BACKGROUND
Polybromo-1 (PBRM1), a targeting subunit of the SWI/SNF chromatin remodeling complex, is mutated at a rate of ~40% in clear cell Renal Cell Carcinoma (ccRCC), second only to VHL. Whether its mutation is correlated with tumor stage is controversial. As other components of the SWI/SNF complex were also reported to be mutated in ccRCC, we aim to examine the protein expression patterns of PBRM1, ARID1A, BRG1, and BRM in ccRCC, and to investigate possible association between their loss of expression and tumor stage, as well as survival. We also included a histone modifier, SETD2, which is recently discovered to be mutated in ~15% of ccRCC.

DESIGN
160 ccRCC, with 40 per tumor stage (1-4), diagnosed at Fox Chase Cancer Center, were used to generate tissue microarray (TMA). Four 1 mm² foci (dots) from different regions of each tumor were selected to represent tumor heterogeneity. Standard immunohistochemistry (IHC) was performed on the TMA slides at Thomas Jefferson University Hospital, and was scored by two pathologists (W. J. and T. P.), with the clinical and staging details blinded. Loss of expression was defined as 0-5% of staining within tumor nuclei in any 1 mm² focus. Discrepancies in scores were resolved by re-review by the two pathologists, and consensus was reached in such cases. Clinical data were also collected and overall survival (OS) and recurrence-free survival (RFS) were calculated. Appropriate statistical analyses were performed (see details in RESULTS).

RESULTS
48/160 (31%), 81/160 (51%), 23/160 (14%), 24/160 (15%), and 61/160 (38%) tumors show loss of PBRM1, ARID1A, SETD2, BRG1, and BRM expressions, respectively. For individual protein, very high percentage of tumors show loss of expression in only a fraction of the four foci, displaying heterogeneity. Striking co-loss patterns among different proteins are also observed.

“Truncal Loss” for individual protein was defined as the most ubiquitous loss of expression in the foci from the same tumor, and “The Only Truncal Loss” if there was no co-loss with other proteins. “Truncal Loss”, like truncal genetic mutation, is most likely an early event in tumorigenesis, therefore, for each individual tumor, phylogenetic tree of protein expression can be constructed (see examples in Figure 1).

Univariate analyses for overall survival (OS) and recurrence-free survival (RFS) were performed using the Cox proportional hazards (PH) model. The following variables showed significant association with RFS. SETD2.Any loss (p=0.018, RR=0.50), BRM.Any loss (p=0.015, RR=1.84); BRG1.Trunctal loss (p=0.033, RR=1.85), and BRM.Trunctal loss (p=0.00028, RR=1.79). For OS, SETD2. Any loss (p=0.013, RR=0.50), PBRM1.Trunctal loss (p=0.004, RR=0.6); BRG1.Trunctal loss (p=0.017, RR=1.97), and BRM.Trunctal loss (p=0.002, RR=1.60). If RR > 1 then it suggests that the risk of death increases with the value of the corresponding variable (as it goes from 0 to 1 or from 0 to 2). A similar interpretation holds when RR < 1. Variables with p <= 0.1 were used in multivariable analyses using the Cox PH model (Table 2 and 3).

CONCLUSIONS
1. Using phylogenetic tree construction and “Truncal Loss” analysis, we identified statistically significant associations between loss of protein expression and tumor stage in SWI/SNF complex components in ccRCC, while the commonly used “Any Loss in Tumor” analysis either failed to do so or did so weakly.

2. We also made the novel findings that “Truncal Loss” of BRG1 or “Any Loss in Tumor” of BRM is significantly associated with better prognosis for OS in multivariate analysis. PBRM1’s association with clinical outcome has been controversial, and our result is consistent with the view that PBRM1 loss is a bad prognosis factor. Consistent with previous reports, “Any Loss in Tumor” of ARID1A or SETD2 is significantly associated with worse prognosis for OS.

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