Higher Stability of Mutant IDH1/2 mRNA As Compared to Wild-Type mRNA in Patients with Acute Myeloid Leukemia

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Introduction

Cellular RNA levels are tightly regulated by very complex nuclear and cytoplasmic processes. The regulation of mutant mRNA in cancer cells is rarely studied. Some studies demonstrated that in vitro studies demonstrated that synonymous mutations had a significant effect on KRAS expression with c.36 T > C (G12G) most strongly inducing KRAS mRNA and protein. In contrast C.36 T > G (G12G) had the opposite effect and significantly decreased KRAS protein expression. Data demonstrated that increased KRAS activity or the loss of wildtype KRAS as dimerization partner for mutant KRAS proteins could impact oncogenicity (Sharma et al. Nature Communications, doi.org/10.1038/s41467-019-10489-2). In addition, reported on the average five-fold higher protein and two-fold higher mRNA of KRAS: G13D mutation levels as compared with wild type in transfection experiments in HCT116 human cell lines (Lampson et al, Curr Biol. 2013 January 7; 23(1): 70–75. doi:10.1016/j.cub.2012.11.031). These data suggest that missense mutations have effects beyond the change in mino acid.

We explored the effects of IDH1/2 mutations on mRNA levels in patients with Acute myeloid leukemia (AML).

Methods

Samples and patients:

DNA and RNA were extracted from a total of 48 peripheral blood and bone marrow samples from patients with acute myeloid leukemia (AML).

The characteristics of the patients with IDH1/2 mutations are listed in the Table below.

	Total number 14
Age: Median (range)	67 (37-83)
Gender: Male	7
No prior therapy	3
Previously treatment: median (range)	1 (1-4)
WBC: Median (range)(10^9/L)	1.9 (1-10.5)
Hgb Median (range)(g/dL)	8.7 (7.7-13.1)
Platelets: Median (range)(10^9/L)	56 (6-275)
PB Blasts Median (range)(%)	9.5 (0-93)
BM blasts Median (range)(%)	36.5 (1-72)
Cytogenetics: Good/Intermediate/poor	1/11/2

DNA and RNA Extraction:

The Agencourt FormaPure Total 96-Prep Kit is used for extracted both DNA and RNA from formalin fixed paraffin embedded human tissue. The Agencourt FormaPure Kits allows us to use a split protocol for extracting both RNA and DNA from the same FFPE lysate.

DNA Library Construction and sequencing

Target enrichment is performed post-UMI assignment using Single primer extension (SPE). The sequencing is conducted using the Illumina NextSeq 550 instrument

RNA Library Construction and Sequencing

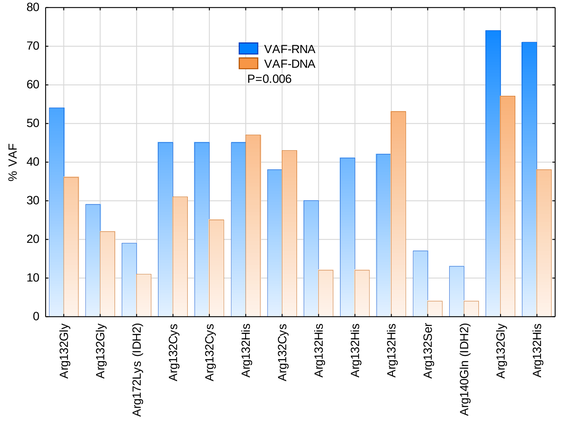
Sample are selectively enriched for 1408 cancer-associated genes using reagents provided in an Illumina® TruSight® RNA Pan-Cancer Panel. Sequencing is performed on Illumina NextSeq 550. Expression levels are measured using FPKM.

Results

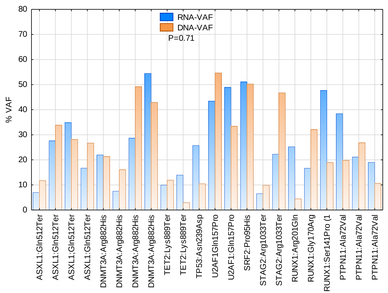
A total of 176 mutations were detected using the DNA panel and 122 mutations using the RNA panel. Some mutations were called by RNA variant caller, but not by DNA variant caller and vice versa.

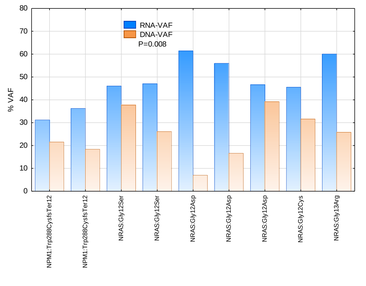
All mutations detected in IDH1 and IDH2 were detected in both DNA and RNA.

The VAF in RNA (median: 41%, range: 13%-74%) was significant higher (P=0.006, Wilcoxon matched pairs test) as compared with DNA (median:28%, range: 13%-74%).

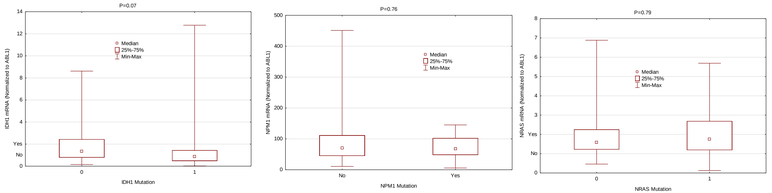


The VAF of the other 31 mutations that were detected in both DNA and RNA varied dependent on the gene. ASXL1, DNMT3A, RUNX1, PTPN11, SRSF2, STAG2 and U2AF1 mutations showed no significant difference between DNA and RNA in VAF (P=0.71). Although the number is small, mutations in NRAS and NPM1 showed significantly higher VAF in RNA as compared with that of DNA (P=0.008).





No significant increase in overall mRNA in mutated vs wild-type cases despite increase in relative mRNA



Conclusion

-Mutant IDH1/2 RNA is relatively more stable in myeloid leukemic cells a compared with the wild-type mRNA. Similarly mutant NRAS and NPM1 mRNAs appear to be more stable as compared with wild -type mRNA.

- No significant difference in VAF between DNA and RNA in cases with ASXL1, DNMT3A, RUNX1, PTPN11, SRSF2, STAG2 and U2AF1 mutations.

-No significant difference in total IDH1, NRAS, or NPM1 mRNA between mutant and wild-type AML cases. This raises the possibility that wild-type mRNA may become unstable in the presence of mutant RNA in some genes.

-This data raises the possibility that mRNA testing might be more sensitive in monitoring minimal measurable disease in patients with IDH1/2, NPM1 and NRAS mutations.