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 cytokine modifications to regulate 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 glasses 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 greater than 50x 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

<table>
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<th>Sex</th>
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<td>M</td>
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Colorectal Adenocarcinomas

N=3621

Mean Age: 60.2 yrs

2. Molecular Profiles of KMT2 Mutated vs WT in CRC

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. 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

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, BRCA1, SDHB, and SDTC. TMB was also significantly higher in the KMT2-mutant MSS tumors.

Figure 4. KMT2-mutated tumors had a statistically higher mean TMB than WT tumors (P<0.0001). 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 pathologic 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=0.0044, data not shown). Several additional genes were more frequently mutated in KMT2 mutant tumors compared to WT tumors: CDH1, 1.7% vs 0.0%, P=0.005; EZH2, 1.7% vs 0.03%, P=0.011; SDRB, 1.7% vs 0.1%, P=0.023; PRKDC, 1.3% 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 the identification of novel targets to improve therapeutic options for CRC patients.

References