Box 1

Patients and clinical samples

A total of paired 74 colorectal neoplasms were included in this study corresponding to 37 patients diagnosed with SCRC at "12 de Octubre" University Hospital (Madrid, Spain). Synchronous CRC refers to more than one pathologically carcinoma diagnosed at the same time or within 6 months of the initial diagnosis [2]. Using the classification based on clonal origin of simultaneous colorectal carcinomas and tumor locations [6]: 8 patients were categorized as MM, 8 belonged to the MP group, 12 individuals were PM and the remaining 9 patients were PP. We excluded all cases associated with CRC and polyposis hereditary syndromes.

From each patient, we carried out molecular analysis of two synchronous neoplasms, with stages and high-quality samples were selected by a pathologist and considered adequate when samples had more than 70% of tumor cells in the specimen. The protocol for DNA isolations was reported previously [6]. Clinicopathological features were collected using a multi-site database. All patients (or a first degree relative in case of death of the index case) provided informed consent, and the study was approved by the Ethics Committee of our Institution.

SNP-array analysis

Formalin-Fixed, Paraffin- Embedded (FFPE) samples were used to perform OncoScan assay (Affymetrix Inc). To analyze CNAs and regions with LOH on the paired samples using OncoScan FFPE assay based on molecular inversion probe technology, the manufacturer's instructions were followed. Briefly, annealing and denaturalization stages were carried out using a GeneAmp PCR system 9700 Thermal Cycler (Life Technologies). The DNA target was hybridized onto the OncoScan array and incubated in a Genechip Hybridization oven 640 (Affymetrix) at 49ºC and 60 rpm for 17 hours. Later, OncoScan arrays washed and stained on a GeneChips Fluidics Station 450 (Affymetrix). Finally, microarrays were scanned using a GeneChip scanner 3000 (Affymetrix). Quality analysis was performed by OncoScan Console software (Affymetrix) and CNV as well as LOH events were identified using Nexus Express for OncoScan 3.1 and Affymetrix OSCHP TruScan (Affymetrix). Detailed data analysis have been previously published [2]. Additionally, overall ploidy was performed applying the Affymetrix OSCHP TruScan and data was included in Gene Expression Omnibus (GEO) database (GSE110026).The chromosomal regions that involved at least 50 SNP markers and were more than 3 Mb long were considered to be regions with LOH. Regions with cnLOH were determined by simultaneous analysis of both CNA and LOH data – a region with LOH and copy number state of 2.

Box 2

DNA sequence analysis

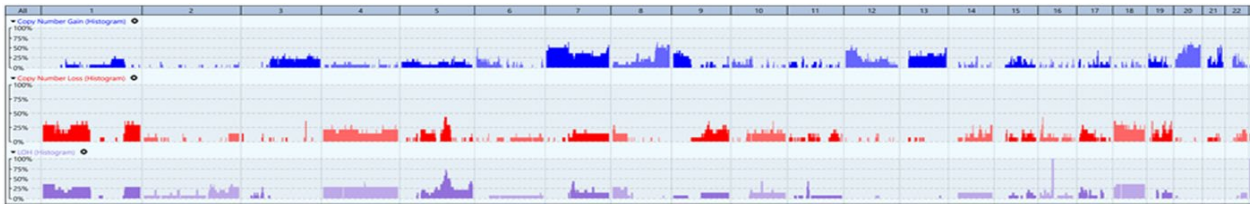
Ampliseq libraries were prepared using Ion AmpliSeq Library Kit 2.0 and the Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies). In order to prepare the library, 5 ng of DNA samples was used following the conditions provided by the manufacturer. To quantify amplified libraries, the Qubit 2.0 Fluorometer and the High Sensitivity Qubit Assay Kit (Life Technologies) were used. Ampliseq libraries were then clonally amplified onto Ion Sphere Particles (ISPs) in an emulsion PCR, which was carried out along with the enrichment in the Ion PGM™ System (Life Technologies) following the manufacturer's instructions. Enriched ISPs were loaded into Ion 318™ v2 chips (Life Technologies) and were sequenced using the Ion PGM™ Hi-Q™ View Sequencing Kit (Life Technologies) in an Ion Torrent PGM instrument (Life Technologies) and according to the user guide. Finally, data analysis was performed using Torrent Suite Software (Life Technologies) where alignment to the human genome (hg19) and base calling were executed by applying caller plug-in. Variants were annotated using Ion Reporter software (Life Technologies). The annotated variants in coding regions and those affecting splicing variants were visually inspected using Integrative Genomics Viewer (IGV; Broad Institute). Filtered variants which were identified as benign were excluded; thus only considering pathogenic variants for this study.

Statistical analysis

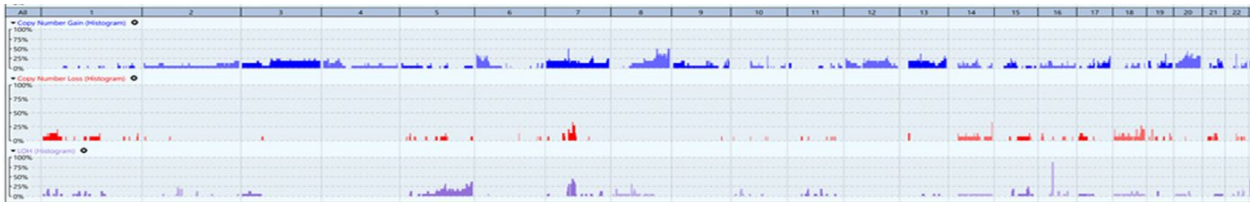
Categorical variables were expressed as number of cases and their percentage, and to compare cases the Pearson's Chi Square (χ^2) test was performed. Continuous variables were expressed as mean values plus/minus standard deviation (SD). Student's t test was done for comparison between two groups, while analysis of variance (ANOVA) (for normal distributions) or the Kruskal-Wallis tests (for nonparametric distributions) were used for comparisons between more than two groups for continuous variables. IBM SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software was used for statistical analyses, and a two-sided P value of<0.05 was considered statistically significant.

Box 3

(a)

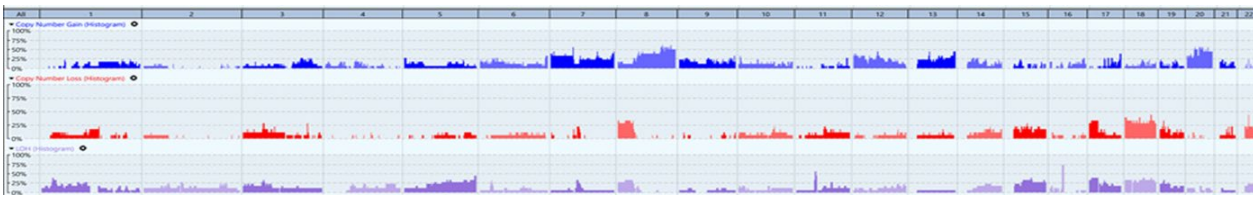


(b)



(c)

(d)



Frequency plots of the genomic profile of CNAs (blue plots represent gain regions and red plots show regions with losses) and LOH (purple plots) in the following SCRC sub-groups: (a) Monoclonal monosegmental, (b) Monoclonal pancolonic, (c) Polyclonal monosegmental, and (d) Polyclonal pancolonic.

Box 4

(a)

(b)

Visualization of the most common regions altered by cnLOH in SCRC via SNP array results. (A) 16p11.2-p11.1 (B) 11p11.2-p11.12.