Microsatellite Stable Colorectal Cancers Stratified by the \(BRAF\) V600E Mutation Show Distinct Patterns of Chromosomal Instability

Catherine E. Bond\(^1,2\), Derek J. Nancarrow\(^3,4\), Leesa F. Wockner\(^5\), Leanne Wallace\(^6\), Grant W. Montgomery\(^6\), Barbara A. Leggett\(^1,2,7\), Vicki L. J. Whitehall\(^1,8\)

1 Conjoint Gastroenterology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, 2 School of Medicine, University of Queensland, Brisbane, Queensland, Australia, 3 Cancer Control Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, 4 Oncogenomics Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, 5 Cancer and Population Studies Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, 6 Molecular Epidemiology Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia, 7 Royal Brisbane and Women’s Hospital, Brisbane, Queensland, Australia, 8 Pathology Queensland, Brisbane, Queensland, Australia

Abstract

The \(BRAF\) (V600E) mutation in colorectal cancers that are microsatellite stable (MSS) confers a poor patient prognosis, whereas \(BRAF\) mutant microsatellite-unchangeable (MSI) colorectal cancers have an excellent prognosis. \(BRAF\) wild type cancers are typically MSS and display chromosomal instability (CIN). CIN has not been extensively studied on a genome-wide basis in relation to \(BRAF\) mutational status in colorectal cancer. \(BRAF\) mutant/MSS (\(BRAF\)mut/MSS) cancers (\(n = 33\)) and \(BRAF\) mutant/MSI (\(BRAF\)mut/MSI) cancers (\(n = 18\)) were compared for presence of copy number aberrations (CNAs) indicative of CIN, with \(BRAF\) wild type/MSS (\(BRAF\)wt/MSS) cancers (\(n = 18\)) using Illumina CytoSNP-12 arrays. \(BRAF\)mut/MSI and \(BRAF\)wt/MSI cancers showed comparable numbers of CNAs/cancer at 32.8 and 29.8 respectively. However, there were differences in patterns of CNA length between MSS cohorts, with \(BRAF\)mut/MSS cancers having significantly greater proportions of focal CNAs compared to \(BRAF\)wt/MSS cancers (\(p < 0.0001\)); whereas whole chromosomal arm CNAs were more common in \(BRAF\)wt/MSS cancers (\(p < 0.0001\)). This related to a reduced average CNA length in \(BRAF\)mut/MSI compared to \(BRAF\)wt/MSI cancers (20.7 Mb vs 33.4 Mb;p < 0.0001); and a smaller average percent of CIN affected genomes in \(BRAF\)mut/MSI compared to \(BRAF\)wt/MSI cancers (23.9% vs 34.9% respectively). \(BRAF\)mut/MSI cancers were confirmed to have low CNA rates (5.4/cancer) and minimal CIN-affected genomes (average of 4.5%) compared to MSS cohorts (\(p < 0.0001\)). \(BRAF\)mut/MSS cancers had more frequent deletion CNAs compared to \(BRAF\)wt/MSS cancers on 6p and 17q at loci not typically correlated with colorectal cancer, and greater amplification CNAs on 8q and 18q compared to \(BRAF\)wt/MSS cancers. These results indicate that comparable rates of CIN occur between MSS subgroups, however significant differences in their patterns of instability exist, with \(BRAF\)mut/MSS cancers showing a ‘focal pattern’ and \(BRAF\)wt/MSS cancers having a ‘whole arm pattern’ of CIN. This and the genomic loci more frequently affected in \(BRAF\)mut/MSS cancers provides further evidence of the biological distinctions of this important cancer subgroup.

Introduction

The \(BRAF\) V600E mutation is present in approximately 10–15% of sporadic colorectal cancer (CRC) \([1]\) and is a hallmark of the serrated neoplastic pathway of CRC, where cancers develop from serrated precursor polyps \([2,3]\). The CpG Island Methylator Phenotype (CIMP) is strongly associated with presence of the \(BRAF\) mutation \([2,4,3]\). In approximately half of these \(BRAF\) mutant cancers, CIMP related methylation and silencing of the DNA mismatch repair gene, \(MLH1\), results in widespread frameshift mutations known as microsatellite instability (MSI). \(BRAF\) mutant/MSI cancers have been well characterized and show typical molecular and clinical features including an excellent patient outcome \([4,6,7,9]\). The remaining \(BRAF\) mutant cancers do not methylate \(MLH1\) and are microsatellite stable (MSS). These \(BRAF\) mutant/MSS cancers have not been as well studied, but importantly confer a very poor patient prognosis \([9,10,11]\).

The majority of sporadic CRC are \(BRAF\) wild type and arise from conventional adenomas that follow a well defined pathway of molecular events leading to cancer \([12]\). These \(BRAF\) wild type cancers are typically MSS and frequently show chromosomal instability (CIN) \([8]\), the presence of which has been correlated with a poor prognosis in these cancers \([13,14,15,16]\). Interestingly, the presence of the \(BRAF\) V600E mutation in MSS cancers confers an even worse prognosis \([9,17]\), however CIN has not been extensively studied on a genome-wide basis in this cancer subgroup.
CIN refers to the rate of acquisition of copy number aberrations (CNAs) where sections of DNA are affected by either deletion or amplification events [18]. CIN can affect whole chromosomes largely through dysfunctional chromosome segregation during mitosis [18,19], and aneuploidy is the stable state of abnormal chromosome numbers [18]. Alternatively CIN can refer to the presence of widespread structural sub-chromosomal rearrangements resulting from incorrect repair of DNA damage [20]. These structural rearrangements can arise through repetitive rounds of breakage and fusion repair cycles leading to complex deletions, amplifications and translocations [21].

Few studies have extensively investigated CIN in the context of BRAF mutational and MSI status. We have previously found comparably high frequencies of LOH events between BRAF mutant/MSS cancers and BRAF wild type cancers at several key genomic loci (18q, 17p, 5q and 8p), that are known to harbour cancer type.

Genetic aberrations that could be contributing to the aggressiveness of these cancers by investigating the extent of CIN on a wide variety of features [9,10,17,22,36]. This study expands on the characterization of CNAs in CRC by identifying different types of CNAs including complex aberrations and copy neutral loss of heterozygosity (cnLOH) events. Several common regions targeted by CNAs in CRC including deletions on chromosomes 17p, 18q, 5q, 8p, 4q and 1p, and amplifications on chromosomes 13q, 20q, 7p, 7q and 8q, have been confirmed through SNP array studies [23,24].

Application of genome-wide single nucleotide polymorphism (SNP) arrays to study the presence of CIN has allowed the identification of different types of CNAs including complex aberrations and copy neutral loss of heterozygosity (cnLOH) events. Several common regions targeted by CNAs in CRC including deletions on chromosomes 17p, 18q, 5q, 8p, 4q and 1p, and amplifications on chromosomes 13q, 20q, 7p, 7q and 8q, have been confirmed through SNP array studies [23,24].

MSI and CIN have previously been considered as two distinct pathways of genomic instability due to findings of MSI cancers being largely diploid [13,25,26]. However, several studies using cytogenetic analysis have found MSI cell lines and cancers to have a considerable presence of chromosomal aberrations, predominantly cnLOH events [27,28,29,30]. Similarly, studies have reported the presence of CIN and CIMP to be inversely correlated [31,32], and the incidence of frequent methylation to be associated with reduced rates and lengths of CNAs [33,34,35]. However, the majority of these studies did not stratify for presence of a BRAF mutation [31,33,35].

We and others have highlighted the importance of the BRAF mutant/MSS cancer type with their correlations with poor patient outcomes and presence of distinct molecular and clinical features [9,10,17,22,36]. This study expands on the characterization of these cancers by investigating the extent of CIN on a genome-wide basis which may help to determine further molecular aberrations that could be contributing to the aggressiveness of this cancer type.

Materials and Methods

Cancer Samples

An initial cohort of 1052 sporadic colorectal cancers and matched normals were obtained from patients following surgery at the Royal Brisbane and Women’s Hospital, Queensland, Australia. Written, informed consent was collected from all patients, and the study was approved under the RBWH and Bancroft Human Research Ethics Committee. Clinical data including patient gender, age, stage at diagnosis (American Joint Committee on Cancer, AJCC), and anatomical site of cancer (with proximal location considered as being proximal to the splenic flexure) was collected where available.

BRAF, p53 and KRAS Mutation, MSI and CIMP Investigations: All cancer samples had previously been investigated for MSI status using the 5 marker panel of the National Cancer Institute (mononucleotide: BAT25, BAT26; dinucleotide: D5S346, D2S123, D17S250) and classified MSI if at least two markers, including at least one mononucleotide marker, were positive. The presence of the BRAF V600E mutation, p53 mutation (across exons 4 to 6), KRAS mutation (at codons 12 and 13), and the CpG Island Methylator Phenotype (using a 5 marker panel consisting of CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1 [5]) had also been previously determined [22,36,37]. The cancers were subsequently divided into three cohorts depending on their MSI and BRAF mutational status: as BRAF mutant/MSS (n = 60), BRAF mutant/ MSI (n = 60) or BRAF wild type/MSS (n = 924).

Single Nucleotide Polymorphism Arrays

From these cohorts, 33 BRAF mutant/MSS, 30 BRAF mutant/ MSI and 18 BRAF wild type/MSS cancers and matched normal samples were chosen for quantification using Picogreen dye, and analysis for genome-wide copy number aberrations (CNAs) with HumanCytoSNP-12v2.1 Single Nucleotide Polymorphism (SNP) arrays (Illumina; San Diego, Ca.) according to the manufacturer’s instructions. The beadchips were scanned using Illumina’s iScan system and the image data was analysed with Illumina’s GenomeStudio version 2011.1.0.24550. The cancer traces were referenced to their matched normal profiles and the boundaries of all somatic copy number aberrations were manually determined and based on human genome build NCBI36/hg19. To account for stromal contamination commonly present in cancer samples, the Simulated DNA Copy Number (SiDCoN) [38] and an automated SiDCoN2 tool which are R script based applications, were used to assign each CNA a log R ratio and B allele frequency score to determine the genotype of each CNA. The SiDCoN application was able to determine the extent of cells that showed an aberrant copy number change for each CNA, including heterogeneous genotypes. Any individual CNA that scored less than 20% of aberrant cellular involvement was excluded from analysis in order to ensure reliable CNA data [38,39]. The CNA s were then converted from Excel to custom data tracks and visualized on the University of California, Santa Cruz’s Genome Browser (http://genome.ucsc.edu/) [40].

Cytogenetics terminology was applied with ‘gains’ and ‘losses’ referring to whole chromosome arm events where a large genomic region was affected and typically consisted of small copy numbers. ‘Amplifications’ and ‘deletions’ referred to CNAs covering sub-chromosomal or focal regions and these potentially involved greater copy numbers [41]. Specific types of deletion and amplification CNAs were analysed with deletion events comprising of loss of heterozygosity (LOH), copy neutral loss of heterozygosity (cnLOH) and homozogous deletion (HD) events, whilst amplification CNAs included 3n and ≥4n (complex) amplification events.

To identify the extent of CNA coverage per chromosome, the length of each CNA was calculated as a fraction of its coverage over the full length of the specific chromosome arm in order to allow for comparisons of CNAs occurring on all chromosome arms with differing lengths [41]. Continuous CNAs covering ≥95% of a chromosomal arm were termed ‘whole chromosome arm’ CNAs; regional CNAs covered between 50–94% of a chromosome arm; and focal events were considered as <50% the length of a chromosome arm in keeping with previously published data [24,41,42,43]. In this study, whole chromosome CNAs (continuous aberrations extending over both chromosome arms) were included in the analysis of whole chromosome arm CNAs as in Beroukhim et al [41] and the Cancer Genome Atlas Network’s characterization of CRC [24]. Minimal common regions (MCRs) were also identified and referred to the smallest genomic loci that contained deletion or amplification copy number changes at the highest frequencies across cancers in each cohort.

Cancer Cell Density in Samples: SiDCoN assisted in estimating the cancer cell density of each sample [39], and those samples that
Statistical Analysis

Significant differences between categorical data were analysed with Pearson’s chi-squared test, or Fisher’s exact test where appropriate. Proportions were tested using a proportion test, and where appropriate these p-values were corrected for multiple comparisons using the Benjamini-Hochberg method. For continuous variables, ANOVA was used to test for a significant difference between groups, and Post-Hoc analysis (using Tukey’s HSD) was performed to explore differences further. For tests within cohorts, either a paired t-test or Wilcoxon’s sign rank test and Friedman’s test of related samples was performed. P values ≤0.05 were considered significant.

Results

Clinical and Molecular Features of Study Cohorts

33 BRAF mutant/MSS (BRAFmut/MSS), 18 BRAF wild type/MSS (BRAFwt/MSS), and 30 BRAF mutant/MSI (BRAFmut/MSI) cancers were analysed. The majority of BRAF mutant cancers derived from the proximal colon, whereas most BRAFwt/MSM cancers were found distally (p < 0.0001) (Table 1). The BRAFmut/MSS cancers presented mostly at advanced stages (AJCC III and IV), compared to BRAFwt/MSS and BRAFmut/MSI cancers (p = 0.03) (Table 1). BRAFmut/MSI cancers conferred a later age of onset compared to MSS cancers (p = 0.0001). Malignantly, the CpG Island Phenotype (CIMP) was predominant in the BRAF mutant cohorts, particularly the BRAFmut/MSI cancers; whereas no BRAFwt/MSI cancers were CIMP high (p < 0.0001) (Table 1). BRAS mutations were present in 28% BRAFwt/MSS cancers and the mutual exclusivity of KRAS with BRAF mutations was confirmed.

Rates of Copy Number Aberrations in Molecular Subgroups

Individual copy number aberrations (CNAs) that had ≥20% cellular involvement as determined by SiDCoN [38,39] were included in the following analysis. Cancers that had less than 40% tumour content as estimated by SiDCoN [39] had substantial evidence of cancer related molecular changes (Table S1 in File S1) [43], suggesting sufficient tumour cellularity to also detect CNAs. Statistical differences in the rate and type of CNAs occurring between cohorts remained valid when analyses were performed with their exclusion (Table S2). Therefore the full cohorts were considered for this investigation.

The MSS cohorts had comparable average rates of CNAs per cancer with a rate of 32.8 per BRAFmut/MSS cancer and 29.8 per BRAFwt/MSS cancer. The BRAFmut/MSI cohort had a significantly lower rate of 5.4 CNAs per cancer (p < 0.0001) (Table 2). The average length of a single CNA in the BRAFmut/MSS cohort (33.4 Mb) was significantly longer than the average CNA length in the BRAFmut/MSS cohort (20.7 Mb) (p < 0.0001), and the BRAFmut/MSI cohort (23.6 Mb) (p < 0.0001) (Table 2). The length of each CNA was considered as a fraction of the length of the specific chromosome arm [41]. This showed significant differences in the average and median chromosome fraction affected by CNAs occurring between cohorts, with the BRAFwt/MSS having the highest fraction of chromosome arm involvement compared to the BRAF mut cohorts (p < 0.0001) (Table 2). This difference in average CNA length corresponded to a greater average percentage of genomes affected by CNAs in the BRAFmut/MSS cohort (34.9%; range 0–80.5%), compared to the BRAFmut/MSI cohort (23.9%; range 0–68.6%). In comparison to MSS cancers, BRAFmut/MSI cancers had a minimal proportion of genome involvement (4.5%; range 0–25.8%) (p < 0.0001) (Table 2). Due to this small extent of CNAs affecting BRAFmut/MSI cancers, the following results will mainly compare the two MSS cohorts.

Deletion and amplification CNAs were considered as a rate of the total number of CNAs occurring within that cohort, as well as the number of events occurring per cancer within each cohort. Across all cohorts, deletion CNAs were more common than amplification CNAs, with deletion events constituting approximately 73% of all
Table 2. Extent of CNAs per Cohort.

|                      | BRAFmut/MSS | Paired t-test within | BRAFwt/MSS | Paired t-test within | P Value between MSS | BRAFmut/MSI | P Value between all 3 cohorts |
|----------------------|-------------|----------------------|------------|----------------------|---------------------|-------------|------------------------------|
|                      | n           |                      |            |                      |                     |             |                              |
| Total Number of CNAs in Cohort | 1084        | -                    | 536        | -                    | 0.36                | 30          | -                            |
| Average Number CNAs per Cancer | 32.8        | -                    | 29.8       | -                    | 0.86                | 5.4         | <0.0001                      |
| Average Length of CNA (Mb) | 20.7        | -                    | 33.4       | -                    | <0.0001             | 23.6        | <0.0001                      |
| Median Fraction of CNA over chromosome arm lengths | 0.09        | -                    | 0.39       | -                    | <0.0001             | 0.02        | <0.0001                      |
| Av. Fraction of CNA over chromosome arm lengths | 0.32        | -                    | 0.50       | -                    | <0.0001             | 0.36        | <0.0001                      |
| Deletion CNAs in Cohort | 815/1084 (75.2%) | -                  | 372/536 (69.4%) | -                  | 0.016 | 120/162 (74.1%) | 0.045 |
| Amplification CNAs in Cohort | 269/1084 (24.8%) | -                  | 164/536 (30.6%) | -                  | 0.0006 | 42/162 (25.9%) | -     |
| Average No. Deletion CNAs per cancer | 24.7 | <0.0001 | 20.7 | 0.0006 | 0.65 | 4.0 | <0.0001 |
| Average No. Amplification CNAs per cancer | 82 | 9.1 | 0.92 | 1.4 | 0.0005 |
| Av. % of Genome Affected by CNAs per cancer | 23.9% | - | 34.9% | - | 0.1 | 4.5% | <0.0001 |
| Av % of Genome Affected by Deletion CNAs per cancer | 18.6% | <0.0001 | 22.7% | 0.018 | 0.56 | 2.2% | <0.0001 |
| Av % of Genome Affected by Amplification CNAs per cancer | 5.2% | 12.2% | 0.012 | 2.2% | <0.0001 |
| Whole Chromosome Arm CNAs in Cohort | 187/1084 (17.3%) | - | 171/536 (31.9%) | - | <0.0001 | 41/162 (25.3%) | <0.0001 |
| Regional CNAs in Cohort | 131/1084 (12.1%) | - | 71/536 (13.2%) | - | 0.5 | 18/162 (11.1%) | 0.70 |
| Focal CNAs in Cohort | 766/1084 (70.7%) | - | 294/536 (54.9%) | - | <0.0001 | 103/162 (63.6%) | <0.0001 |
| Average No. Whole Arm CNAs per cancer | 5.7 | <0.0001 | 9.5 | 0.0091 | 0.04 | 1.4 | <0.0001 |
| Average No. Regional CNAs per cancer | 4.0 | 3.9 | 0.99 | 0.6 | <0.0001 |
| Average No. Focal CNAs per cancer | 23.2 | 16.3 | 0.35 | 3.4 | <0.0001 |
| Average No. Whole Arm Loss CNAs per cancer | 4.8 | <0.0001 | 7.1 | 0.001 | 0.16 | 0.67 | <0.0001 |
| Average No. Whole Arm Gain CNAs per cancer | 0.9 | 2.4 | 0.01 | 0.7 | 0.004 |
The BRAFmut/MSS cohort had a significantly greater average percentage of the genome affected by amplification events than BRAFwt/MSS cancers (12.2% vs 5.2% respectively; \( p = 0.01 \)) (Table 2).

The most frequent deletion events occurring in at least 50% of cancers in both MSS cohorts, involved chromosomes 1p, 4q, 5q, 17p, 18q and 22q. The BRAFwt/MSS cohort had significantly more common deletion events than the BRAFwt/MSS cohort at chromosomes 6p (\( p = 0.02 \)), 6q (\( p < 0.05 \)) and 17q (\( p = 0.02 \)) (Figure 1). Significantly more frequent amplification events occurred in BRAFwt/MSS compared to BRAFmut/MSS cancers at 13q (\( p = 0.0009 \)) and 7q (\( p = 0.006 \)). The BRAFmut/MSS cancers had significantly more frequent amplification events at 8q compared to BRAFwt/MSS cancers (\( p = 0.02 \)) (Figure 1).

The average number of the specific type of either amplification or deletion CNAs per cancer demonstrated the MSS cohorts had similar rates of types of events (Table 2). However the BRAFwt/MSS cancers had significantly longer lengths of all types of deletion and amplification events, except cnLOH CNAs (Figure 2). BRAFmut/MSS had significantly lower rates and shorter lengths of all types of events compared to the MSS cohorts (Table 2, Figure 2).

### Frequency of Copy Number Aberrations According to Length

All CNAs were assessed for the fraction of coverage according to the specific chromosomal arm. Analysis of the length of all CNAs showed the vast majority were either less than 50% or longer than 95% the length of a chromosome arm for each of the three cohorts (Figure S2 in File S1). Therefore, in order to further compare frequencies of CNAs between cohorts, CNAs were considered as either whole arm (\( \geq 95\% \) chromosomal arm length), or focal (as \(< 50\% \) chromosomal arm length [24,41,42,43]). The remaining CNAs (50–94% chromosome arm length) were considered as regional events. Varying the threshold of the focal length from \(< 35\%\) to \(< 65\%\) chromosome arm length, still resulted in the majority of CNAs being kept in either the focal or whole length subsets, and did not alter the statistical significance of important findings (Tables S3A and S3B in File S1).

### Whole Chromosome Arm Copy Number Aberrations

The BRAFwt/MSS cohort had a significantly higher propensity for whole chromosome arm CNA events at 32% compared to the BRAFmut/MSS cohort at 17% \( (p < 0.0001) \) (Table 2). This corresponded to a significantly higher average rate of whole chromosome arm CNAs per cancer in BRAFwt/MSS compared to BRAFmut/MSS cancers \( (p = 0.04) \); and a greater average proportion of genome affected by whole arm events in BRAFwt/MSS compared to BRAFmut/MSS cancers \( (22\% \text{ vs } 12\%, \ p = 0.02) \) (Table 2). The BRAFmut/MSS cohort had the lowest whole arm CNA rate of just 1.4 per cancer \( (p < 0.0001) \); and just 3% of their genome affected by whole arm CNAs \( (p < 0.0001) \) (Table 2). Within both MSS cohorts, the average number of whole arm losses were significantly greater than the average number of whole arm gains \( (BRAFmut/MSS \ p < 0.0001, \ BRAFwt/MSS \ p = 0.001) \). BRAFwt/MSS cancers had significantly more whole arm gain events than BRAFmut/MSS cancers \( (p = 0.01) \) (Table 2).

### Regional Copy Number Aberrations

Rates of regional CNAs were similar between cohorts and whilst they occurred at a lower rate compared to whole arm and focal events, their inclusion allowed for a comprehensive description of CIN across all three cohorts (Table 2). Both MSS cohorts had significantly more regional deletion than amplification events per sample (Table 2).

### Focal Copy Number Aberrations

The BRAFmut/MSS cohort had the highest proportion of focal CNAs at 70.7% of all

---

### Table 2: Cont.

| Cohort       | P Value       | P Value       | P Value       | P Value       |
|--------------|---------------|---------------|---------------|---------------|
| Av. No. Regional Deletion CNAs per cancer | 0.0001        | 0.0006        | 0.0008        | 0.001         |
| Av. No. Regional Amplification CNAs per cancer | 0.0004        | 0.03          | 0.07          | 0.018         |
| Av. % Genome Affected by Whole Arm CNAs | 0.0002        | 0.094         | 0.98          | 0.68          |
| Av. % Genome Affected by Focal CNAs | 6.3%          | 6.6%          | 0.5%          | 0.3%          |

* Adjusted for multiple comparisons. doi:10.1371/journal.pone.0091739.t002
CNAs, whereas the BRAFwt/MSS had significantly less at 54.9% (p < 0.0001). This equated to a rate of 23.2 focal CNAs per BRAFmut/MSS cancer, and 16.3 per BRAFwt/MSS cancer; the BRAFmut/MSI cancers had substantially fewer focal CNAs at 3.4 per cancer (p < 0.0001) (Table 2). There were significantly differing average lengths of focal aberrations per cohort with 6.3 Mb for BRAFmut/MSS, 10.9 Mb for BRAFwt/MSS and 2.3 Mb for BRAFmut/MSI cancers (p < 0.0001).

In all cohorts focal deletion CNAs, predominantly through LOH events, were significantly greater than focal amplification CNAs (BRAFmut/MSS p = 0.004, BRAFwt/MSS p = 0.05) (Table 2). Compared to BRAFwt/ MSS cancers, BRAFmut/MSS cancers showed significantly more frequent focal deletions at 18q (11/33, 33% Vs 1/18, 6%; p = 0.04) predominantly encompassing 18q21.2 which includes the SMAD2 gene locus. Focal amplifications in BRAFmut/MSS cancers were also more common compared to BRAFwt/MSS cancers at 8q (11/33, 33% Vs 1/18, 6%; p = 0.04) predominantly at 8q24.21 covering the Myc locus, and 18q (7/33, 21% Vs 0/18; p = 0.04) affecting 18q11.2 (containing GATA6 and CTAGE).

**Minimal Common Regions (MCRs).** Minimal common regions were considered to include all lengths of CNAs. Both MSS cohorts showed a high rate of cancers (≥40%) with targeted deletion events at several loci previously associated with CRC, such as 18q21.1–18q21.2 (which includes SMAD2, SMAD4, DCC) and 17p13.1 (p53) (Table S4A in File S1). Loci not as commonly associated with CRC were also found to be deleted in a similar proportion of MSS cancers, and included 22q12.1, 22q11.1, 22q13.2, 17p12, 17p11.2, each of which contain several cancer related genes (Table S4A in File S1).

Analysis of MCRs affecting ≥20% of cancers in at least one of the two MSS cohorts revealed several loci where the rates of CNAs differed substantially between cohorts. Although after adjustment for multiple comparisons significance was no longer reached, the BRAFmut/MSS cancers had a high frequency of deletion CNAs compared to BRAFwt/MSS cancers at several loci on 17q and 6p.

**Figure 1. Percentage of cancers per cohort that had an amplification or deletion copy number aberration at each chromosome arm.** Asterisks indicate those chromosome arms where significant differences (p < 0.05) in the rate of CNAs per cancer occurred between MSS cohorts (red for the BRAFmut/MSI cohort and green for the BRAFwt/MSS cohort to indicate which has a significantly greater rate of CNAs per cancer).

doi:10.1371/journal.pone.0091739.g001

**Figure 2. The average length of specific types of deletion and amplification copy number aberrations per cancer.** There were significantly longer lengths for all events (except cnLOH) in the BRAFwt/MSS compared to the BRAFmut/MSS cohort. BRAFmut/MSI cancers had significantly shorter lengths for all types of events compared to MSS cancers (p < 0.0001).

doi:10.1371/journal.pone.0091739.g002
Table 3. Minimal Common Regions (MCRs) affecting ≥20% of cancers in at least one of the BRAFmut/MSS or BRAFwt/MSS cohorts where differences in CNA frequencies were detected between MSS cohorts.

| Chr | Arm start | Chr band start | Chr end position (bp) | Length of MCR (bp) | Type of CNA | % of BRAFmut / MSS, n=33 | % of BRAFwt / MSS, n=18 | p value | Adjusted p value | Potential Cancer Related Genes Involved |
|-----|-----------|----------------|----------------------|--------------------|-------------|--------------------------|--------------------------|---------|-----------------|---------------------------------------|
| 2q  | q37.3     | 240,832,001 q37.3 | 242,518,000 | 1,686,000 | Deletion | 6.1 | 33.3 | 0.02 | 0.27 | GPC1, CAPN10, KIF1A, SEPT2, STK25 |
| 5q  | q34.3     | 165,279,001 q34  | 167,417,000 | 2,138,000 | Deletion | 27.3 | 55.6 | 0.07 | 0.49 | ODZ2 |
| 6p  | p25.1     | 4,134,083 p25.1 | 7,009,966 | 2,875,884 | Deletion | 45.5 | 5.6 | 0.004 | 0.27 | CDYL |
| 6p  | p22.3     | 15,240,001 p22.3 | 15,916,000 | 676,000 | Deletion | 42.4 | 11.1 | 0.03 | 0.34 | JARID2 |
| 6p  | p21.33    | 31,097,001 p21.33 | 31,680,000 | 583,000 | Deletion | 39.4 | 5.6 | 0.01 | 0.27 | MICA, MICB, TNF |
| 6q  | q16.1     | 99,203,001 q16.2 | 100,187,000 | 984,000 | Deletion | 27.3 | 5.6 | 0.08 | 0.49 | CCNC |
| 17q | q22       | 55,950,001 q22 | 57,384,000 | 1,434,000 | Deletion | 57.6 | 22.2 | 0.02 | 0.27 | RNF41, VEZF1, SEPT4, TE9X1, RAD51C, PPM1E, TRIM37, SVA2 |
| 17q | q24.1     | 62,839,470 q24.1 | 63,914,355 | 1,074,886 | Deletion | 51.5 | 22.2 | 0.07 | 0.49 | AXIN2, GNA13 |
| 17q | q24.3     | 68,762,000 q24.3 | 70,569,000 | 1,807,001 | Deletion | 57.6 | 27.8 | 0.02 | 0.27 | SOX9 |
| 17q | q25.1     | 70,650,001 q25.1 | 71,431,000 | 781,000 | Deletion | 54.5 | 22.2 | 0.04 | 0.38 | SOD2, SSTR2, CDC42E4 |
| 7p  | p21.1     | 7,492,001 p21.1 | 20,814,000 | 13,322,000 | Amp | 9.1 | 38.9 | 0.02 | 0.051 | PHF14, ARF4L, ETV1, AGR2/3, BZW2, HDAC5, TWIST1, MACC1, JTG8, ALCB5 |
| 7q  | q21.11    | 81,993,001 q21.11 | 82,532,000 | 539,000 | Amp | 3.0 | 38.9 | 0.002 | 0.012 | CACNA2D3, PCLO |
| 7q  | q36.2     | 154,436,001 q36.3 | 159,119,000 | 4,683,000 | Amp | 3.0 | 38.9 | 0.002 | 0.012 | DPI6, INSI61, SHH, RNF32, MXN1, LMBR1, PTPRN2, NQAPG2, VIPR2 |
| 8q  | q24.21    | 128,085,001 q24.21 | 129,127,000 | 1,042,000 | Amp | 48.5 | 16.7 | 0.035 | 0.079 | MYC, PVTT |
| 8q  | q23.1     | 109,055,001 q23.2 | 109,888,000 | 833,000 | Amp | 45.5 | 16.7 | 0.065 | 0.097 | RSP02, ERF3E |
| 8q  | q24.11    | 117,636,001 q24.11 | 118,097,000 | 461,000 | Amp | 45.5 | 16.7 | 0.065 | 0.097 | EFR3H, UTP23, RAD21 |
| 8q  | q24.22    | 132,704,000 q24.22 | 135,106,000 | 2,402,001 | Amp | 45.5 | 16.7 | 0.065 | 0.097 | EFR3A, PHF20L1, SLA, WISP1, NDRG1, ST3GAL1 |
| Chr Arm | Start position (bp) | End position (bp) | Length of MCR (bp) | Type of CNA | % of BRAF mut / MSS. n = 33 | % of BRAF wt/ MSS. n = 18 | p value | Adjusted p value | Potential Cancer Related Genes Involved |
|--------|---------------------|-------------------|-------------------|-------------|-----------------------------|-----------------------------|---------|-----------------|----------------------------------------|
| 8q     | q22.3               | 100,850,001       | q22.3             | 102,660,000 | 1,810,000                   | Amp                         | 42.4    | 16.7            | VPS13B, RGS22, SPAG1, RNF19A, ANXRD46, SNX1, PARBP1, YWHAZ, ZNF706, GRHL2 |
| 13q    | q14.11              | 40,387,001        | q14.11            | 44,439,000  | 4,052,000                   | Amp                         | 12.1    | 66.7            | FOXO1, ELF1, DGKH, EPSTI7, ENOX1       |
| 13q    | q21.33              | 70,105,001        | q21.33            | 70,950,000  | 845,000                     | Amp                         | 21.2    | 61.1            | KLHL1                                   |
| 18q    | q11.2               | 19,636,000        | q11.2             | 20,838,000  | 1,202,001                   | Amp                         | 21.2    | 0.0             | GATA6, CTAGE, RBBP8, CABLES27           |
| 20p    | p11.21              | 24,267,001        | p11.1             | 25,184,000  | 917,000                     | Amp                         | 9.1     | 44.4            | AURKA, TSHZ2, BCA51, PFDN4, CASS4, BMP7, CTCFL, RAE1, ZBP1, CTCFL, PMEP1 |
| 20q    | q13.2               | 50,823,001        | q13.33            | 56,496,000  | 5,673,000                   | Amp                         | 18.2    | 55.6            |                                         |

(Benjamini-Hochberg method applied for adjusted p values).

doi:10.1371/journal.pone.0091739.t003
including 17q22 (that contains cancer related genes RNF43 and VEZF1), 17q24.3 (SOX9) and 6p25.1 (CD1L) (Table 3). Amplification MCRs were more common in BRAFmut/MSS than BRAFwt/MSS cancers at 8q24.21 (Myc), and 18q11.2 (GATA6, CTAGE) (Table 3).

The BRAFmut/MSI cancers had substantially fewer MCRs than the MSS cohorts, however they did have a comparatively high proportion of cancers (>20%) with focal deletions at 3p14.2 (FHIT), 16p13.3 (RBFOX1) and 20p12.1 (MACROD2) (Table S4C in File 1).

Different Patterns of CIN exist between the BRAFmut/ MSS and BRAFwt/MSS Cancers

Although the MSS cohorts had similar average numbers of CNAs per cancer (Figure 3A, Table 2), the BRAFwt/MSS cancers had the greatest proportion of genome affected by CNAs (Figure 3B). CIN in a typical BRAFwt/MSS cancer predominantly occurred via whole chromosome arm events, whereas CIN in BRAFmut/MSS cancers largely correlated with frequent focal CNAs which resulted in a smaller proportion of genome affected (Figures 3A and 3B).

Figure 3. Average number of copy number aberrations (CNAs) and percentage of genome affected per MSS cohort. A) Average number of CNAs delineated by length per cancer in each MSS cohort. MSS cohorts had a similar number of overall CNAs occurring per cancer, however the BRAFmut/MSS cancers showed a greater number of focal CNAs, with the BRAFwt/MSS cancers having a greater number of whole arm events. BRAFmut/MSI cancers had considerably fewer CNAs of all types. B) Average percentage of genome affected by CNAs delineated by length in each MSS cohort. BRAFwt/MSS cancers had the greatest proportion of genome affected by CNA events, which was due to the higher number of whole arm events in this cohort. BRAFmut/MSS cancers showed a lower proportion of the genome affected by CNAs, which is reflective of the comparably lower rate of whole arm and higher rate of focal events that occurred compared to BRAFwt/MSS cancers.

doi:10.1371/journal.pone.0091739.g003

Figure 4. Heat map showing the distribution of whole chromosome arm and focal copy number aberrations across the cohorts. Sample heterogeneity occurred within cohorts however a focal pattern is evident in the BRAFmut/MSS and a whole arm pattern is present in the BRAFwt/MSS cohort.

doi:10.1371/journal.pone.0091739.g004
Figure 4 shows the genome-wide distribution of CNAs across the three cohorts according to the type and length of event that occurred at a particular chromosome arm. Although the MSS cohorts do show sample heterogeneity, the predominantly focal pattern of CIN is evident in \textit{BRAF} mut/MSS cancers, and this contrasts to the whole chromosome arm pattern seen in \textit{BRAF} wt/MSS cancers.

**Discussion**

This study has shown that \textit{BRAF} mut/MSS colorectal cancers predominantly harbour focal or targeted CNAs, whereas the \textit{BRAF} wt/MSS colorectal cancers have significantly more frequent whole chromosome arm CNAs. This results in a greater average percentage of genome affected by CIN in \textit{BRAF} wt/MSS compared to \textit{BRAF} mut/MSS cancers. \textit{BRAF} wt cancers show a similarly high percentage of the genome affected by whole arm CNAs as a previous report of a large series of different cancer types, including CRC [41]. Comparatively, the \textit{BRAF} mut/MSS cohort has a significantly smaller proportion of their CIN affected genomes covered by whole arm events. Overall these observations identify that \textit{BRAF} mut/MSS cancers represent a more ‘focal pattern’ of CIN, whereas \textit{BRAF} wt/MSS cancers display a ‘whole chromosome arm’ pattern of CIN.

Across all cohorts, the frequency of deletion CNAs exceeded amplification events for all types of CNAs. This difference may reflect a greater selection for deletions which could be tumour promoting and involve more simple mechanisms of acquisition, whereas amplifications may require more complex interactions with homologous and non-homologous chromosomes [44].

Whole chromosome arm CNAs were significantly more common in \textit{BRAF} wt/MSS than in \textit{BRAF} mut/MSS cancers. Whole chromosome arm CNAs can promote tumourigenesis through the gain of oncogenes and loss of tumour suppressors on a large scale [19]. However, whole chromosome arm CIN is also linked with cancer repression where the reverse of cancer promoting effects occur, and there is an overabundant loss of oncogenic factors and gain of tumour suppressive effects [19]. Additionally, increased chromosome copy number can lead to excessive protein production which may place greater metabolic stress on the cancer cell and ultimately reduce their rate of cellular growth and proliferation [19,45,46,47]. Whether the propensity of whole arm CNAs may be contributing to the less adverse nature of \textit{BRAF} wt/MSS cancers compared to \textit{BRAF} mut/MSS cancers through mechanisms described above, may warrant further investigation.

The aggressive \textit{BRAF} mut/MSS cancers had a significantly higher rate of focal CNAs across their genomes. There have been previous reports of early compared to late stage cancers harbouring more whole arm compared to focal CNAs [48], where stage I breast cancers were found to have more frequent whole chromosome arm CNAs compared to stage II/III breast cancers which had smaller, more complex events [49]. Furthermore, a detrimental clinical outcome in melanoma has been associated with a greater frequency of focal CNAs compared to whole chromosome arm events [50]. Potentially these complex, sub-chromosomal events may be facilitating cancer progression by specifically targeting key drivers of tumourigenesis.

Different mechanisms relating to the origin of either whole chromosome or focal CNAs exist. It has been commonly reported that CIN involving whole chromosomes is due to errors relating to chromosome segregation during mitosis [19,51]. These errors are more likely to be those involving dysregulation of kinetochore-microtubule attachments, termed merotely, where a chromosome attached to both spindle poles mis-segregates at anaphase and results in whole chromosome aneuploidy [20]. The ‘focal’ pattern of CIN we have identified in \textit{BRAF} mut/MSS cancers may associate with ‘structural’ CIN which involves structural sub-chromosomal rearrangements including deletions, amplifications and translocations [20]. The causes of these types of structural aberrations may involve dysfunctional repair processes of double strand breaks by homologous recombination and the error prone non-homologous end joining [52]. Potentially many of the particularly complex patterns of structural aberrations may not be driver mechanisms in tumourigenesis but instead could be consequences of these disrupted DNA damage and repair processes. Studies of further genetic abnormalities unique to these specific CRC subgroups that could predispose to their respective patterns of CIN may be warranted.

We have previously found that the CpG Island Methylator Phenotype (CIMP) and CIN can co-exist in \textit{BRAF} mut/MSS cancers [22]. Potentially, the degree of methylation present and the subsequent effects on the extent of chromatin compaction may relate to the different rates of focal and whole arm CNAs observed between \textit{BRAF} mut/MSS and \textit{BRAF} wt/MSS cancers. Regional hypermethylation as present in CIMP positive cancers associates with increased levels of condensed chromatin, whereas widespread hypomethylation is present in cancers with a more open chromatin conformation [53]. This and our previous studies have found a substantial rate of \textit{BRAF} mut/MSS cancers to be CIMP high [22,36], which may confer a more closed chromatin structure in these cancers. Global hypomethylation is well documented in CRC where it can associate with CIN and affects predominantly \textit{BRAF} wild type cancers [54]. A study found in regions with predominantly open or relaxed chromatin, repair mechanisms following double strand breaks were quicker to act due to greater accessibility of repair enzymes to the damaged site and subsequently resulted in less chromosome fragmentation [55]. These findings could help to account for the reduced rate of focal CNAs found in the \textit{BRAF} wt/MSS cohort. The majority of \textit{BRAF} mut/MSS cancers were CIMP high and demonstrated a ‘focal pattern’ of CIN, which may suggest that a condensed chromatin structure contributes to a propensity of focal CNAs.

CIN was evident in the majority of \textit{BRAF} mut/MSI cancers, but affected a much smaller proportion of the genome compared to MSS cancers. Several genomic regions containing fragile sites, such as the \textit{FHIT} gene locus at 3p14.2, \textit{RBFOX1} at 16p13.35 and \textit{MICROD2} at 20p12.1 that were targeted for deletion in MSS cancers, were also relatively commonly deleted in \textit{BRAF} mut/MSI cancers [56,57]. The lower degree of CIN present in MSI cancers may relate to findings that the onset of MSI is an early event in the development of MSI/CIMP positive cancers [58], and as this type of genomic instability is already present, there may be redundancy for the development of further genomic instability through CIN.

As well as distinct variations in the pattern of CIN displayed between the two MSS cohorts, analysis of the minimal common regions (MCRs) of CIN revealed differential rates of either deletion or amplification CNAs occurring at certain genomic loci between them. Many of those more frequent in \textit{BRAF} mut/MSS cancers, for example deletions at 6p23.1-6p21.33 and at specific loci on 17q where several Wnt regulatory genes reside (\textit{RNF43, AXIN2} and \textit{SOX9}) [59,60,61], have not commonly been associated with CRC. Additionally, \textit{BRAF} mut/MSS cancers had a higher frequency of targeted amplification of 8q24.13 that contains the Wnt signalling effector, \textit{Myc}. These Wnt pathway related genes that may be specifically targeted in \textit{BRAF} mut/MSS cancers could be an alternative mechanism in promotion of the Wnt signal in this cancer subtype. Amplification CNAs occurred at 18q11.2 in
**BRAFmut/MSS cancers**, whereas non-specific whole arm deletion events affected this region in **BRAFvet/MSS** cancers. The 18q11.2 locus harbours two genes, GATA6 and CTAG6E that have previously been reported to be amplified and upregulated in gastrointestinal cancers [39,43] including metastatic CRC [62]. Extended studies to better define the loci where greater rates of MCRs occur in **BRAFvet/MSS** compared to **BRAFmut/MSS** cancers may be indicated to ascertain whether these are driver mechanisms that may uniquely promote tumourigenesis in the **BRAFmut/MSS** cohort.

This study has determined that a substantial presence of genome-wide CIN exists in the aggressive **BRAFmut/MSS** cancers of the serrated neoplastic pathway. Significantly different patterns of CIN were found between the two MSS cohorts. **BRAFmut/Wt** MSS cancers were found to harbour frequent focal length CNAs and therefore display a ‘focal pattern’ of CIN suggestive of commonly occurring structural rearrangements. Alternatively, the greater presence of whole arm CNAs in the **BRAFmut/MSS** cancers indicate they have a ‘whole chromosome arm pattern’ of CIN that may be due to dysfunctional mitotic events. Overall these findings suggest that either presence or absence of the **BRAF** V600E mutation could potentially affect subsequent acquisition of genomic instability in these subgroups of CRC. Extended studies to ascertain the clinical impact of the different patterns of CIN identified in these cancer subgroups may be warranted. Additionally, specific loci not as commonly associated with CRC that were more frequently affected by CIN in **BRAFmut/MSS** cancers were found, and this could help in the identification of molecular events that may correlate with the aggressive nature of these **BRAFmut/MSS** colorectal cancers.

### Supporting Information

**File S1** Contains the files: Figure S1, Automatic inclusion of cancer samples with tumour percentage ≥40% as estimated by SiDoCoN. Figure S2. Frequency of copy number aberrations occurring delineated by their fraction of chromosome arm per cohort. Table S1. Verification of the inclusion of cancers that had a tumour percentage ≤40% in this study by analysing the presence of cancer related molecular changes. Table S2. Data and statistical analysis with the exclusion of cancers with <40% tumour content. Table S3. Data and statistical analysis of cohorts when threshold of focal CNA group is changed to <35% chromosome arm length. Table S3B. Data and statistical analysis of cohorts when threshold of focal CNA group is changed to <65% chromosome arm length. Table S4. Minimal Common Regions (MCRs) of copy number aberrations affecting ≥20% of cancers in at least one of the **BRAFmut/MSS** or **BRAFvet/MSS** cohorts. (DOCX)

### Author Contributions

Conceived and designed the experiments: VLJW CEB BAL. Performed the experiments: CEB LW. Analyzed the data: CEB DJN LFJW. Contributed reagents/materials/analysis tools: GWM BAL DJN. Wrote the paper: CEB. Designed the software used in the analysis: DJN.

### References

1. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, et al. (2002) Tumorigenesis: RAS oncoproteins and mismatch-repair status. Nature 418: 934.
2. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, et al. (2004) BRAF mutation is associated with DNA methylation in serrated polyposis and cancers of the colorectum. Gut 53: 1137–1144.
3. Leggett B, Whitehall V (2010) Role of the serrated pathway in colorectal cancer pathogenesis. Gastroenterology 138: 2088–2100.
4. Koinuma K, Shitoh K, Miyakura Y, Furukawa T, Yamashita Y, et al. (2004) Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas. Int J Cancer 108: 237–242.
5. Weißenberger DJ, Siegmund KD, Campan M, Young J, Long TI, et al. (2006) CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 38: 787–793.
6. Jass JR, Do KA, Simms LA, Iino H, Wynter C, et al. (1998) Morphology of colorectal carcinoma patients. Gastroenterology 131: 729–737.
7. Ionov Y, Paulino A, Shihbata D, Peruchó M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colon carcinogenesis. Nature 363: 530–531.
8. Rothe RA, Pelomaki P, Meling GI, Aaltonen LA, Nystrom-Lahni M, et al. (2003) Genomic instability in colorectal cancer: relationship to clinico-pathological variables and family history. Cancer Res 53: 5849–5852.
9. Jassem J, Batail I, Berndt U, Elfgren J, Huggard H, et al. (2012) Combined genomic characterization of human colon and rectal cancer. Nature 487: 330–337.
10. Ionov Y, Peinado MA, Malkhosyan S, Shihbata D, Peruchó M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colon carcinogenesis. Nature 363: 530–531.
11. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, et al. (2002) Tumorigenesis: RAS oncoproteins and mismatch-repair status. Nature 418: 934.
12. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61: 759–767.
13. Sinicrope FA, Rego RL, Halling KC, Foster N, Sargent DJ, et al. (2006) Int J Cancer 118: 1721–1727.
14. Waithera A, Houlston R, Tomlinson I (2006) Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. Gut 57: 941–950.
32. Ogino S, Kawasaki T, Kirkner GJ, Ohishi M, Fuchs CS (2007) 18q loss of heterozygosity in microsatellite stable colorectal cancer is correlated with CpG island methylator phenotype-negative (CIMP-0) and inversely with CIMP-low and CIMP-high. BMC Cancer 7: 72.
33. Savada T, Yamamoto E, Suzuki H, Nojima M, Mansuyama R, et al. (2013) Association between genomic alterations and metastatic behavior of colorectal cancer identified by array-based comparative genomic hybridization. Genes Chromosomes Cancer 52: 140–149.
34. Kozlowska J, Karpinski P, Samida E, Laczmanska I, Misak B, et al. (2012) Assessment of chromosomal imbalances in CIMP-high and CIMP-low/CIMP-0 colorectal cancers. Tumour Biol 33: 1015–1019.
35. Cheng YW, Pincus H, Bacolod MD, Schemmann G, Giardina SF, et al. (2008) Cpg island methylator phenotype associates with low-degree chromosomal abnormalities in colorectal cancer. Clin Cancer Res 14: 6005–6013.
36. Bond CE, Umapathy A, Ramnes I, Greco SA, Zhen Zhao Z, et al. (2012) p53 distinct patterns of genome instability and oncogenesis. Cancer Res 72: 4383–4393.
37. Whitehall VL, Rickman C, Bond CE, Ramsnes I, Greco SA, et al. (2012) Oncogenic PIK3CA mutations in colorectal cancers and polyps. Int J Cancer 131: 813–820.
38. Nancarrow DJ, Handoko HY, Stark MS, Whiteman DC, Hayward NK (2007) SiDCoN: a tool to aid scoring of DNA copy number changes in SNP chip data. PLoS One 2: e1093.
39. Nancarrow DJ, Handoko HY, Smithers BM, Gotley DC, Drew PA, et al. (2008) Genome-wide copy number analysis in esophageal adenocarcinoma using high-density single-nucleotide polymorphism arrays. Cancer Res 68: 4163–4172.
40. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, et al. (2002) The human genome browser at UCSC. Genome Res 12: 996–1006.
41. Bergsikam R, Merrel CH, Porter D, Wei G, Raychaudhuri S, et al. (2010) The landscape of somatic copy-number alteration across human cancers. Nature 463: 899–905.
42. Kim TM, Xi R, Luquette LJ, Park RW, Johnson MD, et al. (2013) Functional genomic analysis of chromosomal aberrations in a compendium of 8000 cancer genomes. Nature 499: 413–419.
43. Dukal AM, Schumacher SE, van Lieshout J, Imamura Y, Fox C, et al. (2012) Gastrointestinal adenocarcinomas of the esophagus, stomach, and colon exhibit distinct patterns of genome instability and oncogenesis. Cancer Res 72: 4383–4393.
44. Fadenberg G, Getz G, Meyerson M, Mirny LA (2011) High order chromatin architecture shapes the landscape of chromosomal alterations in cancer. Nat Biotechnol 29: 1109–1113.
45. Weaver BA, Cleveland DW (2008) The aneuploidy paradox in cell growth and tumorigenesis. Cancer Cell 14: 431–433.
46. Tang YC, Amon A (2013) Gene copy-number alterations: a cost-benefit analysis. Cell 152: 394–405.
47. Williams BR, Prabhu VR, Hunter KE, Glazier CM, Whittaker CA, et al. (2008) Aneuploidy affects proliferation and spontaneous immortalization in mammary gland cells. Science 322: 703–709.
48. Martinec AC, van Wely KH (2010) Are aneuploidy and chromosome breakage caused by a CINgle mechanism? Cell Cycle 9: 2273–2280.
49. Roylande R, Gorman P, Papier T, Wan YL, Ives M, et al. (2006) A comprehensive study of chromosome 16q in invasive ductal and lobular breast carcinoma using array CGH. Oncogene 25: 6534–6535.
50. Hirsch D, Kemmerling R, Davs S, Camps J, Metzger PS, et al. (2013) Chromothripsis and focal copy number alterations determine poor outcome in malignant melanoma. Cancer Res 73: 1454–1460.
51. Jansen A, Medema RH (2012) Genetic instability: tipping the balance. Oncogene 32: 4459–4470.
52. Ohe G, Durante M (2010) DNA double strand breaks and chromosomal aberrations. Cytogenet Genome Res 128: 8–16.
53. Dong G, Nguyen A, Tanaka H, Matsuzaki K, Bell I, et al. (2006) Regional hypermethylation and global hypomethylation are associated with altered chromatin conformation and histone acetylation in colorectal cancer. Int J Cancer 118: 2999–3005.
54. Aruolo M, Balaguer F, Shiu J, Shen Y, Hur K, et al. A high degree of LINE-1 hypomethylation is a unique feature of early-onset colorectal cancer. PLoS One 7: e45337.
55. Mosesso P, Palini F, Pepe G, Pinero, J, Bellacima R, et al. Relationship between chromatin structure, DNA damage and repair following X-irradiation of human lymphocytes. Mutat Res 701: 86–91.
56. Xie T, G DA, Lamb JR, Martin E, Wang K, et al. (2012) A comprehensive characterization of genome-wide copy number aberrations in colorectal cancer reveals novel oncogenes and patterns of alterations. PLoS One 7: e42001.
57. Andersen CL, Lamy P, Thorsen K, Kjeldsen E, Wikman F, et al. (2011) Frequent genomic loss at chr16p13.2 is associated with poor prognosis in colorectal cancer. Int J Cancer 129: 1848–1858.
58. Beggs AD, Domingos E, Abulafi M, Hodgson SV, Tomlinson IP (2013) A study of genomic instability in early preneoplastic colonic lesions. Oncogene 32: 3313–3337.
59. Koo BK, Spitz M, Jordens I, Low YF, Stange DE, et al. (2012) Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. Nature 486: 663–669.
60. Behrens J, Jerchov S, Wurtele M, Grim M, Asbrand C, et al. (1998) Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. Science 280: 596–599.
61. Topol L, Chen W, Song H, Day TF, Yang Y (2009) Sox9 inhibits Wnt signaling by promoting beta-catenin phosphorylation in the nucleus. J Biol Chem 284: 3323–3333.
62. Belaguli NS, Aftab M, Zhang M, Albo D, et al. (2010) GATA6 promotes colon cancer cell invasion by regulating urokinase plasminogen activator gene expression. Neoplasia 12: 856-865.