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## Comprehensive Molecular Characterization of Human Colon and Rectal Cancer

The Cancer Genome Atlas Network

### Summary

To characterize somatic alterations in colorectal carcinoma (CRC), we conducted genome-scale analysis of 276 samples, analyzing exome sequence, DNA copy number, promoter methylation, mRNA and microRNA expression. A subset (97) underwent low-depth-of-coverage whole-genome sequencing. 16% of CRC have hypermutation, three quarters of which have the expected high microsatellite instability (MSI), usually with hypermethylation and *MLH1* silencing, but one quarter has somatic mismatch repair gene mutations. Excluding hypermutated cancers, colon and rectum cancers have remarkably similar patterns of genomic alteration. Twenty-four genes are significantly mutated. In addition to the expected *APC*, *TP53*, *SMAD4*, *PIK3CA* and *KRAS* mutations, we found frequent mutations in *ARID1A*, *SOX9*, and *FAM123B/WTX*. Recurrent copy number alterations include potentially drug-targetable amplifications of *ERBB2* and newly discovered amplification of *IGF2*. Recurrent chromosomal translocations include fusion of *NAV2* and WNT pathway member *TCF7L1*. Integrative analyses suggest new markers for aggressive CRC and important role for *MYC*-directed transcriptional activation and repression.

### Background

The Cancer Genome Atlas (TCGA) project plans to profile genomic changes in 20 different cancer types and has published results on two cancer types<sup>1,2</sup>. We now present results from multidimensional analyses of human colorectal cancer (CRC).

CRC is an important contributor to cancer mortality and morbidity. The distinction between colon and rectum is largely anatomical, but it impacts both surgical and radiotherapeutic management and it may impact prognosis. Most investigators divide CRC biologically into those with microsatellite instability (MSI) (located primarily in the right colon and frequently associated with the CpG island methylator phenotype (CIMP) and hypermutation) and those that are microsatellite-stable (MSS) but chromosomally unstable (CIN).

A rich history of investigations (for a review see<sup>3</sup>) has revealed several critical genes and pathways important to the initiation and progression of CRC<sup>3</sup>. These include the WNT, RAS-MAPK, PI3K, TGF- $\beta$ , P53 and DNA mismatch repair pathways. Large-scale sequencing analyses<sup>4–6</sup> have identified numerous recurrently mutated genes and a recurrent

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chromosomal translocation. Despite that background, we have not had a fully integrated view of the genetic and genomic changes and their significance for colorectal tumorigenesis. Further insight into those changes may enable deeper understanding of the pathophysiology of CRC and may identify potential therapeutic targets.

## Results

Tumor/normal pairs were analyzed by different platforms. The specific numbers of samples analyzed by each platform are shown in Supplementary Table 1.

### Exome sequence analysis

To define the mutational spectrum, we performed exome capture DNA sequencing on 224 tumor/normal pairs (Supplementary Table 2 lists all mutations). Sequencing achieved >20X coverage of at least 80% of targeted exons. The somatic mutation rates varied considerably among the samples. Some had mutation rates  $<1/10^6$  bases, whereas a few had mutation rates  $>100/10^6$ . We separated those cases (84%) with a mutation rate  $<8.24/10^6$  (median number of non-synonymous mutations: 58) and those with mutation rates  $>12/10^6$  (median number of mutations: 728), which we designated as hypermutated (Figure 1).

To assess the basis for the strikingly different mutation rates, we evaluated microsatellite instability (MSI)<sup>7</sup> and mutations in the DNA mismatch repair pathway<sup>8–10</sup> genes *MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6* and *PMS2*. Among the 30 hypermutated tumors with a complete data set, 23 (77%) had high levels of MSI (MSI-H). Included were 19 with *MLH1* methylation, 17 of which had high CpG island methylation phenotype (CIMP). By comparison, the remaining seven hypermutated tumors, including the six with the highest mutation rates, lacked MSI-H, CIMP or *MLH1* methylation but usually had somatic mutations in one or more mismatch repair genes or *Pole* aberrations rarely seen in the non-hypermutated tumors (Figure 1).

### Gene mutations

Overall, we identified 32 somatic recurrently mutated genes (defined by MutSig<sup>11</sup> and manual curation) in the hypermutated and non-hypermutated cancers (Figure 1B). After removal of non-expressed genes, there were 15 and 17, respectively, in the hypermutated and non-hypermutated cancers (Figure 1B, see Supplementary Table 3 for complete list). Among the non-hypermutated tumors, the eight most frequently mutated genes were *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *TCF7L2* and *NRAS*. As expected, the mutated *KRAS* and *NRAS* genes usually had oncogenic codon 12/13 or 61 mutations, whereas the remaining genes had inactivating mutations. *CTNNB1*, *SMAD2*, *FAM123B* and *SOX9* were also mutated frequently. *FAM123B* (*WTX*) is an X-linked negative regulator of *WNT* signaling<sup>12</sup>, and virtually all its mutations were loss-of-function. Mutations in *SOX9*, a gene important in cell differentiation in the intestinal stem cell niche<sup>13,14</sup>, have not been associated previously with human cancer, but all nine mutated alleles in the non-hypermutated CRCs were frameshift or nonsense mutations. Tumor suppressors *ATM* and *ARID1A* also had a disproportionately high number of frameshift or nonsense mutations. *ARID1A* mutations have recently been reported in CRC and many other cancers<sup>15,16</sup>.

In the hypermutated tumors, *ACVR2A*, *APC*, *TGFBR2*, *MSH3*, *MSH6*, *SLC9A9* and *TCF7L2* were frequent targets of mutation (Figure 1B), along with mostly *BRAF* V600E mutations. However, two genes that were frequently mutated in the non-hypermutated cancers were significantly less frequently mutated in hypermutated tumors: *TP53* (60 vs 20%,  $p < 0.0001$ ), and *APC* (81% vs 51%,  $p = 0.0023$ , both Fisher's exact test). Other genes, including *TGFBR2*, were recurrently mutated in the hypermutated cancers, but not in the non-hypermutated samples. These findings suggest that hypermutated and non-hypermutated tumors progress through different sequences of genetic events.

As expected, hypermutated tumors with *MLH1* silencing and MSI-H exhibited additional differences in mutational profile. When we specifically examined 28 genes with long mononucleotide repeats in their coding sequences, we found that the rate of frameshift mutation was 3.6-fold higher than the rate of such mutation in hypermutated tumors without *MLH1* silencing, and 50-fold higher than in non-hypermethylated tumors (Supplementary Table 2).

### Classification of tumors based on mutation rate and methylation pattern

As mentioned above, patients with colon and rectal tumors are managed differently<sup>17</sup>, and epidemiology also shows differences between the two<sup>17</sup>. An initial integrative analysis of MSI status, somatic copy number alterations (SCNAs), CIMP status and gene expression profiles of 132 colonic and 62 rectal tumors enabled us to examine possible biological differences between tumors in the two locations. Among the non-hypermutated tumors, however, the overall patterns of changes in copy number, CIMP, mRNA and miRNA were indistinguishable between colon and rectal carcinomas (Figure 2). Based on that result, we merged the two for all subsequent analyses.

Unsupervised clustering of the promoter DNA methylation profiles of 236 colorectal tumors revealed four subgroups (Supplementary Methods; Supplementary Figure 1). Two of the clusters contained tumors with elevated rates of methylation and were classified as CIMP-high (CIMP-H) and CIMP-low (CIMP-L), as previously described<sup>18</sup>. The two non-CIMP clusters were predominantly from tumors that were non-hypermutated and derived from different anatomic locations. mRNA expression profiles separated the colorectal tumors into three distinct clusters (Supplementary Figure 2). One significantly overlapped with CIMP-H tumors ( $p = 3 \times 10^{-12}$ ) and was enriched with hypermutated tumors; the other two clusters did not correspond with any group in the methylation data. Analysis of miRNA expression by unsupervised clustering (Supplementary Figure 3) identified no clear distinctions between rectal cancers and non-hypermethylated colon cancers.

### Chromosomal and sub-chromosomal changes

257 tumors were profiled for somatic copy-number alterations (SCNAs) with Affymetrix SNP 6.0 arrays. Of those tumors, 97 were also analyzed by low depth-of-coverage (low-pass) whole-genome sequencing (WGS). As expected, the hypermutated tumors had far fewer SCNAs (Figure 2). No difference was found between MSI and MSS hypermutated tumors (Supplementary Figure 4). We used the GISTIC algorithm<sup>19</sup> to identify likely gene targets of focal alterations. There were several previously well-defined arm-level changes,

including gains of 1q, 7p/q, 8p/q, 12q, 13q, 19q, and 20p/q<sup>6</sup> (Supplementary Figure 4; Supplementary Table 4). Significantly deleted chromosome arms were 18p/q (including *SMAD4*) in 66% of the tumors and 17p/q (including *TP53*) in 56%. Also significantly deleted genes were 1p, 4q, 5q, 8p, 14q, 15q, 20p, and 22q.

We identified 28 recurrent deletion peaks (Supplementary Table 4; Supplementary Figure 4), including genes like *FHIT*, *A2BP1* and *WWOX* with large genomic footprints located in potentially fragile sites of the genome, in near-diploid hypermutated tumors. Other focal deletions involved tumor suppressor genes such as *SMAD4*, *APC*, *PTEN* and *SMAD3*. A significant focal deletion of 10p25.2 spanned four genes, including *TCF7L2*, which was also frequently mutated in our dataset. A gene fusion between adjacent genes *VTIIA* and *TCF7L2* through an interstitial deletion was found in 3% of CRCs and is required for survival of CRC cells bearing the translocation<sup>4</sup>.

There were 17 regions of significant focal amplification (Supplementary Table 4). Some of them were superimposed on broad gains of chromosome arms. Included were a peak at 13q12.13 near the peptidase gene *USP12* and ~500kB distal to the CRC candidate oncogene *CDK8*; an adjacent peak at 13q12; a peak containing *KLF5* at 13q22.1; and a peak at 20q13.12 adjacent to *HNF4A*. Peaks on chromosome 8 included 8p12 (which contains the histone methyl-transferase *WHSC1L1*, adjacent to *FGFR1*) and 8q24 (which contains *MYC*). An amplicon at 17q21.1, found in 4% of the tumors, contains seven genes, including the tyrosine kinase *ERBB2*. *ERBB2* amplifications have been described in colon, breast and gastric/esophageal tumors, and breast and gastric cancers bearing these amplifications have been treated effectively with the anti-ERBB2 antibody trastuzumab<sup>20–22</sup>.

One of the most common focal amplifications, found in 7% of the tumors, is gain of a 100–150 kb region of chromosome arm 11p15.5. It contains the genes encoding insulin (*INS*), insulin-like growth factor 2 (*IGF2*), and tyrosine hydroxylase (*TH*), as well as miR-483, which is embedded within *IGF2* (Figure 3a). We found elevated expression of *IGF2* and miR-483 but not of *INS* and *TH* (Figure 3b–c). Immediately adjacent to the amplified region is *ASCL2*, a transcription factor active in specifying intestinal stem cell fate<sup>23</sup>. Although *ASCL2* has been implicated as a target of amplification in CRC<sup>23–25</sup>, it was consistently outside the region of amplification and its expression was not correlated with copy-number changes. These observations suggest that *IGF2* and miR-483 are candidate functional targets of 11p15.5 amplification. *IGF2* overexpression through loss of imprinting has been implicated in the promotion of CRC<sup>26,27</sup>. MiR-483 may also play a role in CRC pathogenesis<sup>28</sup>.

A subset of tumors (15%) without *IGF2* amplification also had dramatically higher levels (as much as 100X) of *IGF2* gene expression, an effect not attributable to methylation changes at the *IGF2* promoter. To assess the context of *IGF2* amplification/overexpression, we systematically looked for mutually exclusive genomic events using the MEMo method<sup>29</sup>. We found a pattern of near exclusivity (corrected  $p < 0.01$ ) of *IGF2* overexpression with genomic events known to activate the PI3-K pathway (mutations of *PIK3CA/PIK3R1* or deletion/mutation of *PTEN*, Figure 3c, and Supplementary Table 5). The *IRS2* gene, whose product links IGF1R, the receptor for IGF2, with PI3-K, is on chromosome 13, which is

frequently gained in colorectal cancer. Those cases with the highest *IRS2* expression were mutually exclusive of the cases with *IGF2* overexpression ( $p=0.04$ ) and also lacked mutations in the PI3-K pathway ( $p=0.0001$ ) (Figure 3c). Those results strongly suggest that the IGF2/IGF1R/IRS2 axis signals to PI3-K in CRC and imply that therapeutic targeting of the pathway could act to block PI3-K activity in this subset of patients.

## Translocations

To identify novel chromosomal translocations, we performed low-pass, paired-end, whole-genome sequencing on 97 tumors with matched normals. In each case we achieved sequence coverage of ~3–4X and a corresponding physical coverage of 7.5–10X. Despite the low genome coverage, we detected 250 candidate inter-chromosomal translocation events (range 0–10/tumor). Among those events, 212 had one or both breakpoints in an intergenic region, whereas the remaining 38 juxtaposed coding regions of two genes in putative fusion events, of which 18 were predicted to code for in-frame events (Supplementary Table 6). We found three separate cases in which the first two exons of the *NAV2* gene on chromosome 11 are joined with the 3' coding portion of *TCF7L1* on chromosome 2 (Supplementary Figure 5). *TCF7L1* encodes TCF3, a member of the TCF/LEF class of transcription factors that heterodimerize with nuclear  $\beta$ -catenin to enable  $\beta$ -catenin-mediated transcriptional regulation. Intriguingly, in all three cases, the predicted structure of the NAV2-TCF7L1 fusion protein lacks the TCF3  $\beta$ -catenin binding domain. This translocation is similar to another recurrent translocation identified in CRC, a fusion in which the amino terminus of VTI1A is joined to TCF4 that is encoded by *TCF7L2*, deleted or mutated in 12% of non-hypermuted tumors and a homolog of *TCF7L1*<sup>4</sup>. We also observed 21 cases of translocation involving TTC28 located on Chromosome 22 (Supplementary Table 6). In all cases the fusions predict inactivation of TTC28, which has been identified as a target of p53 and an inhibitor of tumor cell growth<sup>30</sup>. Eleven of the 19 (58%) gene-gene translocations are validated by either obtaining PCR products and in some case sequencing the junction fragments (Supplementary Figure 5).

## Altered pathways in CRC

Integrated analysis of mutations, copy-number, and mRNA expression changes in 195 tumors with complete data enriched our understanding of how some well-defined pathways are deregulated. We grouped samples by hypermutation status and identified recurrent alterations in the WNT, MAPK, PI3K, TGF- $\beta$  and p53 pathways (Figure 4, Supplementary Figure 6, Supplementary Table 1).

We found that the WNT signaling pathway was altered in 93% of all tumors, including biallelic inactivation of *APC* (Supplementary Table 7) or activating mutations of *CTNNB1* in ~80% of cases. There were also mutations in *SOX9* and mutations and deletions in *TCF7L2*, as well as the DKK family members and *AXIN2*, *FBXW7* (Supplementary Figure 7), *ARID1A* and *FAM123B/WTX* (the latter a negative regulator of WNT/ $\beta$ -catenin signaling<sup>12</sup> found mutated in Wilm's tumor<sup>31</sup>). A few mutations in *FAM123B/WTX* have been described in colorectal cancer<sup>32</sup>. *SOX9* has been suggested to play a role in cancer, but no mutations have previously been described. The WNT receptor Frizzled (*FZD10*) was overexpressed in ~17% of samples, in some instances at levels 100X normal. Altogether, we found 16

different altered WNT pathway genes, confirming the importance of that pathway in CRC. Interestingly, many of those alterations were found in tumors that harbor *APC* mutations, suggesting that multiple lesions affecting the WNT signaling pathway confer selective advantage.

Genetic alterations in the PI3K and RAS-MAPK pathways are common in CRC. In addition to IGF2 and IRS2 overexpression, we found mutually exclusive mutations in *PIK3R1* and *PIK3CA* as well as deletions in *PTEN* in 2%, 15% and 4% of non-hypermutated tumors, respectively. We found that 55% of non-hypermutated tumors have alterations in *KRAS*, *NRAS* or *BRAF*, with a significant pattern of mutual exclusivity (Supplementary Figure 6, Supplementary Table 1). We also evaluated mutations in the ERBB family of receptors because of the translational relevance of such mutations. Mutations or amplifications in one of the four genes are present in 22/165 (13%) non-hypermutated and 16/30 (53%) hypermutated cases. Some of the mutations are listed in the COSMIC database<sup>33</sup>, suggesting a functional role. Intriguingly, recurrent V842I ERBB2 and V104M ERBB3 mutations were found in four and two non-hypermutated cases, respectively. Mutations and focal amplifications of ERBB2 (Supplementary Figure 6) should be evaluated as predictors of response to agents that target those receptors. We observed co-occurrence of alterations involving the RAS and PI3K pathways in a third of tumors (Figure 4; Fisher's exact test  $p = 0.039$ ). These results suggest that simultaneous inhibition of the RAS and PI3K pathways may be required to achieve therapeutic benefit.

The TGF- $\beta$  signaling pathway is known to be deregulated in colorectal and other cancers<sup>34</sup>. We found genomic alterations in *TGFBR1*, *TGFBR2*, *ACVR2A*, *ACVR1B*, *SMAD2*, *SMAD3* and *SMAD4* in 27% of the non-hypermutated and 87% of the hypermutated tumors. We also evaluated the p53 pathway, finding alterations in *TP53* in 59% of non-hypermutated cases (mostly biallelic, Supplementary Table 8) and alterations in ATM, a kinase that phosphorylates and activates p53 following DNA damage, in 7%. Alterations in those two genes showed a trend towards mutual exclusivity ( $p = 0.016$ ) (Figure 4, Supplementary Figure 6, Supplementary Table 1).

We integrated copy number, gene expression, methylation and pathway data using the PARADIGM software platform<sup>35</sup>. The analysis revealed a number of novel characteristics of CRC (Figure 5A). For example, despite the diversity in anatomical origin or mutation levels, nearly 100% of these tumors have changes in MYC transcriptional targets, both those promoted by and those inhibited by MYC. These findings are consistent with patterns deduced from genetic alterations (Figure 4) and suggest an important role for MYC in the CRC. The analysis also identified several gene networks altered across all tumor samples and those with differential alterations in hypermutated vs. non-hypermutated samples (Supplementary Table 7, Supplemental Data on the TCGA publication webpage).

Since most of the tumors used in this study were derived from prospective collection, survival data are not available. However, the tumors can be classified as aggressive or non-aggressive on the basis of tumor stage, lymph node status, distant metastasis and vascular invasion at the time of surgery. We found numerous molecular signatures associated with tumor aggressiveness, a subset of which is shown in Figure 5B. They include specific focal



amplifications and deletions, and altered gene expression levels, including those of *SCN5A*<sup>36</sup>, a reported regulator of colon cancer invasion (full list: Supplementary Tables 10–11). Association with tumor aggressiveness is also observed in altered expression of miRNAs and specific somatic mutations (*APC*, *TP53*, *PIK3CA*, *BRAF*, and *FBXW7*; Supplementary Figure 8B). Mutations in *FBXW7* (38 cases) and distant metastasis (32 cases) never co-occurred ( $p = 0.0019$ ). Interestingly, a number of genomic regions have multiple molecular associations with tumor aggressiveness that manifest as “clinically-related genomic hotspots”. Examples of this are the region 20q13.12, which includes a focal amplification and multiple genes correlating with tumor aggression, and the region 22q12.3, containing *APOL6*<sup>37</sup> (Supplementary Figures 8–9).

## Discussion

This comprehensive integrative analysis of 224 colorectal tumor/normal pairs provides a number of insights into the biology of CRC and identifies potential therapeutic targets. To identify possible biological differences in colon and rectum tumors we found, in the non-hypermuted tumors, irrespective of their anatomical origin, the same type of copy number, expression profile, DNA methylation and miRNA changes. Over 94% of them had a mutation in one or more members of the WNT signaling pathway, predominantly in *APC*. However, there were some differences between tumors from the right colon and the remaining sites. Hypermethylation was more common in the right colon, and three quarters of hypermutated samples came from the same site, although not all of them had MSI (Figure 2). Why most of the hypermutated samples come from the right colon and why there are two classes of tumors at this site is not known. The origins of the colon from embryonic midgut and hindgut may provide an explanation. Since the survival of patients with high MSI cancers are better and these cancers have hypermutation, mutation rate may be a better prognostic indicator.

Whole exome sequencing and integrative analysis of genomic data provided further insights into the pathways that are dysregulated in CRC. We found that 93% of non-hypermuted and 97% of hypermutated cases had deregulated WNT signaling pathway. Novel findings included recurrent mutations in *FAM123B*, *ARID1A* and *SOX9* and very high levels of overexpression of the WNT ligand receptor Frizzled 10. To our knowledge, *SOX9* has not previously been described as frequently mutated in any human cancer. *SOX9* is transcriptionally repressed by WNT signaling, and the *SOX9* protein has been shown to facilitate  $\beta$ -catenin degradation<sup>38</sup>. *ARID1A* is frequently mutated in gynecological cancers and has been shown to suppress Myc transcription<sup>39</sup>. Activation of WNT signaling and inactivation of the TGF- $\beta$  signaling pathway are known to result in activation of *MYC*. Our mutational and integrative analyses emphasize the critical role of *MYC* in CRC. We also compared our results with other large-scale analyses<sup>6</sup> and find many similarities and few differences in mutated genes (Supplementary Table 3).

Our integrated analysis revealed a diverse set of changes in TCF/LEF encoding genes suggesting additional roles for TCF/LEF factors in CRC beyond being passive partners for  $\beta$ -catenin.

Our data suggest a number of therapeutic approaches to CRC. Included are WNT signaling inhibitors and small-molecule  $\beta$ -catenin inhibitors that are showing initial promise<sup>40–42</sup>. We find that several proteins in the RTK/RAS and PI3K pathways including IGF2, IGFR, ERBB2, ERBB3, MEK, AKT and mTOR could be targets for inhibition.

Our analyses show that non-hypermethylated adenocarcinomas of the colon and rectum are not distinguishable at the genomic level. However, tumors from the right/ascending colon were more likely to be hypermethylated and to have elevated mutation rates than were other CRCs. As has been recognized previously, activation of the WNT signaling pathway and inactivation of the TGF- $\beta$  signaling pathway, resulting in increased activity of MYC, are nearly ubiquitous events in CRC. Genomic aberrations frequently target the MAPK and PI3-K pathways but less frequently target receptor tyrosine kinases. In conclusion, the data presented here provide an unprecedented resource for understanding this deadly disease and identifying possibilities for treating it in a targeted way.

## Methods Summary

Tumor and normal samples were processed by either of two Biospecimen Core Resources (BCRs), and aliquots of purified nucleic acids were shipped to the genome characterization and sequencing centers (Supplementary Methods). The BCRs provided sample sets in several different batches. To assess any batch effects we examined the mRNA expression, miRNA expression and DNA methylation data sets using a combination of cluster analysis, enhanced principal component analysis, and analysis of variance (Supplementary Methods). Although some differences among batches were detected, we did not correct them computationally because the differences were generally modest and because some of them may reflect biological phenomena (Supplementary Methods).

We used Affymetrix SNP 6.0 microarrays to detect copy-number alterations. A subset of samples was subjected to low pass (2–5X) whole genome sequencing (Illumina HiSeq), in part for detection of SCNA and chromosomal translocations<sup>43,44</sup>. Gene expression profiles were generated using Agilent microarrays and RNA-Seq. DNA methylation data were obtained using Illumina Infinium (HumanMethylation27) arrays. DNA sequencing of coding regions was performed by exome capture followed by sequencing on the SOLiD or Illumina HiSeq platforms. Details of the analytical methods used are described in Supplementary Methods.

All of the primary sequence files are deposited in dbGap and all other data are deposited at the Data Coordinating Center (DCC) for public access (<http://cancergenome.nih.gov/>). Data matrices and supporting data can be found at [http://tcga-data.nci.nih.gov/docs/publications/coadread\\_2012/](http://tcga-data.nci.nih.gov/docs/publications/coadread_2012/). The data can also be explored via the ISB Regulome Explorer (<http://explorer.cancerregulome.org/>) and the cBio Cancer Genomics Portal (<http://cbioportal.org>). Descriptions of the data can be found at <https://wiki.nci.nih.gov/x/j5dXAg> and in Supplementary Methods.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.



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## Appendix

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**Author Contributions:** The TCGA research network contributed collectively to this study. Biospecimens were provided by the Tissue Source Sites and processed by the Biospecimen Core Resource. Data generation and analyses were performed by the Genome Sequencing Centers, Cancer Genome Characterization Centers, and Genome Data Analysis Centers. All data were released through the Data Coordinating Center. Project activities were coordinated by the NCI and NHGRI Project Teams. **Project Leaders:** Raju Kucherlapati and David A. Wheeler; **Writing Team:** Todd Auman, Adam J. Bass, Timothy A. Chan, Lawrence Donehower, Angela Hadjipanayis, Stanley R. Hamilton, Raju Kucherlapati, Peter W. Laird, Matthew Meyerson, Nikolaus Schultz, Ilya Shmulevich, Joshua M. Stuart, Joel Tepper, Vesteinn Thorsson, David A. Wheeler. **Mutations:** Michael S. Lawrence, Lisa R. Trevino, David A. Wheeler, Gad Getz; **Copy-Number and Structural Aberrations:** Alex H. Ramos, Adam J. Bass, Angela Hadjipanayis, Peng-Chieh Chen; **DNA Methylation:** Toshinori Hinoue; **Expression:** J. Todd Auman; **miRNA:** Gordon Robertson, Andy Chu; **Pathways:** Chad J. Creighton, Lawrence Donehower, Ted Goldstein, Sam Ng, Jorma de Ronde, Chris Sander, Nikolaus Schultz, Joshua M. Stuart, & Vesteinn Thorsson.

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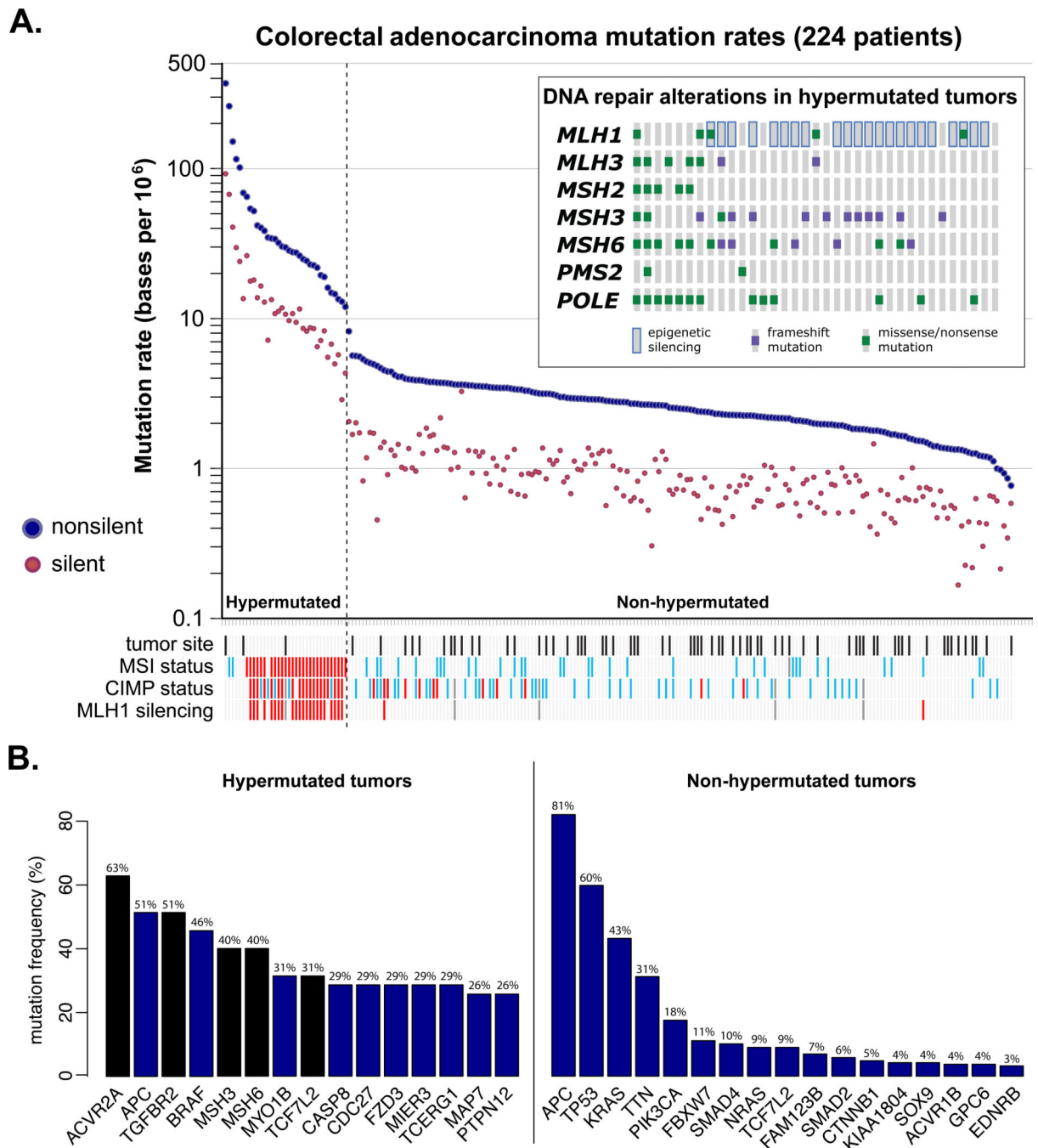
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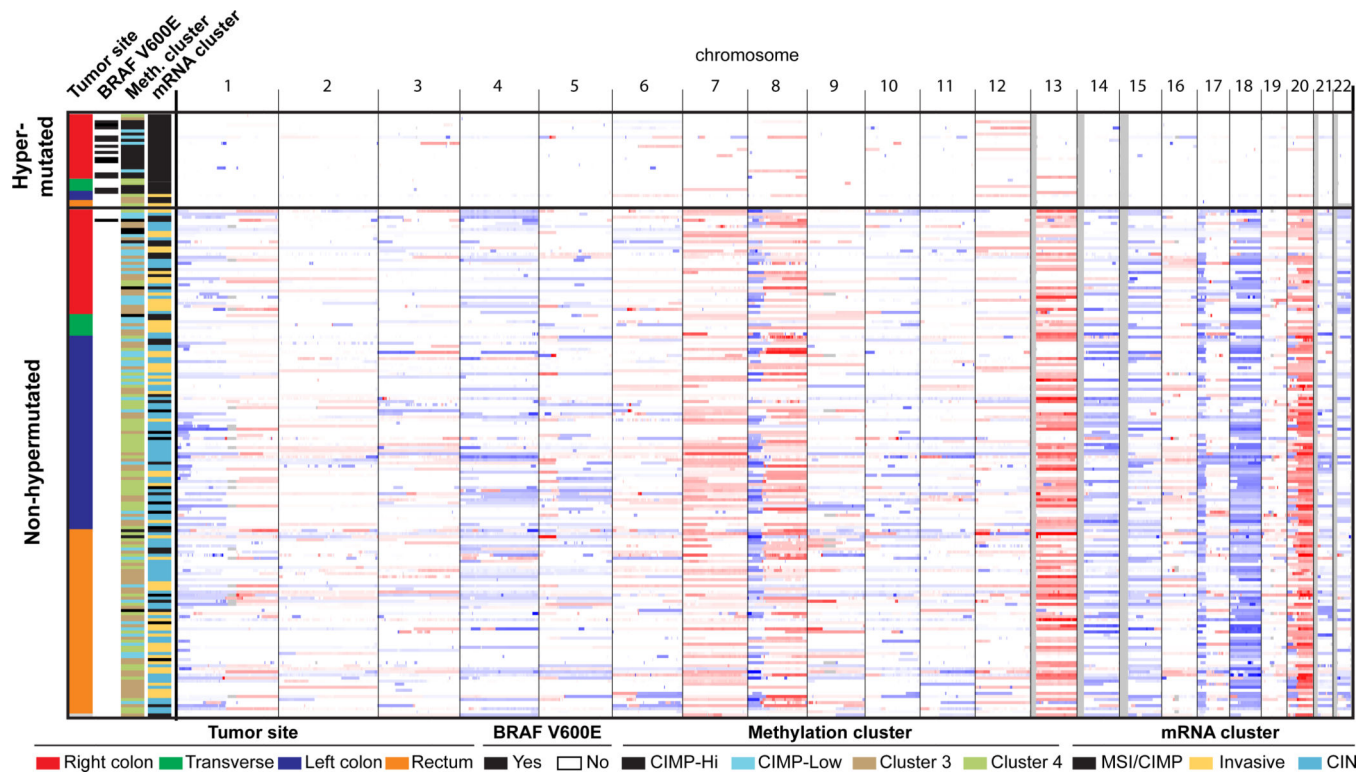
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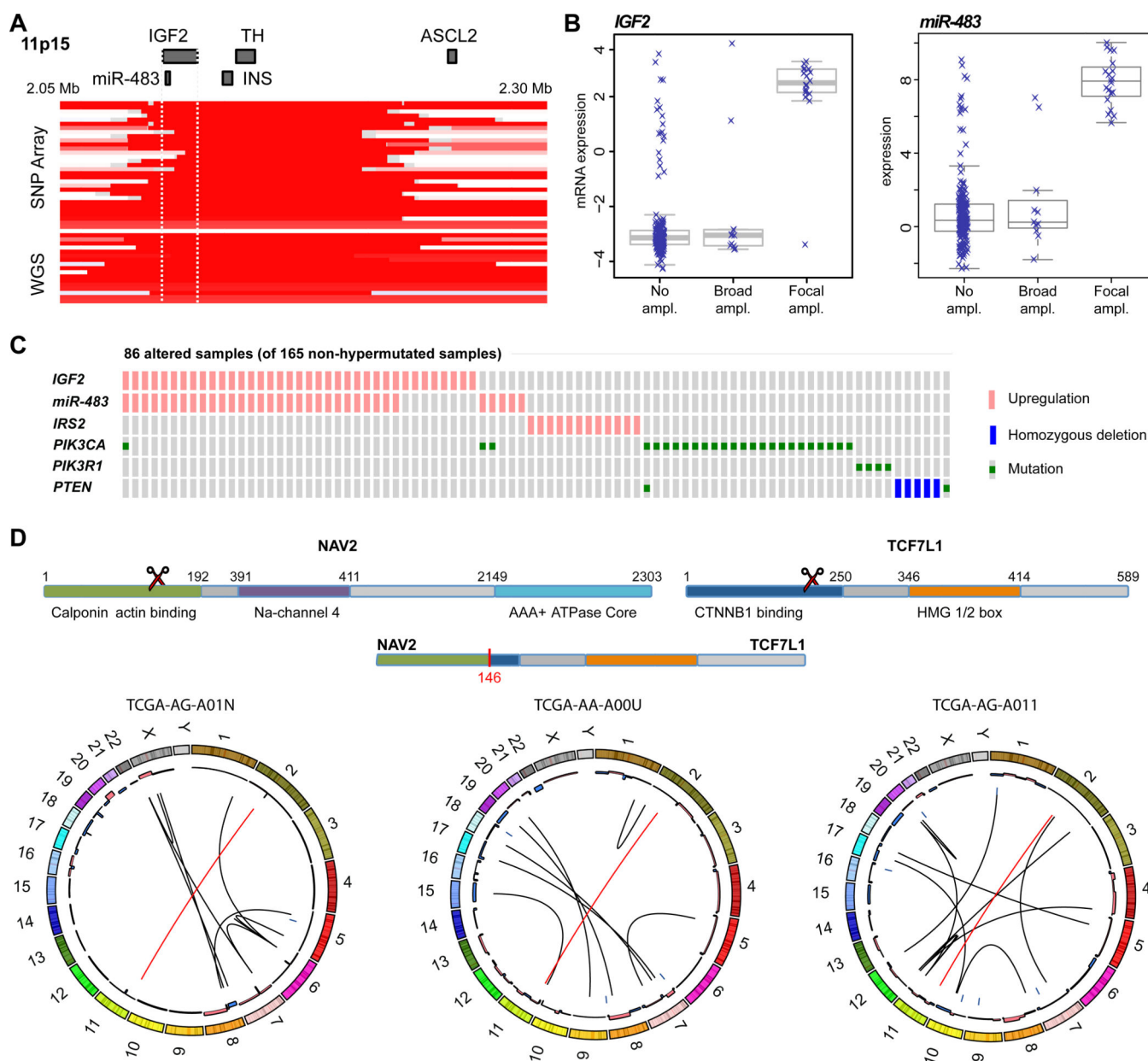
**Figure 1. Mutation frequencies in human CRC**

**A.** Mutation frequencies in each of the tumors. Note a clear separation of hypermutated and non-hypermutated samples. **Inset:** Mutations in mismatch repair genes and *POLE* among the hypermutated samples. The order of the samples is the same as in Figure 1A. **B.** Significantly mutated genes in non-hypermutated and hypermutated tumors. Blue bars represent genes identified by MutSig and genes in black bars are identified by manual examination of sequence data.



**Figure 2. Integrative analysis of genomic changes in 195 CRC tumors**

Hypermutated tumors have near diploid genomes and are highly enriched for hypermethylation, CIMP expression phenotype, and *BRAF* V600E mutations. Non-hypermutated tumors originating from different sites are virtually indistinguishable from each other based on their copy-number alteration patterns, DNA methylation, or gene expression patterns. Copy-number changes of the 22 autosomes are shown in shades of red for copy-number gains and shades of blue for copy-number losses.

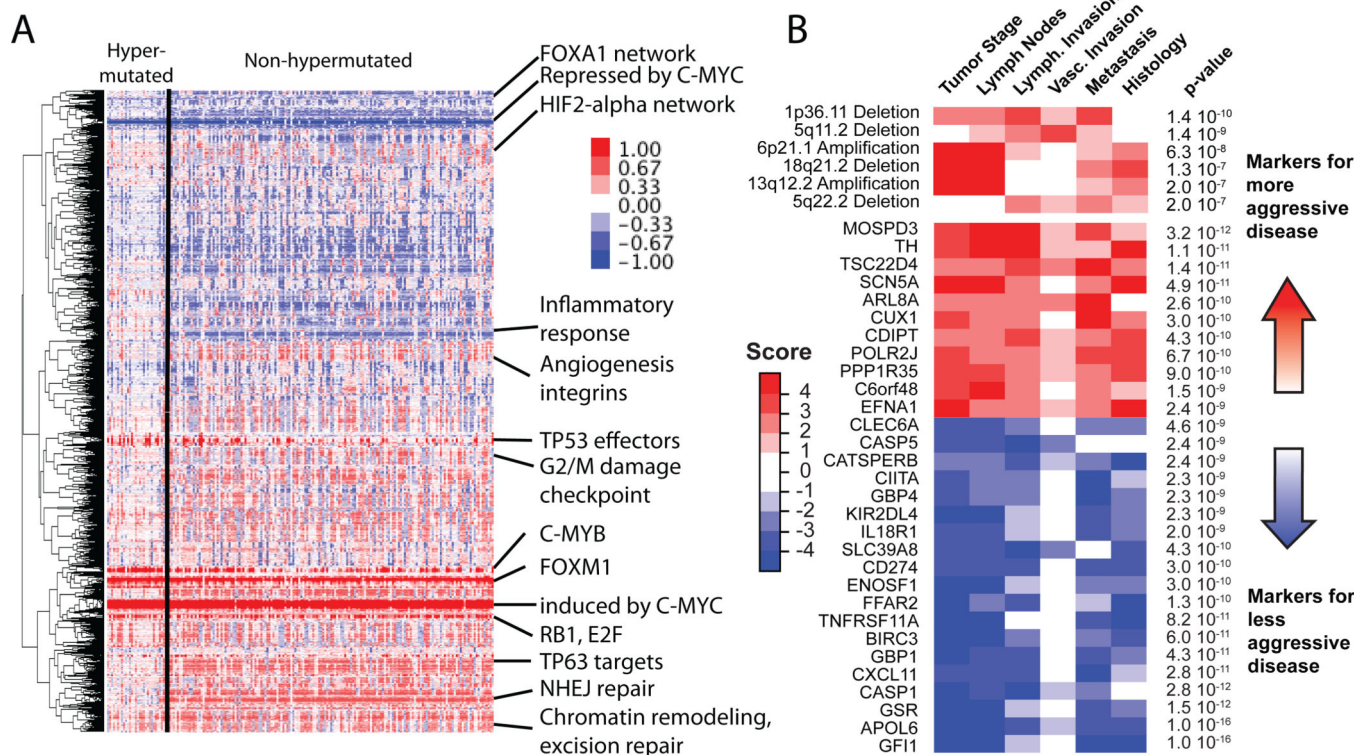


**Figure 3. Copy number changes and structural aberrations in CRC**

**A.** Focal amplification of 11p15.5. Segmented DNA copy-number data from SNP arrays and low pass whole genome sequencing are shown. Each row represents a patient; amplified regions are shown in red. **B.** Correlation of expression levels with copy number changes for IGF2 and miR-483. **C.** IGF2 amplification and over-expression are mutually exclusive of alterations in PI3K signaling genes. **D.** Recurrent NAV2-TCF7L2 fusions. The structure of the two genes, locations of the breakpoints leading to the translocation and circular representations of all rearrangements in tumors with a fusion are shown. The red line lines represent the NAV2-TCF7L2 fusions, black lines indicate other rearrangements. The inner ring represents copy-number changes (blue = loss, pink = gain).







**Figure 5. Integrative analyses of multiple data sets**

**A.** Clustering of genes and pathways affected in colon and rectum tumors deduced by PARADIGM analysis. Blue = under-expressed relative to normal and red = overexpressed relative to normal. Some of the pathways deduced by this method are shown on the right. **B.** Gene expression signatures and SCNAs associated with tumor aggression. Molecular signatures (rows) that show statistically significant association with tumor aggressiveness according to selected clinical assays (columns) are displayed in color, with red indicating markers of tumor aggressiveness, and blue the markers of less aggressive tumors. Significance is based on the combined  $p$ -value from the weighted Fisher's method, corrected for multiple testing. Color intensity and score is in accordance with the strength of an individual clinical-molecular association, and is proportional to  $\log_{10}(p)$ , where  $p$  is  $p$ -value for that association. To limit the vertical extent of the figure, gene expression signatures are restricted to combined  $p$ -value  $p < 10^{-9}$ , SCNAs to  $p < 10^{-7}$  and features are shown only if they are also significant in the subset of non-MSI-H samples (the analysis was performed separately on the full data as well as on the MSI-H and non-MSI-H subgroups).