



HL

AACR 2017 | Poster 2448/5 – Deep exome sequencing reveals recurrent somatic mutations in Classical Hodgkin Lymphoma

 Terri Penfold | Apr 07, 2017

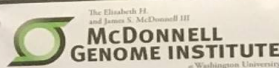
On Monday 3rd April during this year's American Association for Cancer Research (AACR) annual meeting, a poster (2448 / 5) by Felicia Gomez, from Washington University, St. Louis, MO, *et al.* titled "Deep exome sequencing reveals recurrent somatic mutations in Hodgkin's Lymphoma" was presented.

The group hypothesized that recurrent somatic mutations can be recognized in Hodgkin Reed-Sternberg (HRS) cells, taken from biopsies of bulk Hodgkin Lymphoma (HL), using ultra-deep exome sequencing.

Key Highlights:

- In total, using 63 samples from 31 patients, 7.04×10^{12} bases were sequenced; 1.10×10^{11} average bases per sample
- Mean depth of coverage achieved in both tumor and normal samples is $>1,000x$; tumor coverage range = 50–17,498; normal coverage range = 50–22,837
- Mutations identified in 31/32 patients
- The most currently mutated genes = *SOCS1* (11 mutations), and *TNFAIP3* and *IGLL5* (7 mutations each)
- Mutations in *TNFAIP3* were frameshift deletions, nonsense, and splice site mutations (all potentially truncating)
- Identified other mutations known to be mutated in cHL = *B2M*, *STAT6*, *ITPK*, *GNA13*, *BCL7A*, and *CREBBP*

The poster concluded by stating that recurrent mutations in cHL can be identified using ultra-deep sequencing of bulk tumor tissue. The group identified previously unreported mutations which require validation, as well as known mutations. This data improves our understanding of the pathogenesis of cHL.



McDONNELL GENOME INSTITUTE
Washington University

Deep Exome Sequencing Reveals Recurrent Somatic Mutations in Hodgkin's Lymphoma

The Elizabeth H. and Ronald S. McDonnell III

Felicia Gomez¹, Kilannin Krysiak¹, Zachary Skidmore¹, Cody Ramirez¹, Nancy L. Bartlett¹, Amanda F. Cashen¹, Friederike Kreisel¹, Eric Duncavage¹, Malachi Griffith¹, Todd A. Fehniger¹, Obi L. Griffith¹

¹ Washington University School of Medicine in St. Louis, St. Louis, MO

Introduction

High-throughput sequencing provides important insights into cancer pathogenesis and has changed the ways in which therapies are conceptualized. The application of genomic technologies to cancers that are characterized by rare cell populations continues to be a challenge. When DNA is extracted from bulk tumor samples a mixture of malignant and non-malignant cells are present. This mixture is particularly problematic in cancers defined by rare cell populations because it further complicates the task of identifying true somatic mutations. Classic Hodgkin lymphoma (cHL) exemplifies this challenge. The pathologic hallmarks of this disease are malignant Hodgkin and Reed-Sternberg (HRS) cells. HRS cells have a particularly low abundance within an affected lymph node, generally comprising $1-30\%$ of the tumor microenvironment. Because of the rarity of HRS cells, a limited number of studies have described genomic events that characterize this cancer. These studies have examined cHL cell lines, germline DNA and, recently, a small number of flow-sorted primary HRS-cells.¹ Here, we hypothesized that recurrent somatic mutations can be identified in HRS cells using bulk lymphoma biopsy samples and ultra-deep whole exome sequencing.

Methods

Samples

Fresh frozen tumor (lymph node)/normal (skin) pairs from 31 patients with cHL (32 samples) were examined. These patients are described in Table 1.

Sequencing

To identify genomic variants deep exome sequencing was performed. Three KAPA libraries were constructed per sample. Libraries were pooled, size selected, and captured using an OTT v1000 capture reagent. Pools were sequenced across eight lanes of an Illumina instrument.

Sequence alignment was performed using the Genome Mapping System². Briefly, paired-end reads were aligned to GRCh38 using the SpeedSeq pipeline.³ SNVs were identified using free variant callers⁴⁻⁶. Indels were identified using free variant callers⁴⁻⁶. All different variant callers⁴⁻⁶. All SNVs and indels were annotated using Ensembl build 79.

Data Analysis

Variants were filtered using the following criteria: min. 50x coverage, min. 0.5% tumor VAF, max. 5% normal VAF, and a min. of 5 variant supporting reads in the tumor. We excluded non-coding sites (Figure 1). We removed sites with an adjusted allele frequency $\geq 0.1\%$ in ExAC⁷. All variants were further filtered against all pooled matched normals. SNVs were removed if they had > 4 variant reads and $> 1\%$ VAF in ≥ 2 normal samples. INDELS were removed if they had > 3 variant reads and $> 1\%$ VAF in ≥ 3 normal samples. SNVs and INDELS with ≥ 3 variant supporting reads with a VAF of 0.5% in ≥ 2 libraries were included. Remaining sites were visually inspected using IGV⁸. Following all filters and manual review 1052 sites remained. Using these sites, we required all genes to be mutated ≥ 2 times the dataset. This final filter left 252 sites in 100 genes (Table 2).

	Remaining	Filtered	Total Filtered
Total Variants	1226791	0	0
Include Chromosome 1-22, X, and MT	1192606	34185	34185
Exclude only Coding Sites	7630	1184976	1219311
Exclude sites with normal VAF $\geq 5\%$	7563	67	1219228
Exclude sites with tumor VAF $\leq 0.5\%$	7543	20	1219248
Exclude sites with ≤ 5 variant supporting reads in the tumor	6862	681	1219929
Exclude sites with $\leq 50x$ in normal	6596	266	1220195
Exclude sites with $\leq 50x$ in tumor	6591	5	1220200
Exclude sites with $\leq 0.3\%$ Allele Frequency in ExAC	5385	1206	1221408
Exclude Sites that Failed Normal Filter	4930	453	1221861
Exclude Sites that Failed Per Library Filter	4788	142	1222003
Sites Added From Re-Calling Variants	4822	34	1221969
Sites the Passed Manual Review (1089 Sites Reviewed)	1052	37	1221932
Genes Mutated ≥ 2 times	752	800	1222732

Table 2. Filtering Strategies. Following all filters and manual review 252 sites in 100 genes remain. One patient sample was not manually reviewed and is excluded from further analysis.




Figure 2. Mutation Landscape. Top 6 mutated genes are shown (38 sites mutated in ≥ 4 samples). Columns are ordered by mutation presence in the shown for patients who have multiple mutations in one gene. One mutation type is shown for patients who have multiple mutations in one gene. The depicted type legend. The histogram describes the frequency of mutations in the adjacent gene.

Results

A total of 7.04×10^{12} bases were sequenced across all 63 samples (31 patients) with an average of 1.10×10^{11} bases per sample. Following the initial filters (minimum coverage, tumor VAF, minimum variant supporting reads, and coding site annotation) the mean depth of coverage achieved in both the tumor and normal samples is $> 1000x$ (tumor coverage range = 50 - 17,498; normal coverage range = 50 - 22,837; Figure 1).

We report preliminary data on mutations identified in 31 of the 32 patients. *TNFAIP3* (7 mutations), *SOCS1* (11 mutations) and *IGLL5* (7 mutations) are the most recurrently mutated genes. We identified mutations other genes that are known to be mutated in cHL including *B2M*, *STAT6*, *ITPK*, *GNAI3*, *BCL2A*, and *CREBBP*.⁹⁻¹⁴ Several of the mutations identified in *SOCS1*, *IGLL5*, and *TNFAIP3* have been previously identified and are present in COSMIC¹⁵ (Figures 4). *TNFAIP3* is a tumor suppressor gene that is thought to promote cHL through modulating A20 protein expression. Mutations in *TNFAIP3* are associated with A20 inactivation. All mutations identified here are potentially truncating (i.e. frame shift deletions, nonsense, and splice site mutations). These mutations may effect the A20 expression. Further analysis is necessary to predict *TNFAIP3* mutation effects. *IGLL5* has been identified in DLBCL¹⁶, but is not well described in cHL. Further work is needed to understand how the mutations identified here relate to cHL pathogenesis.

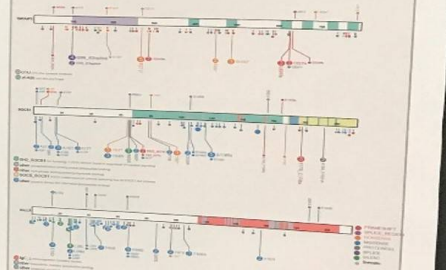


Figure 4. Observed mutations in *TNFAIP3*(7), *SOCS1*(11), and *IGLL5* (7) on top COSMIC (Catalogue of Somatic Mutations in Cancer) found in hematopoietic tumors; IGLL5:112 mutations; SOCS1:90 mutations. Amino acid positions are indicated by numbers within the peptide representation.

Conclusions and Future Directions

These data indicate that ultra deep sequencing of bulk tumor tissue can detect recurrent somatic mutations in cHL. We have identified previously reported cHL mutations and potential novel mutations that require validation, thereby improving our understanding of cHL pathogenesis. These results suggest that ultra deep exome sequencing is a useful discovery tool for rare tumor cell populations in bulk samples.

Figure 3. Coverage of all sites after initial filters. Coverage is comparable between tumors and normals.

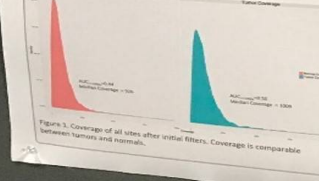


Figure 3. Coverage and Variant Allele Frequency (VAF) are shown. These data suggest coverage is sufficient for low VAF sites. Data indicate that sample purity is $\geq 10\%$.

Table 1. Patient Description

Characteristic	Value
Total Patient Number	31
Female	81.6%
Male	48.4%
Age (Median)	37
Age Range	18-69
History	
Nodular Sclerosis	87.74%
Mixed Cellularity	9.35%
Other	6.45%
NA - no information	22.58%
Stage	
II	0.0%
III	45.2%
IV	22.6%
Unknown	25.8%
Early Stage Category	
Favorable	6.5%
Unfavorable	35.5%
Unknown	3.2%
Advanced Stage	48.4%
Bulky Disease	
No	67.7%
Yes	29.0%
Unknown	3.2%
EBV Status	
No	41.9%
Yes	32.9%
NA - no information	45.2%
Symptoms	
Yes	48.4%
No	48.4%
NA - no information	3.2%

Reference:

- Gomez F. et al. Deep exome sequencing reveals recurrent somatic mutations in Hodgkin's Lymphoma [Poster]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; 2017. Poster nr [2448 / 5].

© 2018 Scientific Education Support Ltd. This PDF is provided for personal use only. For wider or commercial use, please seek permission from secretariat@scientificeducationssupport.com and attribute the source as: <<http://www.lymphomahub.com/medical-information/aacr-2017-poster-24485-deep-exome-sequencing-reveals-recurrent-somatic-mutations-in-classical-hodgkin-lymphoma>>