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Mutation landscape of AML patients with compromised ASXL1-Cohesin interactions

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Background

ASXL1 and the cohesin complex (*STAG2*, *RAD21*, *SMC1A*, and *SMC3*) are commonly mutated chromatin regulators with significant clinical implications in AML. The ASXL1-cohesin interactome regulates gene expression through chromatin accessibility via *ASXL1*'s cohesin binding motif (CBM) (See figures 1; 4). *ASXL1* variants are most commonly located in the ASXM1 domain and onwards, and characteristically lead to loss of the PHD domain (see figures 1; 4). Gain-of-functions in truncated *ASXL1* are suggested to increase catalytic activity of *BAP1,* which binds the ASXH domain at AA 351 (See figure 4), and gain an interaction with *BRD4,* which binds between the ASXN and ASXH domains, to drive H3K4Me3 and H2AK119Ub.

Methods

2463 suspected AML patient bone marrow, peripheral blood, or FFPE tissue samples were evaluated using an all exon amplicon-based 27 gene NGS panel. Patients with a VAF ≤10% in *ASXL1* were excluded to avoid reporting artifacts, particularly in variant c. 1934dup. Statistics were performed using Fisher's exact test.

Results

Mutations in *STAG2*-mutated patients were enriched for sAML, as evidenced by the higher number of mutations in *ASXL1*, *SRSF2, and BCOR* (associated with sAML) compared to *NPM1, DNMT3A, and PTPN11* (pAML) (see figure 3). *STAG2* mutations were found in 173 samples representing 93.5% of cohesin mutations. Of all *ASXL1* mutations (VAF 10.1– 54.5%; median 32.2%) 4.0% occurred in the CBM. While 23.5% of samples with mutations outside the *ASXL1* CBM had concomitant mutations in *STAG2*, none of the 18 samples with CBM mutations (VAF 11.3 – 51.7%; median 42.5%) had any mutations in cohesion members (P = 0.0174). The proportion of *BCOR* (27.8% vs 9.2%; P = 0.024) and *CEBPA* (27.8% vs 8.2%; p = 0.016) mutated patients in the CBM+ group was significantly higher than the CBM- group. *JAK2* (16.7% vs 5.4%), *KRAS* (22.2% vs 13.6%), *EZH2* (22.2% vs 13.6%), and *RUNX1* (38.9% vs 27.7%) mutations were also higher, though not significantly in this group (see figure 3). Mutations throughout all of *ASXL1*, the 13 amino acids after the CBM, and hotspot variants all had *STAG2* mutations at a frequency of 20.9-44.4%, further suggesting mutual exclusivity.

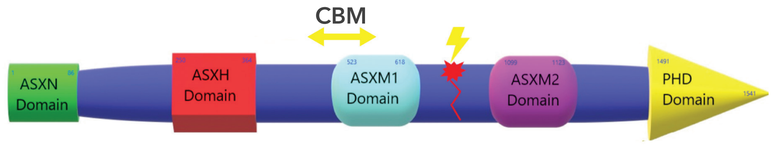

Figure 1: Characterization of ASXL1 variants

ASXL1 variants largely consist of nonsense and frameshift mutations, which lead to loss of the PHD domain, and usually the ASXM2 domain. These mutations lead to changes in ASXL1's function, and also lead to a gain-of-function through a novel interaction with BRD4. ASXL1 has been shown to complex with BAP1 (at AA 351) and cohesin (somewhere between AA 401 – 587). This complex regulates the 3D architecture of chromatin, and in the case of myeloid malignancies, favors the transcription of hematopoietic genes (vs. ones that favor differentiation) via open and closed chromatin configurations. Cohesin members are frequently co-mutated with ASXL1 (On average ~22.5 – 25.7% of the time), with some variants (e.g. R693*) having co-mutation frequencies as high as 44%. Our data shows that mutations in ASXL1's cohesin binding motif (CBM), a 186 AA stretch, and cohesin members are mutually exclusive events. In comparison, even the 13 AA's after the CBM had cohesin mutations (or in this case, STAG2 mutations) at a similar frequency that you seen on average. Collectively, this suggests that mutations in cohesin members are disfavored events when this ASXL1-Cohesin interaction is impaired.

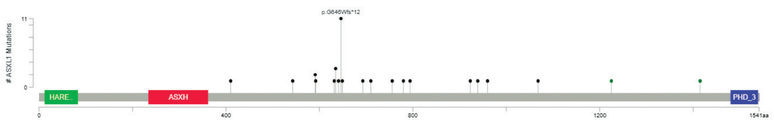

Figure 2: Distribution of ASXL1 variants identified in this study

The height of each lollipop represents the number of cases with this variant. Diagram produced by cbioportal.

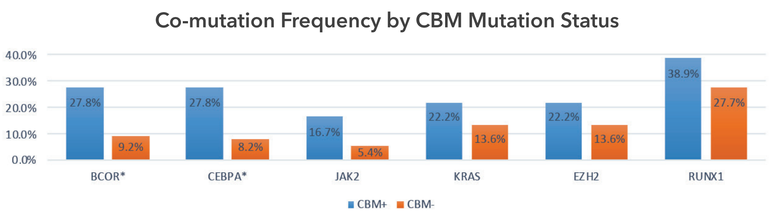

Figure 3: CBM-mutated and non-mutated patients display different co-mutation frequencies, reflecting different players in the biology of chromatin accessibility in this subset of patients

BCOR and CEBPA had significantly different co-mutation frequencies in CBM+ vs CBM- patients (p=0.024 & 0.016, respectively). Of all the other co-mutants, *JAK2, KRAS, EZH2, and RUNX1* had notable differences in their mutation frequencies. However, these frequencies could be attributed to either randomness or a weak signal attributed to our smaller sample size. Further studies may shine light on this phenomenon.

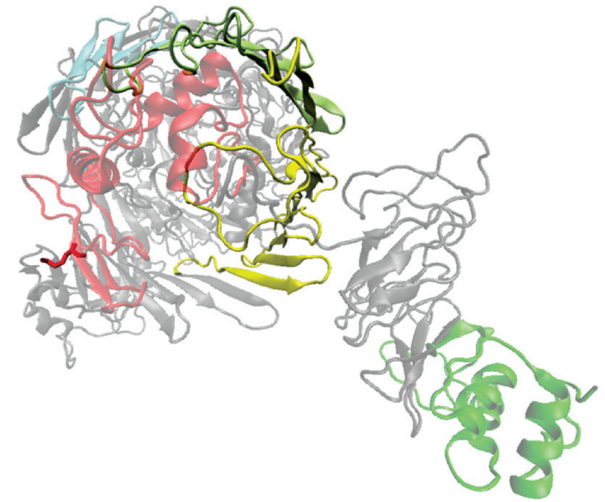

Figure 4: 3D Protein structure of an ASXL1 molecule lacking its PHD Domain

A ribbon structure of *ASXL1* with its ASXN (green), ASXH (red), and ASXM1 (olive green and teal) domains color-coded along with the CBM that cohesin ring complexes with. This binding motif is situated on the anterior aspect of the *ASXL1* protein in the beginning of the ASXM1 domain (in front of the olive green and teal beta sheets depicted in the structure) and possibly reflects that *ASXL1* binds to the inner portion of the cohesin ring structure. However, further studies will be needed to confirm this. The 3D structure was produced in VMD while the PDB was produced by the Zhang Laboratory at University of Michigan using threading (LOMETS).

Key Points

- *ASXL1* & cohesin are major chromatin regulators selective for sAML with a well established protein-protein interaction (See figure 4)
- Mutations in *STAG2 and ASXL1*'s CBM were mutually exclusive events and harbored different co-mutation frequencies
- In **compromised *ASXL1* CBM cases, *BCOR* and *CEBPA* transcriptional** regulators were significantly more mutated, but in cases of *ASXL1* mutation outside the CBM, cohesin mutations were preferred
- These results suggest alternative chromatin accessibility mechanisms driving leukemogenesis that are evolutionarily favored