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LETTER TO THE EDITOR

Comparative proteogenomic analysis of right-sided colon cancer, left-sided colon cancer and rectal cancer reveals distinct mutational profiles

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Abstract

Right-sided colon cancer (RCC) has worse prognosis compared to left-sided colon cancer (LCC) and rectal cancer. The reason for this difference in outcomes is not well understood. We performed comparative somatic and proteomic analyses of RCC, LCC and rectal cancers to understand the unique molecular features of each tumor sub-types. Utilizing a novel in silico clonal evolution algorithm, we identified common tumor-initiating events involving APC, KRAS and TP53 genes in RCC, LCC and rectal cancers. However, the individual role-played by each event, their order in tumor development and selection of downstream somatic alterations were distinct in all three anatomical locations. Some similarities were noted between LCC and rectal cancer. Hotspot mutation analysis identified a nonsense mutation, APC R1450* specific to RCC. In addition, we discovered new significantly mutated genes at each tumor location. Further in silico proteomic analysis, developed by our group, found distinct central or hub proteins with unique interactomes among each location. Our study revealed significant differences between RCC, LCC and rectal cancers not only at somatic but also at proteomic level that may have therapeutic relevance in these highly complex and heterogeneous tumors.

Keywords: Right-sided colon cancer, Left-sided colon cancer, Rectal cancers, Clonal evolution, Proteomics, Hotspot mutations

Often grouped as one disease, right-sided colon cancer (RCC, originating from cecum, ascending colon, hepatic flexure) and left-sided colon cancer (LCC, originating from splenic flexure, descending colon, sigmoid colon) represent clinically distinct entities with significant differences in their prognosis and treatment outcomes [1, 2]. Therefore, given their anatomic continuity, the reason for these clinical differences presumably lie at the molecular level delineated by embryological origin. Previous studies have sought to identify these differences by analyzing significantly mutated genes and RNA expression [3, 4]. However, molecular differences including significant specific amino acid alterations (hot spots), proteomic differences and order of mutations in clonal evolution of these tumors have not been studied. We used somatic and proteomic data of colorectal cancers from The Cancer Genome Atlas (TCGA) [4, 5], Memorial Sloan Kettering Cancer Center (MSKCC) [6] and The Cancer Proteome Atlas (TCPA) [7] to study proteogenomic differences in these tumors (See Additional files 1 and 2).

Results and discussion

Clonal evolution trajectories

Understanding the mutational timing and evolutionary trajectory of tumors is key to investigate the molecular
Fig. 1 (See legend on next page.)
underpinnings of cancer development and progression. Thus, we applied the PiCnIc (Pipeline for Cancer Inference) algorithm to our data to study ensemble-level cancer progression models and predict the evolutionary mutational trajectories between RCC, LCC and rectal cancers in the TCGA cohort (see Additional file 3). All three cancer locations had mutations in APC, TP53 and KRAS, possibly reflecting common initiating somatic events (Fig. 1). However, there were differences in the hierarchical groupings of mutations that surrounded these events.

In RCC ($n = 135$; Fig. 1a; Additional file 4: Table S1), APC somatic mutations and TP53 somatic mutations were independent events. APC somatic mutations ‘selected’ for KRAS mutations or amplifications. APC somatic mutations also ‘selected’ for SMAD4 somatic mutations or deletions, BRAF mutations and amplification. KRAS and BRAF showed mutual exclusivity. Interestingly, alterations in FBWX7, TCF7L2, and PIK3CA also selected for SMAD4 somatic mutations. Unlike RCC, alterations in PIK3CA and IGF2 amplification and ERBB2 alterations, significant differences were also noted between these two sites. Taken altogether, our results show non-adherence to the established Vogelstein linear progression model of colorectal cancer progression from normal mucosa to adenoma to carcinoma [8].

Further, our data suggest that RCC, LCC and rectal cancers have distinct mutational behavior in the context of their evolutionary trajectories, mutational timing during cancer development and progression. However, initial events such as mutation in the gatekeeper gene, APC, appear to be similar in colorectal cancers irrespective of location.

**Mutation hotspot analysis**

We studied somatic mutations at the residue sites that can disrupt functional protein domains leading to tumorigenesis and clonal evolution via selective pressure (see Methods in Additional file 3). We found APC R1450* to be a significant mutation specifically enriched in RCC (12–15%) compared to LCC (1%) and rectal tumors (1%) in both the TCGA and MSKCC datasets ($p < 0.001$, Fig. 2a). To our knowledge, this is the first report to describe the APC R1450* mutation as being predominantly located in RCC. This particular hotspot in APC is exclusively a truncation mutation and lies within the MCR domain (residues 1282–1581; [9]) of the protein, which is a highly mutated area. The resulting truncated mutant conserves beta-catenin binding sites (15 AA repeats) but loses all three axin-binding sites (SAMP repeats) and microtubule interaction via EB1 and PdZ domains. Unlike APC R1450*, the frequency of other mutations within this region is relatively similar among the TCGA and MSKCC data sets. The relative frequencies of non-R1450* mutations within the MCR domain of APC for RCC were 63 and 64% in the TCGA and MSKCC data sets, respectively, for LCC 52 and 51%, respectively, and for rectal cancers 64% vs 58% (which did not meet statistical significance, $p = 0.35$). APC R1450* mutations are mutually exclusive from beta-catenin destruction complex genes suggesting that they may be
Fig. 2 a shows the frequency of APC hotspot the R1450 residue in (i) right-sided colon cancers, (ii) left-sided colon cancers and (iii) rectal cancers in TCGA (left) and MSKCC (right) datasets. Y-axis represent total number of mutations at each residue. b shows the mutual exclusivity of APC R1450* (APC_1450) compared to other genes of β-Catenin destruction complex in RCC. “APC_MCR” represents other APC mutations within the MCR region that are not at the 1450 residue. The bar plot above the oncplot represents total mutations in each sample.
Fig. 3 (See legend on next page.)
early events in right-sided colon cancer tumorigenesis (Fig. 2b). Given the recent findings by Zhang et al. demonstrating the efficacy of TASIN-1 (small molecule inhibitor) in a murine xenograft model of human colorectal cancer harboring a truncation mutation (A1309*) similar to APC R1450* suggests that this mutation may be viable therapeutic target [10]. In addition, we performed significantly mutated gene analysis and discovered newer driver genes at each location (see Additional file 5: Figure S1).

Proteogenomic analysis
Using The Cancer Proteome Atlas (TCPA) data, we examined RCC, LCC and rectal cancers by proteomic cancer co-expression subnetworks using association estimators methodology previously described by our group (see Methods in Additional file 3). Interestingly, no common protein emerged as having a centralized role (hub protein) across all 3 cancer locations. Within protein-protein interaction networks, several hub proteins, and their respective interactomes, were found to be unique to each of the locations (Fig. 3, see Additional file 6: Table S4).

Several hub proteins that might have a major role in RCC were identified: BAP1 (tumor suppressor gene) CASP8 (apoptosis) PCNA (DNA repair) NRAS (RTK-RAS pathway) PEA15 (apoptosis and RET signaling) DVL3 (cell proliferation and ATM-dependent DNA damage response) and PDPK1 (growth regulation) (Fig. 3a). The potentially significant hub proteins in LCC were: BAP1, BAK1 (apoptosis and prognostic in breast cancer) COG3 (protein glycosylation/golgi function) CCNB1 (mitosis and prognosis in breast cancer) SRSF1 (RNA splicing and prognosis in small cell lung cancer) DIRAS3 (tumor suppressor gene) and LCK (resistance to apoptosis) (Fig. 3b). Hub proteins unique to rectal cancers were: IGFI1R (proliferation, invasion, migration), TSC1 (cell growth) BRCA2 (DNA repair) and COP5 (multiple pathways) (Fig. 3c).

BAP1 was found to have a prominent role in both RCC and LCC. Although there are several conserved interactions, the BAP1 interactome of LCC diverges from that of RCC. Among the conserved interacting proteins are: BRD4, ADAR, GAB2, SLCA15, EIF4G1, ERCC5 and TP53BP1, BRD4, ADAR, MSH6, FOXM1 and XRCC5. Specific to LCC, BAP1 showed interactions with ERCC1, PRKCA, GATA6, JAK2, RAD51, TSC1, RSC1, NOTCH1, BCL2, KIT, PRKCD, CDH2, ARID1A, ASNS, SQSTM1 and DVL3. Specific to RCC, BAP1 was noted to interact with CDH1, MAPK14, MRE11A, MET, YAP1, STK11, ERBB3, PIK3CA, PXN, CHEK1, CTNNB1, STAT5A, EEF2K, G6PD, COG3, RBM15, BCL2A1, SYK, RELA and ANXA1.

Our results suggest BAP1 may have an essential role in carcinogenesis of colon cancer with conserved as well as divergent evolutionary interactions with other proteins in RCC and LCC that are largely absent in rectal cancers.

A somewhat surprising observation from this analysis is that the protein hubs and their interactomes are distinct for each of the anatomically defined tumor sites examined. Further, these protein signatures are not necessarily concordant with the somatic tumor profiles. Identifying alterations in tumor DNA and RNA have been of paramount importance. Clarifying post-transcriptional events and protein-protein interactions will also be highly relevant to understanding the variations in tumor biology and clinical behavior of these tumors. Prospective studies are needed to validate our findings and their implications in the clinical outcomes.

Additional files

**Additional file 1:** Patients demographics from TCGA. (DOCX 19 kb)

**Additional file 2:** Inclusion and exclusion criteria for somatic mutation analysis. (DOCX 433 kb)

**Additional file 3:** Methods section. (DOCX 52.9 kb)

**Additional file 4:** Tables S1-S3. PicNiC statistics (bic and aic) for RCC, LCC and rectal cancers. (XLSX 43 kb)

**Additional file 5:** Somatic mutation analysis for RCC, LCC and rectal cancers. (DOCX 43 kb)

**Additional file 6:** Proteomics pathway and gene level analysis results. (XLSX 47 kb)

**Abbreviations**
AIC: Akaike information criterion; BIC: Bayesian information criterion; CAPRI: Cancer progression inference algorithm; KDE: Kernel density estimator; LCC: Left colon cancer; MSKCC: Memorial Sloan kettering cancer center; PicNic: Pipeline for cancer inference; PMID: Pubmed identifier; RCC: Right colon cancer; TCGA: The cancer genome atlas; TCPA: The cancer proteome atlas

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Availability of data and materials
TCGA somatic mutation data for colorectal cancers can be obtained from the GDC legacy archive and Broad CDAC Firehose. Proteomic data can be downloaded from The Cancer Proteome Atlas (TCPA). The datasets used and analyzed in the current study are also available from the corresponding author in response to reasonable requests.

Authors’ contributions
RI and AH contributed to the design of study, involved in acquisition, analysis and interpretation of data, involved in drafting the manuscript and revising it critically for important intellectual content. ZA, OMT, CE, AK, PC, JC, KK, NM, BD involved in data acquisition, analysis and interpretation of data and involved in drafting the manuscript. LSC, SR, RM, BE, TP, AH, JS were involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate
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Competing interests

Consent for publication
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