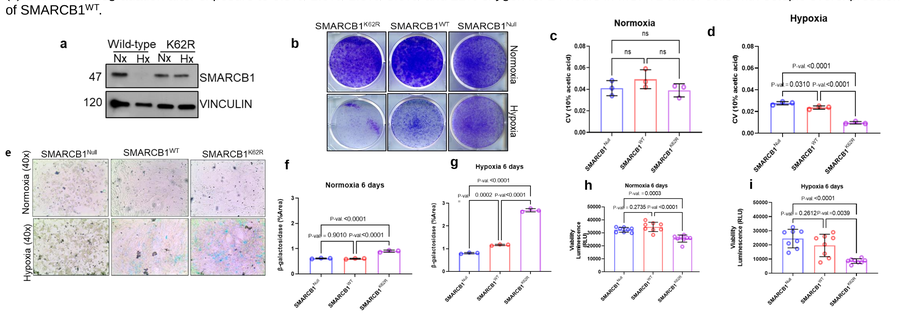


Figure 3. SMARCB1-deficient tumors are resistant to hypoxic stress, while SMARCB1-proficient tumors are sensitive to hypoxic stress. (a) Western blotting analysis of MSRT1 cells overexpressed with either SMARCB1WT or SMARCB1K62R. (b) Clonogenic assay of MSRT1 tumors overexpressed with either SMARCB1Null , SMARCB1WT , and SMARCB1K62R grown in either normoxia or hypoxia. (c-d) CV was dissolved in 10% acetic acid and quantified as shown above. (e) Representative images of β-galactosidase staining of MSRT1 cells overexpressed with SMARCB1Null, SMARCB1WT, and SMARCB1K62R. (f-g) Quantification of β-galactosidase using FIJI ImageJ software. (h-i) Luminescent (RLU) activity indicating cell viability in MSRT1 with SMARCB1Null, SMARCB1WT, and SMARCB1K62R after prolonged exposure to normoxia and hypoxia. P-values were calculated with Student's t-test in Prism GraphPad.

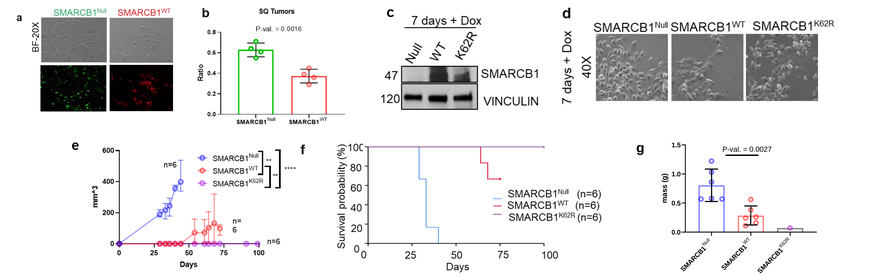


Figure 4. SMARCB1-deficient tumor cells are more resistant to hypoxia and expand under hypoxic conditions. (a) Microscopic images of SMARCB1Null mouse renal tumors (GFP) and SMARCB1WT cells (GFP/RFP) prior to subcutaneous injection into NSG mice. (b) Flow cytometry evaluation of SMARCB1Null and SMARCB1WT tumor cells that were mixed in a 1:1 ratio and injected subcutaneously into NSG mice. (c) Immunoblotting analysis of SMARCB1 protein expression in RMC219-tet-empty vector (SMARCB1Null) and RMC219-tet-inducible SMARCB1WT and RMC219-tet-inducible SMARCB1K62R after 7 days of treatment with 2 μg of Doxycycline (DOX). (d) Corresponding microscopic images of RMC219-tet-inducible cells showing that cells are healthy prior to subcutaneous injection in NSG mice. (e) Growth curve of subcutaneous tumors in NSG mice. (f) Kaplan-Meier survival curve of RMC219 subcutaneous tumors. For time-to-event event-free survival analysis, 200 mm3 was set as the endpoint. (g) Final tumor mass of RMC219 subcutaneous tumors.

Results (continued)

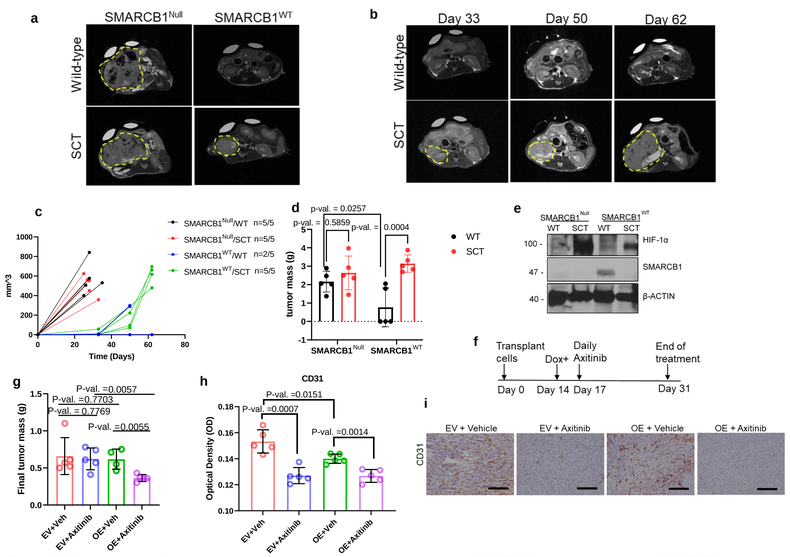
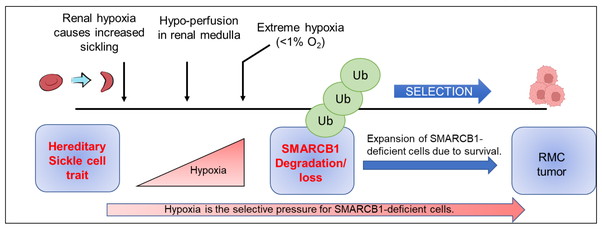


Figure 5. Sickle cell trait promotes tumor growth and aggressiveness in SMARCB1-deficient tumors. **SMARCB1-deficient tumors are resistant to anti-VEGF (vascular endothelial growth factor) inhibitors that cause induce hypoxia.** (a-b) Representative coronal T2-weighted MRI sections of tumor-bearing mice after orthotopic injection of MSRT1-SMARCB1^{Null} and MSRT1-SMARCB1^{WT} tumor cells into right kidney of wild-type mice (n=5) and mice with sickle cell trait (n=5). (c) Growth curve of primary kidney tumors. Tumor volume was quantified using coronal T2-weighted MRI sections at various timepoints after initial orthotopic injection of cells. ImageJ was used to calculate the tumor volume from T2-weighted MRI sections. (d) Final tumor mass of primary kidney tumors. (e) Immunoblotting analysis of SMARCB1 protein expression in orthotopic kidney tumors. (f) Axitinib (an anti-VEGF inhibitor) treatment schedule. (g) Final tumor mass of RMC219-tet-inducible cell lines that were injected subcutaneously (SQ) into NSG mice. Each dot represents one mouse. (h) and corresponding quantification of the optical density of CD31 IHC analysis. (i) Representative IHC image of CD31 staining of subcutaneous tumors treated with Axitinib. Data are expressed as mean value ± SD, with *P* value calculated by student's *t* test.

Future Direction

- Elucidate the E3 ubiquitin ligase involved in ubiquitinating SMARCB1.
- Investigate the role of the SWI/SNF complex in hypoxia response.



Schematic 1: The pathogenesis of renal medullary carcinoma.

Conclusion

- SMARCB1 is degraded via ubiquitin-mediated proteasome degradation pathway during extreme hypoxic stress.
- SMARCB1-deficient kidney cells are resistant to hypoxia stress and maintain viability and growth compared to SMARCB1-proficient counterparts.
- Impairing the degradation of SMARCB1 with lysine residue mutation K62 decreases cell viability and increases senescence under hypoxia stress, suggesting that SMARCB1-deficiency is selected for and required for survival under hypoxia stress.

Reference

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