A Model Linking Sickle Cell Hemoglobinopathies and SMARCB1 Loss in Renal Medullary Carcinoma
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Abstract
Renal medullary carcinoma (RMC) is a highly aggressive malignancy that predominantly afflicts young adults and adolescents with sickle hemoglobinopathies. It is characterized by complete loss of expression of the chromatin remodeler and tumor suppressor SMARCB1. Despite therapy, the outcomes of patients with RMC remain very poor, highlighting the need to understand the etiology of this cancer, and develop new diagnostic, preventive, and therapeutic strategies. A key knowledge gap in RMC biology is why sickle hemoglobinopathies predispose to the development of this cancer. We propose a model wherein the extreme conditions of hypoxia and hypertonicity of the renal medulla, combined with regional ischemia induced by red blood cell sickling, activate DNA repair mechanisms to drive deletions and translocations in SMARCB1, which is localized in a fragile region of chromosome 22. This mechanism would explain the linkage between RMC and sickle hemoglobinopathies, as well as the age dependence and predilection of RMC toward the right kidney.

Significance: This perspective proposes an integrated and testable model of renal medullary carcinoma pathogenesis. Insights provided by this model can additionally inform other malignancies arising from the renal medulla and/or associated with loss of the SMARCB1 tumor suppressor gene. Clin Cancer Res; 24(9): 2044-9. ©2018 AACR.

Introduction
Our evolving understanding of the molecular defects that occur in renal cell carcinomas (RCC) has provided valuable insights into the etiology of the different variants of this malignancy. As for other types of cancer, understanding the molecular events in the pathogenesis of RCC variants can reveal novel mechanisms driving oncogenesis and can in turn provide insights into other malignancies that share key disease characteristics.

Renal medullary carcinoma (RMC) is a very rare malignancy, comprising <0.5% of all RCCs (1). Originally described in 1995 (2), RMC is predominantly found in young (median age, 28 years) African Americans with sickle cell trait, as well as sickle cell disease and other sickle hemoglobinopathies (3, 4). RMC is refractory to targeted therapies used in other RCCs, and <5% of patients survive longer than 36 months (1, 4).

The pathogenesis of RMC remains obscure, as are the reasons why sickle cell hemoglobinopathy predisposes to risk of this disease (1). RMC is characterized (100% of cases) by complete loss of expression of SMARCB1 (1, 5), a component of the SWI/SNF chromatin remodeling complex (6). Inactivation of SMARCB1 in RMC occurs primarily due to inactivating translocations and/or deletions (7, 8). Herein, we propose a model that takes into account the extreme hypoxic and hypertonic environment of the renal medulla, where these tumors arise, and that incorporates a mechanism by which red blood cell (RBC) sickling, and the accompanying ischemia, can drive key events involved in SMARCB1 deletions/translocations in the pathogenesis of RMC.

All Sickle Hemoglobinopathies Are Associated with RMC
The vast majority (>85%) of RMC cases have been described in patients with sickle cell trait (3, 4), which has led to the suggestion that other genetic or environmental factors increase RMC risk in individuals with sickle cell trait (AS genotype), but not sickle cell disease (SS genotype). However, considering that the population genotype rates are 8.3% for AS and 0.15% for SS (9) among African Americans, and the risk for RMC is equal between AS and SS, we would expect the ratio of AS to SS among African Americans with RMC to be approximately 55:1. Indeed, a recent systematic review reported that 156 patients with RMC had AS and 4 had SS resulting in a ratio of 39:1 favoring AS over SS (3), which is not statistically different than the predicted allele frequency (P = 0.53). An additional line of evidence comes from a recent study by Anazoeze and colleagues reporting 2 cases of RCC among 3596 patients with sickle cell disease in Nigeria over a median follow-up of 10 years (10). Current best estimates in the United States over a 10-year period point to an RMC prevalence of 1/39,000 among individuals with sickle cell trait (3). Therefore, assuming there was no misclassification (Anazoeze and colleagues did not report whether loss of SMARCB1 expression was tested, ref. 10),
a prevalence of RMC in the range of 2/3,596 (1/1,798) over a 10-year period among individuals with sickle cell disease would again favor those with sickle cell trait. Although statistical confidence around these comparisons is hindered by the low number of reported cases, we can nevertheless conclude that there is no evidence that patients with sickle cell trait are at greater risk for RMC than patients with sickle cell disease.

The Renal Medulla Is a Hypoxic and Hypertonic Environment

In addition to sickle cell trait/disease, RMC has been described in other sickle hemoglobinopathies such as sickle beta thalassemia (SB) and sickle cell hemoglobin C (SC) disease (3, 4), but never in hemoglobinopathies not associated with RBC sickling. This raises the question: What is it about RBC sickling that predisposes to RMC?

Under normal conditions, the renal inner medulla is the most hypoxic and hypertonic tissue in the human body, with a partial pressure of oxygen (pO₂) in the range of 10 to 20 mm Hg and interstitial osmolarity of ~1,200 mOsm/L (Fig. 1; refs. 11, 12). Such extreme hypoxia and hypertonicity allow the kidneys to concentrate urine via the countercurrent multiplication process (11, 12). Within this environment, the RBCs of individuals with sickle hemoglobinopathies, including sickle cell trait, will sickle (13). As a result, microvascular occlusions occur, further increasing the hypoxic environment and causing concomitant acidosis, which will further exacerbate sickling and increase blood viscosity (14). Indeed, sickle cells are often found in the renal medulla of individuals with sickle cell trait despite the absence of sickling in the peripheral blood (13), and sickled RBCs are a frequent finding in RMC nephrectomy specimens from patients with sickle cell trait (2).

It is well established that the high interstitial NaCl concentration of the renal medulla produces numerous DNA double-strand breaks (DSB) in inner medulla cells (15, 16). DNA DSBs are prerequisites for translocations and deletions (17), and indeed,

Translational Relevance

Renal medullary carcinoma (RMC) is a highly aggressive malignancy that notably afflicts only patients with sickle hemoglobinopathies, is characterized by loss of expression of SMARCB1, and has a predilection toward the right kidney. We propose a testable model that connects these three defining features and demonstrates that RMC is not specific to sickle cell trait and should instead be thought of as a disease of all sickle hemoglobinopathies. Key translational insights provided by this model are as follows: (i) SMARCB1, a tumor suppressor altered in multiple malignancies, is located at a hotspot for deletion and translocations; (ii) the extreme hypoxia and hypertonicity within the renal medulla produce, and dysregulate the repair of, DNA double-strand breaks; and (iii) the right-sided laterality of RMC and of other tumors arising from the renal medulla such as collecting duct carcinoma may be due to differences in vascular anatomy.

Figure 1.
The renal cortex is isosmotic to plasma, whereas the renal medulla becomes progressively more hypertonic up to ~1,200 mOsm/L in the inner medulla. In addition, the medulla becomes progressively more hypoxic with a partial pressure of oxygen (pO₂) as low as 7 mm Hg in the inner medulla. These extreme conditions result in RBC sickling even in patients with sickle cell trait. Furthermore, the high NaCl concentration in the inner medulla produces DNA DSBs and simultaneously inactivates DNA repair pathways that would have otherwise repaired these lesions.
translocations and deletions are the most common mechanisms for inactivation of SMARCB1 in RMC (7, 8). Furthermore, chronic hypoxia leads to repression of the RAD51 and BRCA1 pathways associated with high-fidelity homologous recombination (HR) and induces a switch to nonhomologous end joining (NHEJ) repair pathways (17–19). Classic NHEJ (cNHEJ) is thought to mainly produce small insertions and deletions and is regulated by the p53-binding protein 1 (53BP1) pathway (17, 20, 21). Alternative NHEJ (aNHEJ) is regulated by poly (ADP-ribose) polymerase 1 (PARP-1) and, when HR is deficient, serves as a backup repair pathway that can rescue resected DSBs and repair broken replication forks at the cost of chromosomal stability (20–23). The lower fidelity of aNHEJ is more likely to produce deletions and translocations—the most frequent alterations observed in SMARCB1 in RMC.

Another striking feature of normal renal inner medulla cells is that the interstitial hypertonicity also suppresses the DNA damage response that would otherwise repair the NaCl-induced DSBs (15, 24). As long as these cells are exposed to high NaCl, DSB repair remains inhibited even if radiation is utilized to produce additional DSBs (15, 25). However, DNA damage repair is quickly reactivated when NaCl concentration is lowered to iso-osmolarity (15, 16, 25). Of note, RBC sickling produces microcirculatory ischemia that regionally reduces the interstitial osmolarity of the renal inner medulla (26, 27). This process begins during childhood for individuals with either sickle cell trait or disease and ultimately leads to complete inability to concentrate urine (isosthenuria; ref. 27). However, this phenomenon is not seen in hemoglobinopathies not associated with sickling, such as hemoglobin C disease (CC) and trait (AC; ref. 27).

We propose that the regional microinfarctions in the inner medulla due to RBC sickling result in perturbations of interstitial osmolality that reactivate DNA DSB repair. To repair these breaks under the hypoxic conditions of the inner medulla (which is further exacerbated by RBC sickling), cells must utilize the aNHEJ repair pathway instead of HR. We hypothesize that use of this low-fidelity repair pathway increases the risk of translocations and deletions, especially near chromosomal fragile sites.

Figure 2 illustrates each component of the proposed model of RMC pathogenesis. This model predicts that RMC precursor cells in the hypoxic renal medulla microenvironment will utilize the aNHEJ backup pathway (characterized by increased PARP-1 activity, ref. 20) more than the HR (characterized by increased RAD51 and BRCA1 activity, ref. 20). However, while cNHEJ suppresses translocations in mice, it has recently been found to produce oncogenic chromosomal translocations in humans (21, 28). Therefore, an alternative possibility would be that the cNHEJ (characterized by increased 53BP1 activity, ref. 20) is the main driver of the chromosomal translocations in RMC after the suppression of HR. Defining the role of PARP-1 compared with RAD51/BRCA1 and 53BP1 in the pathogenesis of RMC would be a next step in determining whether cNHEJ or aNHEJ acts as the driver for the SMARCB1 deletions and translocations that characterize these tumors.

SMARCB1 Is Located in a Fragile Chromosome Region

SMARCB1 is located at 22q11.2, a known hotspot for de novo deletions and translocations. This site harbors a series of 8 low-copy repeats (LCR) interspersed throughout that region (Fig. 3;
Hagen established that the right renal artery is longer than the left, while viscosity from RBC sickling in the medullary vasa recta. It is well pronounced venous stasis produced by compression of the left renal vein between the aorta and the superior mesenteric artery (34). It is also a region known for cancer-associated translocations. One of the most well-characterized translocations, and a hallmark of chronic myeloid leukemia, is the fusion between the breakpoint cluster region and the proto-oncogene ABL tyrosine-protein kinase (BCR-ABL1) due to a reciprocal t(9;22)(q34;q11) translocation (32). The BCR gene is located in close proximity to SMARCB1 in the 22q11.2 region (Fig. 3). As shown in the model for RMC pathogenesis (Fig. 2), we hypothesize that the proximity of SMARCB1 to this vulnerable region of chromosome 22, combined with age- and sickling-induced activation of low-fidelity aNHEJ repair, facilitates deletion and translocation of SMARCB1 and drives development of this disease.

### Why RMC Develops More Frequently in the Right Kidney

RMC tends to occur more frequently in the right (~70% of cases) than the left kidney (3, 4). This can appear counterintuitive because the left kidney is slightly larger than the right (33), and hematuria in sickle cell trait patients arises from the left kidney 4 times more frequently than the right (34). However, hematuria in sickle cell trait patients is thought to be due to the more pronounced venous stasis produced by compression of the left renal vein between the aorta and the superior mesenteric artery (34).

The mechanism we propose for RMC pathogenesis postulates that the predisposing condition is not venous stasis, but rather regional ischemia due to reduced blood flow and increased viscosity from RBC sickling in the medullary vasa recta. It is well established that the right renal artery is longer than the left, while both arteries have similar diameters (35). The Hagen–Poiseuille equation is a commonly used simplified equation to describe blood flow through nontortuous and straight cylindrical vessels of circular cross-section (36):

$$\Delta P = \frac{8 \eta Q L}{\pi r^4}$$

where $\Delta P$ is the pressure difference across a circuit, $Q$ is the flow rate, $\eta$ is the blood viscosity, $L$ is the vessel length, and $r$ is the vessel radius. Based on this equation, assuming equal radius and pressure differences, the flow rate will be inversely proportional to the length of the vessel. Thus, the longer length of the right renal artery will result in reduced blood flow in the right medulla compared with the left, further exacerbating regional microinfarctions that predispose to RMC. This has also been postulated to be the cause of the much higher frequency of cocaine-induced renal infarctions in the right kidney compared with the left kidney (37).

### Insights into the Origins of Other Malignancies

The proposed model can inform other malignancies that share key characteristics with RMC. SMARCB1 inactivation by structural changes as opposed to point mutations is also found to be the case in at least two other malignancies known to be driven by SMARCB1 loss: malignant rhabdoid tumors (MRT) and epithelioid sarcomas (38, 39). Collecting duct carcinoma (CDC) is another highly aggressive malignancy that arises from the renal inner medulla and demonstrates a predilection for the right kidney (40). Similarly to RMC, blood supply differences between the two kidneys may play a role in the pathogenesis of CDC.

Patients with sickle hemoglobinopathies may also be predisposed to a very rare RCC variant characterized by the fusion of anaplastic lymphoma kinase (ALK) with vimentin (VCL; ref. 41). All three of the VCL–ALK RCC cases that have thus far been described in the literature arose from the renal medulla (two were right-sided and one was left-sided) of children with sickle cell trait and demonstrated intact SMARCB1 expression (41–43). Our model predicts that the renal inner medulla of individuals with sickle hemoglobinopathies will be more susceptible to chromosomal structural alterations, such as the VCL–ALK translocation, due to the activation of error-prone repair pathways. We also anticipate...
that, as the number of reported VCL–ALK RCC cases grows, these malignancies will be found to share with RMC the same predilection toward the right kidney. Unclassified RCC with medullary phenotype (RCCU-MP) is a very rare RMC variant characterized by the loss of SMARCB1 by immunohistochemistry in patients without sickle hemoglobinopathies (44). This entity is considerably more rare than typical RMC, occurs in older patients (median age of 39 years), and does not appear to favor the right kidney based on the small number of cases reported thus far (44). It remains to be determined whether RCCU-MP occurs later in life due to the attenuated onset of regional ischemias in the renal inner medulla of affected individuals or due to a completely different etiopathogenic mechanism. Toward this goal, it will be important to delineate whether SMARCB1 is inactivated in RCCU-MP by deletions and translocations or by other mechanisms of gene silencing.

Conclusions and Future Directions

We have proposed a testable model that accounts for the unique features associated with RMC pathogenesis. Each of the components will require rigorous evaluation and refinement as our knowledge of RMC, and of the role of SMARCB1 defects in these and other tumors, increases. In addition, it will be important to elucidate how environmental and interindividual genetic/epigenetic factors impact the pathways proposed in this model to increase or attenuate the risk of RMC. For example, different haplotype distributions within AS populations could produce phenotypic differences that could affect the frequency and severity of regional ischemia in the renal medulla and thus modulate the risk for RMC. In addition, sex differences in the frequency of microvascular occlusions due to sickling (45, 46) may explain why men are twice as likely to be affected by RMC than women (3, 4). It is anticipated that investigations aimed at testing various aspects of this model will also pave the way toward the earlier diagnosis, better management, and prevention of RMC, and perhaps other tumors associated with defects in SMARCB1 and/or arising from the renal medulla, such as MRTs and CDCs.

Disclosure of Potential Conflicts of Interest

N. M. Tannir is a consultant/advisory board member for Pfizer, Bristol-Myers Squibb, Oncoreta, Eisai Medical Research, Novartis Pharmaceutical Corp., and Nektar Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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