Molecular differences between colorectal cancers with mutations in histone modifiers genes vs wild-type (WT) tumors.

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Background

The first evidence of histone modification in CRC was discovered in 2005 [1] and since then, other studies suggested that histone alterations can lead to a dysregulation of oncogenic pathways. For example, oncogenic RAS pathways can affect histone covalent modifications to regulare the expression of target genes like Cyclin D1 or E-cadherin [2].

Disruption of epigenetic regulation in CRC, particularly aberrant histone methylation mediated by histone methyltransferases (HMTs) and demethylases (HDMs), correlate with poorer survival rates in mCRC. DNA methylation and histone acetylation have been already addressed for drug design and several DNA methyltransferases and histone deacetylases inhibitors are FDA-approved anti-cancer drugs (e.g., azacitidine, decitabine, vorinostat, romidepsin and belinostat). Indeed, epigenetics provides promising new targets for anticancer therapy. Numerous phase I/II trial are now enrolling patients to test the safety and efficacy of drugs targeting histone modifiers in solid tumors, including CRC [3].

Initial results of current clinical trials will probably guide the future clinical development of new histone methylation modifiers and different therapeutic indications [4].

Herein, we aimed to highlight the molecular differences between CRC harboring pathological mutations in genes involved in histone modification (*KMT* family) versus wild-type tumors. We also investigated whether an association existed between histone modification and well-known molecular and clinical CRC features, such as *KRAS*, MSI, *BRAF* status, sidedness, gender, age.

Methods

- IHC was performed on FFPE sections of glass slides. PD-L1 testing was performed using the SP142 anti-PD-L1 clone (Ventana, Tucson, AZ).
- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq (592-genes)/MiSeq platform (44-gene) (Illumina, Inc., San Diego, CA). All variants were detected with greater than 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of greater than 500 and an analytic sensitivity of 5%.
- Microsatellite instability (MSI) was examined by counting number of microsatellite loci that were altered by somatic insertion or deletion counted for each sample. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions to generate a sensitivity of > 95% and specificity of > 99%.
- Tumor mutational burden (TMB) was estimated from 592 genes (1.4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations.
- Chi-square and Wilcoxon Rank were used for comparative analyses using R version 3.5.0.

Results

1. Patient Demographics

Sex	N	% of Total
F	1713	47%
M	1908	53%

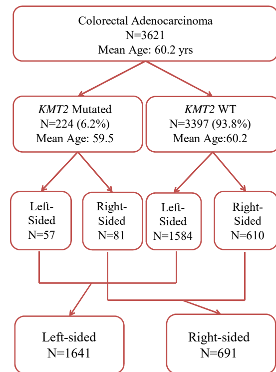


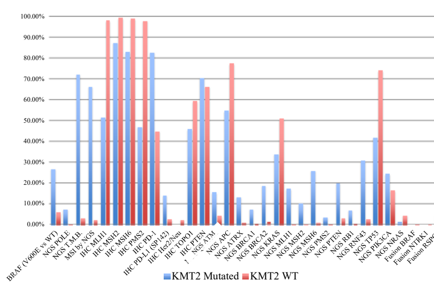
Figure 1. A total of 3621 CRC were identified from a retrospective database of tumor specimens that underwent comprehensive molecular profiling as part routine clinical care. Tumors were classified by sidedness of tumor origin.

Right-sided tumors had a statistically higher rates of *KMT2* mutations compared to left-sided (11.7% vs 3.4%, P=0.001, significant after multiple test correction).

Distribution of *KMT2* Mutations

Gene	N
KMT2A	41
KMT2C	87
KMT2D	154

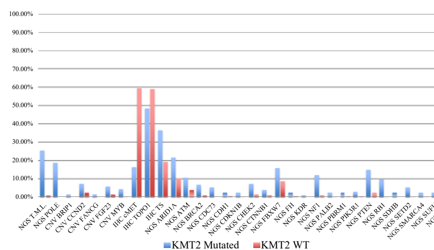
2. Molecular Profiles of *KMT2* Mutated vs WT in CRC



Biomarker	p-value	Q-value
BRAF (V600E vs WT)	<0.001	<0.001
NGS POLE	<0.001	<0.001
NGS T.M.B.	<0.001	<0.001
MSI by NGS	<0.001	<0.001
IHC MLH1	<0.001	<0.001
IHC MSH2	<0.001	<0.001
IHC MSH6	<0.001	<0.001
IHC PMS2	<0.001	<0.001
IHC PD-1	<0.001	0.001
IHC PD-L1 (SP142)	<0.001	<0.001
IHC TOPO1	<0.001	0.001
IHC PTEN	0.248	0.929
NGS ATM	<0.001	<0.001
NGS APC	<0.001	<0.001
NGS ATRX	<0.001	<0.001
NGS BRCA1	<0.001	<0.001
NGS BRCA2	<0.001	<0.001
NGS KRAS	<0.001	<0.001
NGS MLH1	<0.001	<0.001
NGS MSH2	<0.001	<0.001
NGS MSH6	<0.001	<0.001
NGS PMS2	<0.001	<0.001
NGS PTEN	<0.001	<0.001
NGS RB1	<0.001	<0.001
NGS RNF43	<0.001	<0.001
NGS TP53	<0.001	<0.001
NGS PIK3CA	0.003	0.015
NGS NRAS	0.042	0.207

Figure 2. *KMT2* mutations were strongly associated with MSI-H (P<0.0001), TMB-high (P<0.0001), *BRAF* mutation (P<0.0001), PD-L1 overexpression (P<0.0001). All were significant after multiple test correction. Numerous other genes significantly co-mutated with *KMT2* include a number of tumor suppressors. Interestingly, *APC*, *TP53* and *KRAS* were significantly more mutated in the *KMT2* wild-type cohort.

3. Molecular Profiles of KMT2 Mutated vs WT in MSS CRC



Biomarker	p-value	Q-value
NGS T.M.B.	<0.001	<0.001
NGS POLE	<0.001	<0.001
CNV BRIP1	0.001	0.025
CNV FANCG	0.095	0.971
CNV FGF23	0.04	0.557
CNV MYB	<0.001	0.002
IHC TOPO1	0.092	0.97
IHC TS	0.001	0.011
NGS ATM	0.006	0.101
NGS BRCA2	<0.001	0.001
NGS CDC73	<0.001	<0.001
NGS CDKN1B	0.032	0.472
NGS CHEK2	0.001	0.012
NGS CTNNB1	0.092	0.97
NGS NF1	<0.001	<0.001
NGS PALB2	0.023	0.378
NGS PBRM1	0.063	0.818
NGS PIK3R1	0.084	0.961
NGS PTEN	<0.001	<0.001
NGS RB1	<0.001	<0.001
NGS SDHB	<0.001	<0.001
NGS SETD2	<0.001	<0.001
NGS SMARCA4	0.024	0.378
NGS SUFU	<0.001	<0.001
NGS TP53	0.001	0.025

Figure 3. In order to exclude whether MSI was the cause of the molecular differences observed between *KMT2* mutated (n=74) and WT tumors (n=3248), we analyzed only tumors characterized as MSS by NGS. Several genes remained significantly more mutated in *KMT2*-mutant MSS tumors compared to *KMT2* wild-type including *BRCA2*, *NF1*, *RB1*, *SDHB*, and *SETD2*. TMB was also significantly higher in the *KMT2*-mutant MSS tumors.

4. TMB in CRC tumors with or without a *KMT2* Mutation (A) and according to tumor sidedness (B)

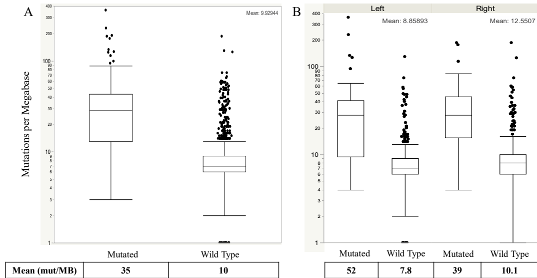


Figure 4. KMT2-mutated tumors had a statistically higher mean TMB than WT tumors (P=0.001). This difference remained significant when comparing mutated vs WT tumors from specific locations, i.e. within left-side (P=0.001) and right-side (P=0.001). Additionally, within the *KMT2* WT cohort, right-sided tumors had a higher TMB compared to the left-sided ones (P=0.001). However, TMB did not differ between sides within the *KMT2* mutant population.

When cases with a pathogenic POLE mutation, known to cause hyper-mutation, were removed, TMB-H remained more frequent in tumors with *KMT* mutations (8.3% vs 0.9%, P=0.004, data not shown). Several additional genes were more frequently mutated in *KMT* mutated tumors compared to WT tumors (*CDC73,* 1.7% vs 0.0%, P=0.003; *KDR,* 1.7% vs 0.03%, P=0.011; *SDHB,* 1.7% vs 0.1%, P=0.023; *PRKDC*, 1.7% vs 0.1%, P=0.046; *CHEK2*, 5.6% vs 1.4%, P=0.049). However, none were statistically significant after adjusting for multiple testing.

Conclusions

Our findings provide the first exploratory data on *KMT* genes mutations and their association with clinical and molecular features in CRC, in a large population of patients with extensive genetic testing.

Further investigations are warranted to elucidate the association between MSI-H and histone modifications, potentially leading to a better understanding of epigenetic alterations in CRC and to the identification of novel targets to improve therapeutic options for CRC patients.

References

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