LOSS OF EXPRESSION OF SWI/SNF CHROMATIN REMODELING COMPLEX COMPONENTS AND SETD2 ARE HETEROGENEOUS, WIDESPREAD, AND CO-OCCUR IN CLEAR CELL RENAL CELL CARCINOMA

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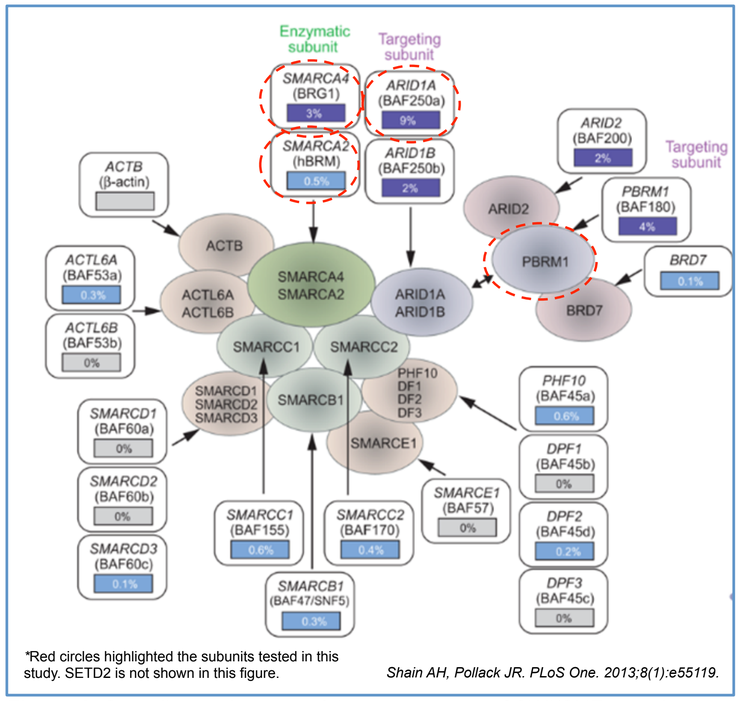
BACKGROUND

The subunits of the SWI/SNF chromatin remodeling complex have one of the highest mutation rate in human cancers (see Figure 1). Polybromo-1 (PBRM1), a subunit of the SWI/SNF complex, is the second most mutated gene (40%) in clear cell Renal Cell Carcinoma (ccRCC). ARID1A, another subunit that competes with PBRM1 for binding to the complex, was infrequently mutated. The protein expressions of BRG1 and BRM (two catalytic subunits of the complex), and SETD2, a histone modifier (10-15% mutation rate), are not known in ccRCC. In this study we examined their protein expressions by immunohistochemistry (IHC) in tissue microarrays (TMAs), and investigated the prevalence of loss of expression, heterogeneity, and the potential co-loss patterns of these proteins.



*Red circles highlighted the subunits tested in this study. SETD2 is not shown in this figure.

Shain AH, Pollack JR. PLoS One. 2013;8(1):e55119.

Figure 1. Overall mutation rate of the components of SWI/SNF complex in human cancers.

DESIGN

160 ccRCC tumors diagnosed at Fox Chase Cancer Center were used to generate tissue microarray (TMA), with 40 tumors from each pathologic stage (1-4). For each tumor, 4 different foci (1 mm^2 each) representative of morphologic heterogeneity were selected. IHC studies using PBRM1, ARID1A, SETD2, BRG1, and BRM antibodies were performed using standard protocol, and the slides were reviewed independently 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 mm^2 focus. Discrepancies in scores were resolved by pathologic re-review by the two pathologists, and consensus was reached. Fisher's exact test was used for statistical analysis, and a p value <0.05 was considered statistically significant.

RESULTS

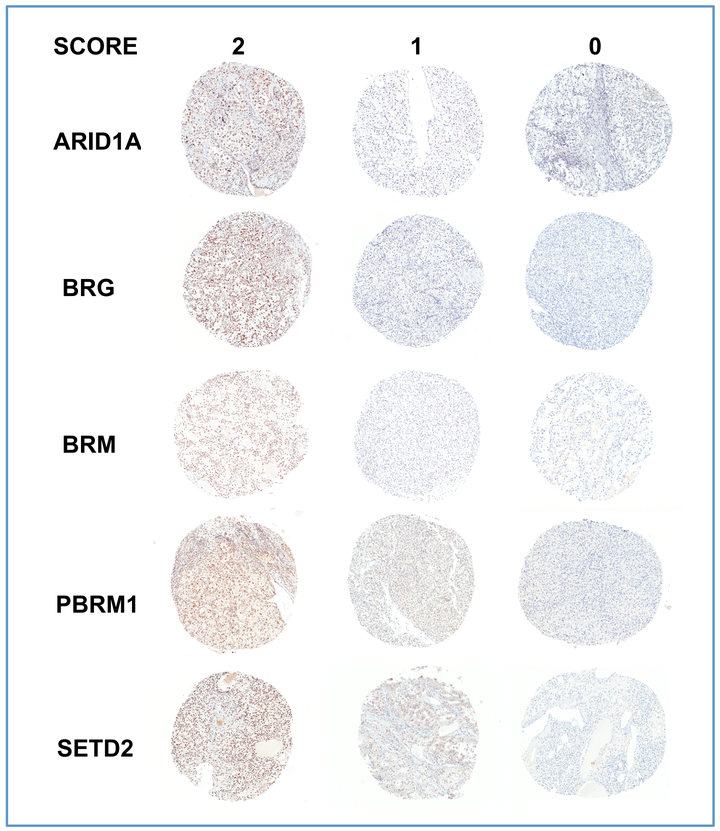


Figure 2. Examples of immunostains for components of chromatin remodeling complex pathway in ccRCC.

- Wide-spread loss of expression was identified in the proteins analyzed (see Table 1).
- Very high percentage of tumors displays heterogeneity in loss of expression, since frequently < 4 foci from the same tumor had loss of a certain protein.

Table 1. Wide-spread loss of expression of the proteins tested by IHC.

PROTEIN	LOSS OF EXPRESSION
BRG1	15% (24/160)
BRM	38% (61/160)
PBRM1	31% (49/160)
ARID1A	51% (81/160)
SETD2	14% (23/160)

- Strikingly, novel co-loss patterns were identified among these proteins.
  - ❖ ARID1A loss almost always accompanied PBRM1 loss.
  - ❖ BRM loss almost always accompanied BRG1 loss, PBRM1 loss or Arid1A loss
  - ❖ SETD2 loss almost always occurred with the loss of one or more of the other four proteins.
- The 2-sided p values for the above associations (Fisher's exact tests) were all <0.0001, and the strong positive associations were true when tumor foci or tumors were compared.

CONCLUSIONS

The importance of the SWI/SNF complex and related pathways are highlighted by the wide-spread loss of protein expression in its components. The loss of protein expression of BRG1 and especially BRM in ccRCC are novel findings. As mutational analysis may be expensive and rarely covers hundreds of samples, routine IHC method provides a relatively quick and reliable method to assess tumor heterogeneity in large number of cases. It is also striking to find the co-losses of SWI/SNF components in ccRCC. As genetic interactions play powerful roles in tumorigenesis, these findings will provide insights and guidance to basic research and therapeutics.

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ACKNOWLEDGEMENT

This study was partially supported by an NIH RO1 grant awarded to HFY.