BIOINFORMATIC ANALYSES FOR CLONAL HEMATOPOIESIS IN A FAMILY WITH GERMLINE TP53 AND XAF1 MUTATIONS

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Background

For an estimated 28 generations, a Brazilian family has possessed a haplotype that contains TP53-R337H and/or XAF1-E134* alleles. TP53-R337H refers to the arginine to histidine substitution at amino acid position 337 of p53, a tumor suppressor. XAF1-E134* refers to the mutation of the X-linked inhibitor of apoptosis where the glutamic acid at position 134 is changed to a stop codon. Both of these mutations result in the malfunction of vital genes that prevent tumor proliferation. Clonal hematopoiesis (CH) is defined as the clonal expansion of blood cell populations as a result of somatic mutations in leukemia-associated genes, resulting in increased risk for acute myelogenous leukemia (AML) and coronary artery disease. The Gawad Lab used an AML panel to conduct deep targeted sequencing of genes associated with clonal hematopoiesis (CH) using blood samples from family members whose genomes harbor TP53-R337H, XAF1-E134*, or neither variant.

Objectives

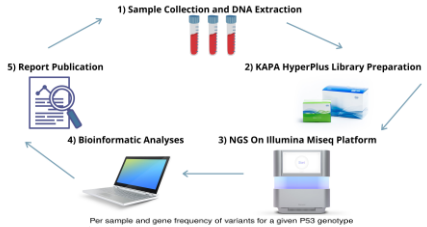
The relationship between TP53-R337H and XAF-E134* CH has not been previously studied. The aim of this research was to gain an understanding of how the germline TP53-R337H and XAF-134* alleles impacts a patient's risk for developing CH. The genes from a Brazilian family known to possess the mutated allele were sequenced to determine if they have an inherited risk for developing CH.

Hypothesis

- Individuals who possess the germline TP53-R337H and XAF-134* alleles have a higher risk of developing CH.
- Having a germline TP53-R337H and XAF-134* allele will increase the risk of developing CH as a result of somatic mutations in specific genes.

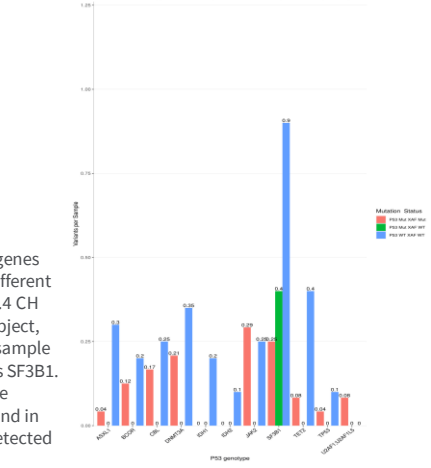
Methods

Samples for this study were obtained from 48 family members aged 47-85 at the Hospital Erasto Gaertner in Brazil. Sample DNA was isolated from mononuclear blood cells. To prepare sequencing libraries and to enrich regions associated with CH and AML, a KAPA HyperPlus Kit was used on the isolated samples. After the sequencing libraries were prepared, the samples were sequenced on an Illumina NovaSeq 2000. Reads were aligned by BWA and variants were called using GATK and CleanDepSeq. RStudio was used to sort and filter the variant data. To limit the number of false positives in the samples, the following parameters were set in RStudio: base frequency >0.02 and < 0.3; reads > 20; and frequency in the general population < 1%.



Results

The filtered data yielded 106 genetic variants in 11 different genes known to be associated with CH. Overall, for subjects with different P53/XAF1 genotypes, there were an average of 1.3, 3.0, and 0.4 CH variants per sample for the Mut/Mut, WT/WT, and Mut/WT subject, respectively. The graph on the right depicts the variants per sample across the CH genes. The most commonly mutated gene was SF3B1. In addition, some genes appear to show a correlation with the subject genotype, including IDH1/2 mutations only being found in subjects that are P53 WT, and U2AF1 mutations only being detected in subjects that are P53 and XAF1 mutant.



Conclusions

- There does not appear to be an overall increased risk of CH in patients with germline P53-R337H.
- Some genes show correlated CH mutation patterns based on the subject's germline P53 and XAF1 genotypes.
- Additional work is needed to continue to refine the analysis pipelines.

Next Steps

While the preliminary results of this study have provided new insights into any potential interactions between germline P53-R337H and CH, additional analyses need to be completed prior to making any conclusions. This includes improvements to the analysis pipelines, correlating our findings with the age of the subject, and looking at other genetic alterations in those blood cells, such as whole chromosomal gain or loss.