Cytogenomic Changes in Sporadic Colorectal Cancer and Surrounding Nonneoplastic Tissues: The Relevance of Subtelomeric Copy Number Variations

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Abstract

The purpose of this study was to investigate the relevance of subtelomeric cytogenomic changes in patients with sporadic colorectal cancer (CRC) using multiplex ligation-dependent probe amplification (MLPA) and single nucleotide polymorphism arrays. The results revealed pathogenic genomic alterations in the *TNFRS18* (1p), *CHL1* (3p), *TRIML2* (4q), *FBXO25* (8p), *NKX3-1* (8p), *RECQL4* (8q), *DOCK8* (9p), *ZMYND11* (10p), *KDM5A* (12p), *PSPC1* (13q), *ADPRTL2* (14q), *MTA1* (14q), *DECR2* (16p), *GAS8* (16q), *THOC1* (18p), *CTDP1* (18q), *SOX12* (20p), *ADRM1* (20q), *UCKL1* (20q), *OPRL1* (20q), *IL17RA* (22q), and *SYBL1* (Xq) genes. We detected copy number variations (CNVs) with frequencies greater than 40% in the probes located in 20q, which contains very important genes in the study of tumors. These findings showed instability in the tumor genome and altered regions associated with cell migration, transcription activation, apoptosis, and immune system deregulation. Unexpectedly, we detected concomitant pathogenic CNVs in tumors and surrounding tissues. Our data suggest that characterizing the genomic CRC profile is an important contribution to better understanding instability as a mechanism of carcinogenesis in CRC patients.

Introduction

Investigations of cytogenomic changes are focused on genomic variations, such as copy number variations (CNVs) and architecture at the microscopic and submicroscopic levels. These encompass several pathogenic conditions, including many of the most common cancers [1, 2].

Genomic arrays are a useful tool for studying slight variations between whole genomes and play a role in research into both constitutional disorders and cancer [1, 3]. Different cytogenomic techniques have been used to detect these changes, including multiplex ligation-dependent probe amplification (MLPA) and single nucleotide polymorphism (SNP) arrays [3, 4]. MLPA is a useful method based on polymerase chain reaction (PCR) principles for the detection of different genomic abnormalities (aneuploidies, gene deletions or duplications, subtelomeric rearrangements and methylation status), allowing the detection of specific deletions, and duplications in regions of interest, whereas arrays can assess the CNVs and SNPs present in the whole genome of a patient in a single reaction [3, 5]. MLPA probes are evenly distributed over the investigated region, and the specific or overlapping genomic locations depended on the design of each set assay for a MLPA probe [1, 2, 4].

The study of genomic variations related to hereditary colorectal cancer (CRC) can provide strong clues to individual's sporadic CRC development predisposition [5, 6]. The presence of pathogenic CNVs may also disturb gene dosage and be a relevant source of cytogenomic instability in human cancers [1].

Despite extensive knowledge of chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) pathways in CRC, less is known about the role of genomic changes [2].

In sporadic cancer, CIN is characterized by gains and losses of small genomic segments or entire chromosomal arms, which are mainly caused by chromosome breaks [5, 6]. CIN can result from defects in chromosomal segregation, telomere instability, or the response to DNA damage [2]. Approximately 85% of CRC cases have accumulated chromosomal imbalances and runs of homozygosity (ROH), which lead to altered expression of tumor suppressor genes and oncogenes [2–7]. MSI is a hypermutable event caused by the loss of DNA mismatch repair activity, whereas a CIMP is produced by hypermethylation of promoter CpG island sites [7, 8]. Both mechanisms result in the inactivation of several tumor-related genes in CRC tissues [8–10].

The cytogenomic instability of cancer cells is also characterized by a high rate of telomere loss and double-strand breaks in subtelomeric regions; therefore, cytogenomic instability could contribute to chromosomal instability and tumor cell progression in CRC tissues [11]. Authors agree that ROH are an important genetic clue for understanding tumorigenesis and that the SNP array is currently the best resolution method for analyzing these changes [12].

The aim of this study was to investigate the role of subtelomeric genomic changes in neoplastic and nonneoplastic surrounding colorectal tissues in patients with sporadic CRC.

Materials And Methods

Study Participants

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of Faculdade de Medicina do ABC (808.569, approval date 26/09/2014).

We collected 33 CRC samples from patients with a mean age of 63 years old (49 to 84 years), including 16 males and 17 females, from the Division of Gastrointestinal Surgery of ABC Medical School (Santo André, São Paulo, Brazil). All patients underwent curative or palliative surgical resection of CRC by conventional or laparoscopic access. The information obtained from each patient included gender, age, family history of cancer, previous treatments with neoadjuvant therapy, primary tumor site, surgery performed, tumor histologic grade, metastasis (presence vs. absence), and staging [13].

Adult patients of both genders with the confirmed histological diagnosis of CRC confirmed by histopathological exam according WHO criteria [13, 14], age greater than 49 years old, and high quality of extracted DNA were included regardless of ethnicity.

Patients who had undergone neoadjuvant therapy (chemotherapy or radiotherapy); patients with familial adenomatous polyposis, hereditary CRC syndromes, colorectal neoplasia other than carcinoma, or inflammatory bowel disease; patients with synchronous or metachronous tumors elsewhere; and those who had incomplete anatomopathological data were excluded. The criterion for exclusion of suspected family cancer for patients was the existence of at least 3 first-degree family members and age of less than 50 years old with CRC or another related cancer, such as cancer of endometrium, stomach, kidney, ureter, biliary, or small intestine [14].

After applying the inclusion and exclusion criteria, we selected 10 patients (7 women and 3 men) and excluded 23. The mean age was 62.1 years (49 to 84 years). Primary tumors were located in the left colon in 5 cases, the right colon in 4, and the rectum in 1. The operations performed were rectosigmoidectomy in 4 cases, right colectomy in 4, and left colectomy in 2. Patients' characteristics are shown in Table 1.

Collection and Classification of Samples

To obtain patients' DNA, 5 ml of peripheral blood was collected immediately before the beginning of the surgical procedure, and tissue samples were collected at the time of surgery for tumor resection. Two tissue samples (approximately 1 cm^3 in size) were obtained: a sample of the macroscopically neoplastic region of the tumor, and another sample of the distant surrounding colorectal tissue located within 10 cm cranially of the tumor. Tissues were preserved in 400 μL of RNAlater® Stabilization Solution (Ambion® Life Technologies, Austin, Texas, Unites States).

Other samples of the areas considered to be tumoral and nontumoral were obtained, embedded in paraffin, stained with hematoxilin and eosin, and subjected to microscopic examination to confirm the presence or absence of neoplasia.

Cytogenomic Techniques

Following the manufacturer's instructions, we obtained genomic DNA from blood and tissues using the QIAamp® Midi DNA Blood and Tissue Kit (QIAGEN, Hilden, Mettmann, Germany) and stored it at -20 °C. DNA concentration and purity were evaluated using a spectrophotometer (NanoDrop 2000, Thermo Scientific, Wilmington, Delaware, United States), Qubit 2.0 fluorimeter (Invitrogen, Carlsbad, California, United States) and agarose gel electrophoresis (2%).

MLPA

All DNA samples were analyzed using MLPA according to the manufacturer's recommendations (MRC- Holland, Amsterdam, Netherlands). Blood samples without cytogenomic changes were used to standardize the MLPA reaction and as a control in all assays with tumor and nonneoplastic surrounding tissue samples.

The P036 and P070 kits were used to investigate CNVs in subtelomeric regions. The P413-B1 (CCR- LOSS) and P146-B1 (CCR-GAIN) kits were used for the detection of specific cytogenomic changes. Probes locations for each kit used in MLPA are shown in Supplemental Material 1. Details of the identified regions and probes are available at www.mlpa.com.

Amplified products were analyzed using the ABI 3500 Genetic Analyzer (Life Technologies) and GeneMarker® software (Softgenetics, LLC, State College, Pennsylvania).

The uttermost area of every one piece was related with controls samples. The results were considered altered when the peak-to-height ratio was fewer than 0.75 (deletion) or superior than 1.25 (duplication).

SNP Arrays

SNP arrays were used only in selected cases to confirm the MLPA results. We evaluated 8 samples of tumor tissue and surrounding nonneoplastic tissue from patients ID 13, ID 14, ID 23 and ID 26 (Table 1), with 4 paired samples for each patient that were analyzed as independent tests.

Array assays were performed using the Infinium CytoSNP-850K v1.2 BeadChip by iScan scanner (Illumina, San Diego, California, United States) following the manufacturer's recommendations. Infinium array technology has high specificity for SNP targets and provides comprehensive coverage of chromosomal abnormalities, CNVs, and ROH.

Raw data were analyzed using BlueFuse-™ Multi v4.4 (Illumina, Inc., San Diego, California, United States). Genomic positions are provided as mapped for the construction of the GRCh37/hg19 genome. The construction of the Circos plot was carried out according to the guidelines found at 2 sites (http://circos.ca/intro/genomic_data/ and https://training.galaxyproject.org/training-material/topics/visualisation/tutorials/circos/tutorial.html#copy-number-variation).

The results were compared with the following CNV databases: the Genomic Variants Database (http://projects.tcag.ca/variation/), the Chromosomal Imbalance and Phenotype Database in Humans Using Resources Ensembl (http://decipher.sanger.ac.uk/), and the UCSC Genome Bioinformatics (database (http://genome.ucsc.edu).

Results

MLPA results

The genomic imbalances found in samples of tumor tissue and tissue surrounding nonneoplastic tissue of 10 patients whose CRC was operated on using the MLPA technique as well as the imbalances correlations with the functions of genes, are shown in Table 2.

The analysis of subtelomeric kits revealed deletions and duplications in the *KDM5A* (12p), *TNFRS18* (1p), *IL17RA* (22q), *MTA1* (14q), *GAS8* (16q), *CHL1* (3p), *PSPC1* (13p), *SOX12* (20p), *SYBL1* (Xq), *CDC16* (13q), and *RABL2B* (22q) genes (Table 2).

The specific CRC kit results of the tumor samples showed genomic gains in the long arm of chromosome 20, including probes located in the *ADRM1*, *OPRL1*, *MAPRE1*, *TPX2*, and *ZNF217* genes; genomic losses in the short arm of chromosome 8, including markers in the *NKX3* and *LPL* genes; and alterations in the long arm of chromosome 13, including the *RB1*, *DACH*, *PSPC*, and *ZMYM2* genes. The percentage graph, which shows the most relevant genomic changes found by the MLPA technique in all patients with CRC is shown in Fig. 1.

The protein networks associated with the relevant genes identified in our study by using the STRING database and the genetic ontology classification (confidence score > 0.9) is shown in Fig. 2.

The main cellular pathways were cellular apoptosis, natural killer (NK)-cell-mediated cytotoxicity pathway, cell cycle pathway, cell-adhesion-molecules, interleukin-4 and interleukin-13 signaling, regulation of TP53 activity signaling pathway by interleukins, immunoregulatory interactions between lymphoid cells and nonlymphoid cells, and cytokine signaling in immune system.

Snp Array Results

The SNP array results showed several different pathogenic CNVs, mainly associated with tumorigenesis and tumor necrosis factors, and corroborated the genomic instability in these tissues. The array results confirmed the MLPA findings and unexpectedly revealed genomic alterations in the surrounding nonneoplastic tissue, which should be free from changes (Tables 3 and 4). SNP array results of ROH in surrounding tissue are shown in Supplemental Material 1.

Discussion

Our investigation revealed cytogenomic changes associated with both the pathogenesis and the progression of CRC. Additionally, it was possible to distinguish the complex and heterogeneous molecular characteristics of tumor tissues, clearly revealing the genomic instability profile characteristic of these samples. We also revealed the presence of pathogenic CNVs in surrounding nonneoplastic tissues, which were not detectable in histopathological investigations. This may indicate an initial tumorigenesis process for these patients.

Short-arm loss of chromosome 18 and long-arm duplications of chromosome 20 are commonly associated with CIN in preliminary genomic events in CRC [4, 6, 8, 10], and our results showed both types of genomic alterations: deletions in 18p11.3 involving the *THOC1* gene, and duplications in 20q13 involving the *UCKL1* gene. Previous studies have also associated deletions near the terminal regions located at 18p and 18q with the malignant progression of adenomas to adenocarcinomas [15–18], suggesting that subtelomeric genomic changes may be closely involved in the CRC process. Others have reported the association of structural alterations on chromosome 20 with instability in CRC [6, 8, 10].

Our results also showed concomitant genomic changes, such as *THOC1* gene losses in 18p11.3 and *UCKL1* gene duplications in 20q13.3 in both tumoral and nontumoral tissues. Silencing of the *THOC1* gene inhibits the proliferation of some cell cancer strains, while the *UCKL1* gene is related to maintaining a high cell proliferation rate in tumors [17, 19–21]. Furthermore, chromosome 20 is a genomic target of extreme importance in tumors [17]. We identified several genomic alterations associated with chromosome 20, including ROH regions and duplication and deletion of whole arms in one patient. Duplications in the 20q subtelomeric region are a marker of unfavorable prognosis in patients with CRC [22].

Lake et al. [23] studied the relationship of tumorigenesis in primary and metastatic conjunctival melanomas in their analysis of metastatic samples, finding that the gene *OPRL1* (20q13.33) was frequently excluded. In our study, we recognized a genomic gain in the long arm of chromosome 20, including at a probe located in the *OPRL1* by using MLPA. Additionally, they found by use of MLPA and SNP arrays that the *ADRM1* gene (20q13.3), located on chromosome 20, was duplicated in colorectal tumor tissue. This gene encodes a plasma membrane protein that participates in cell adhesion and dysregulation of this protein has been implicated in carcinogenesis by inducing interferon gamma in some cancer cells [17, 22]. Fejzo et al. [24] have shown that the ubiquitin proteasome ADRM1 receptor is amplified in cancers, including gastric, ovarian and colonic cancer. This *ADRM1* gene directs the protein levels of specific oncogenes, resulting in an increase in metastatic potential [24].

Some genomic changes not previously described in CRC were identified in our study, such as duplication of *ADAP1* (7p22.3), *DMRT1* (9p24.3), and *DOCK8* (9p24.3), which may be important in the study of sporadic CRC pathophysiology, considering that all these genes are involved in cell cycle control. The *ADAP1* gene codes a protein related to Arf6 signaling events and the

B cell receptor signaling pathway. The *DOCK8* gene has been identified as a putative gene associated with the progression of brain tumors, especially gliomas [9].

In addition to the CNVs detected in *THOC1* and *UCKL1*, we found other genomic abnormalities concomitant in neoplastic and nonneoplastic surrounding tissues, including duplications in *TNFRSF18* and deletions of the *MTA1* and *DECR2* genes [25]. The *TNFRSF18* gene, a member of the tumor necrosis factor receptor superfamily, plays a key role in the self-regulation of cellular apoptosis and the immune system, coding and regulating T cells [25]. The MTA1 protein plays important roles in cell signaling processes, chromosomal remodeling and transcription that in turn participate in the progression, invasion and growth of metastatic cells [26].

The *FBXO25* gene encodes a member of the F-box protein family, a subunit of an ubiquitin protein ligase complex hat functions in phosphorylation-dependent ubiquitination. A connection exists between CNVs and tumor suppressor genes such as *FBXO25* with negative regulation of gene expression [27], which may help in further understanding the behavior of tumorigenesis and cancer progression. In the present investigation, we detected a deletion in the *FBXO25* gene (8p23.3) in the tumor tissue using both techniques.

In this investigation, other genomic alterations detected in tumor samples included duplications in the *PSPC1* gene (13q12.1), which encodes protein tyrosine kinase 6 and is involved in determining oncogenic subcellular translocations. Its positive regulation of *PSPC1* is related to a prometastatic activator associated with a poor prognosis, while its negative regulation suppresses activated metastases and is a potential marker of improved cancer therapy outcomes [28].

In particular, duplication in 1p36 located in the *TNFRSF18* gene in both tumoral and nontumoral tissues, was a relevant finding of our study. The tumor necrosis factor receptor-associated factor-6 protein (TRAF-6), which is encoded by one of the *TNFRSF18* family genes, was abnormally expressed in positive CRC tissues and was closely linked to patient's prognosis [29–31]. Thus, *TNFRSF18* amplification deserves special attention to clarify its clinical significance in this patient's profile [29–31].

In the present investigation, SNP array results of the surrounding nonneoplastic tissue samples from patients with metastases revealed different structural variations at 1q42.3, comprising the *LYST* gene, which has been previously associated with cancer and autoimmune diseases. *LYST* has been associated with immunodeficiency syndromes and with impaired cytotoxic lymphocytic function, especially among NK cells, which are very important in the defense against tumor growth [32].

When the amount of DNA damage is greater than the DNA repair capability, a checkpoint-signaling pathway is activated [29, 33–37]. One of the first steps in the cellular response to DNA damage induced by exogenous agents is DNA repair protein activation [35–37]. The literature suggests that CNVs may be important genetic variants that explain tumor heterogeneity and genetic instability in CRC [38–40]. Therefore, we suggest that these CNVs are initialization markers of cellular abnormality. Accordingly, our results demonstrate tumor cells characterized by different CNVs in subtelomeric regions, clearly indicating the presence of CIN confirmed by SNP test array results.

In sporadic tumors, CIN is characterized by gains and losses of small genomic segments or whole arms, that are mainly caused by chromosomal breaks [4, 6, 8, 41]. CIN can result from defects in chromosomal segregation, instability or loss of telomeres, or errors during a response to DNA damage. Double-strand breaks are a common mechanism in tumor instability, and usually occur through nonhomologous end joining [5, 7, 8, 38]. Furthermore, subtelomeric regions are more susceptible to rearrangement and have recently been implicated in a genomic rearrangement event known as chromothripsis, which has been reported in some types of cancer [41–44]. The aberrant chromosomal architecture -i.e., the variation of small insertions or deletions leading to major chromosomal changes such as premitotic defects, stress in replication and telomeric fusions- has an important role in CIN and is usually found in cancer genomes [6, 41, 45–48]. Studies have reported aneuploidies of whole chromosomes in 70% of colorectal tumors [45, 46]. Importantly, CIN may disturb the cell environment and immune signaling. The inhibition of the immune vigilance system has revealed altered expression of several genes involved in adaptive immunity and/or associated with cytotoxic cells and NK cells, suggesting a decrease in the level of immune cells

and functioning as an immunosuppressant [45–48] Notably, Bakhoum et al. [48] discussed the complex effects of CIN as a central driver of tumor evolution based on genomic copy number heterogeneity. In our investigation, most of the patients studied (such as patients 23 and 26) had metastases and several genomic alterations detected by MLPA and an array with several CNVs in subtelomeric regions, indicating the presence of CIN.

Approximately 85% of CRC cases demonstrated chromosomal and ROH imbalances, which led to changed expressions of tumors and oncogene suppressor genes [45–50]. We observed several insertions and deletions and ROH in the array results in surrounding nonneoplastic tissue. There results indicate there was an unstable microenvironment growing around the tumor.

Our findings suggest the need for a more detailed molecular investigation of the altered genomic regions, considering an expanded study of the genomic profile in a larger population, using this model to confirm the discovery of relevant prognostic markers for this disease.

In conclusion, our findings show the importance of characterizing the genomic CRC profile to understand tumor initiation and progression in sporadic CRC patients. Structural variants may lead to chromosomal instability and directly influence genic transcription through genetic dosing that occurs via exclusions or gene amplifications. The identification of the concomitant CNVs in the tumors and surrounding nonneoplastic tissues, which should hypothetically be free of changes, opens the possibility for the use of MLPA and/or SNP array assays for early diagnosis in CRC cases.

Declarations

Authors' Contributions

All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript. MR prepared the samples, performed cytogenomic analysis, and wrote the paper analysis; EAZ and YGO performed genotype-phenotype correlations and created the graphics and images; FARM, TVMMC and ATD discussed the results; AMAAM conducted the anatomopathological analyses; JGD helped create the graphics and images; KCT performed manuscript preparation and critical revision; BB, and JW provided the samples, clinically assessed the patients, and critical revision; and LDK designed and coordinated the study.

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Competing Interests

The authors declare no competing interests.

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of Faculdade de Medicina do ABC (808.569, approval date 26/09/2014).

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Tables

Table 1
Clinical and anatomopathological characteristics of patients operated on colorectal cancer.

ID	Gender	Age	Diagnosis	Site of carcinoma	Tumor grading	Metastatic Site
12	Female	76	pT3, pN2a, low grade moderately differentiated adenocarcinoma	Right colon	Low	-
13	Male	72	pT3, pN2a, moderately differentiated adenocarcinoma	Sigmoid colon	Low	Liver
14	Male	68	pT3, invasive and moderately differentiated tubular adenocarcinoma	Rectum	Low	Liver
17	Female	49	pT3, moderately differentiated adenocarcinoma	Sigmoid colon	Low	-
18	Male	55	pT4, conventional moderately differentiated adenocarcinoma	Sigmoid colon	Low	-
20	Female	84	pT3, moderately differentiated adenocarcinoma, signet ring cells	Right colon	Low	-
22	Female	49	pT4, poorly differentiated adenocarcinoma with mucus secretory foci	Descending colon	High	Ovary, stomach and peritoneum
23	Female	63	pT3, invasive moderately differentiated adenocarcinoma	Right colon	Low grade	Peritoneum
26	Female	57	pT4, Invasive moderately differentiated adenocarcinoma	Sigmoid colon	High grade	Uterus, appendix and terminal ileum
30	Female	48	pT4, high histological degree adenocarcinoma, pT4, signet ring cells	Right colon	High grade	Peritoneum

*ID-sample identification

Table 2

Genomic imbalances found in samples of tumor and surrounding nonneoplastic tissues of patients operated on colorectal cancer using MLPA and correlation with gene function.

Tissue	Genomic position	Gene	Genomic change	Gene Function*
Tumor	**1p36.3**	*TNFRSF18*	del/dup	Signaling pathway mediated by tumor necrosis factor
Surrounding	**1p36.3**	*TNFRSF18*	dup	
Surrounding	**2p25.3**	*ACP1*	del	Ester hydrolysis and intrauterine growth
Tumor	**3p26.3**	*CHL1*	del	Cellular proliferation
Surrounding	**3p26.3**	*CHL1*	del	
Tumor	**7p22.3**	*ADAP1*	dup	Regulation of GTPase activity; signaling pathway of the cell surface receiver
Surrounding	**7p22.3**	*ADAP1*	dup	
Tumor	**8p21.2**	*NKX3-1*	del	Tumor progression
Surrounding	**8p21.2**	*NKX3-1*	del/dup	
Tumor	**8p23.3**	*FBXO25*	del	Ubiquitination of proteins
Surrounding	**8p23.3**	*FBXO25*	del	
Tumor	**8q24.3**	*RECQL4*	dup	Encodes DNA helicase
Surrounding	**8q24.3**	*RECQL4*	dup	
Tumor	**9p24.3**	*DMRT1*	dup	Mitotic proliferation regulator in germ cells
Tumor	**9p24.3**	*DOCK8*	dup	Memory T-cells proliferation
Surrounding	**9p24.3**	*DOCK8*	del	
Tumor	**9q34.3**	*EHMT1*	dup	Methylation of histones and chromatin change
Tumor	**10p15.3**	*ZMYND11*	del	Tumor suppressor and transcription regulator
Surrounding	**10p15.3**	*ZMYND11*	del	
Tumor	**10q26.3**	*ECHS1*	del	Fatty acids β- oxidation in mitochondrial matrix
Tumor	**11q25**	*NCAPD3*	del/dup	Condensation of mitotic chromosomes and histones methylation
Tumor	**12q24.33**	*ZNF10*	del/dup	Transcriptional repressor
Surrounding	**12q24.33**	*ZNF10*	del	
Tumor	**13q12.1**	*PSPC1*	dup	Nucleotide binding, tumor progression
Surrounding	**13q12.1**	*PSPC1*	del	
Tumor	**13q12.1**	*ADPRTL1*	del	DNA and inflammatory processes repair
Tumor	**13q34**	*CDC16*	dup	Cell cycle and proliferation
Tumor	**14q11.2**	*HEI10*	del	Cell cycle regulator
Surrounding	**14q11.2**	*HEI10*	del	
Tumor	**14q11.2**	*ADPRTL2/PARP2*	del/dup	DNA repair, regulation of apoptosis, and maintenance of genomic stability
Surrounding	**14q11.2**	*ADPRTL2/PARP2*	del	

Tissue	Genomic position	Gene	Genomic change	Gene Function*
Tumor	**14q32.3**	*MTA1*	del/dup	Cancer invasion and metastasis
Surrounding	**14q32.3**	*MTA1*	dup	
Tumor	**16p13.3**	*DECR2*	del	Fatty acid degradation
Surrounding	**16p13.3**	*DECR2*	dup	
Tumor	**17q25.3**	*TBCD*	dup	Protein folding
Surrounding	**17q25.3**	*SECTM1*	dup	Cellular signaling
Tumor	**18p11.3**	*THOC1*	del	Apoptosis
Surrounding	**18p11.3**	*THOC1*	del	
Tumor	**18q23**	*CTDP1*	del	Protein dephosphorylation
Tumor	**19q13.4**	*CHMP2A*	del	Cellular maintenance
Surrounding	**19q13.4**	*CHMP2A*	del	
Tumor	**19p13.3**	*CDC34*	dup	Ubiquitination of proteins
Surrounding	**19p13.3**	*CDC34*	dup	
Tumor	**20p13**	*SOX12*	del/dup	DNA link; transcription from the RNA-polymerase promoter
Surrounding	**20p13**	*SOX12*	dup	
Tumor	**20p13**	*ZCCHC3*	del/dup	Poly-A RNA Link
Surrounding	**20p13**	*ZCCHC3*	dup	
Tumor	**20q13.3**	*ADRM1*	del	Cell adhesion
Surrounding	**20q13.3**	*ADRM1*	dup	
Tumor	**20q13.3**	*UCKL1*	dup	Pathway involvement that synthesizes CTP from citidine
Surrounding	**20q13.3**	*UCKL1*	dup	
Tumor	**20q13.3**	*OPRL1*	del/dup	Neuropeptide link
Surrounding	**20q13.3**	*OPRL1*	dup	
Tumor	**21q11.2**	*STCH*	del	ATP link
Surrounding	**21q11.2**	*STCH*	del	
Tumor	**21q11.2**	*RBM11*	del	Regulation of alternative splicing during the germ cells and neurons differentiation
Surrounding	**21q11.2**	*RBM11*	del	
Surrounding	**21q22.3**	*S100B*	del	Calcium-dependent protein binding
Tumor	**Xp22.33 / Yp11.32**	*SHOX*	dup	Development of skeletal system and transcription from RNA-polymerase II
Tumor	**Xq28/Yq12**	*SYBL1/ VAMP7*	del/dup	Epigenetic regulation
Surrounding	**Xq28/Yq12**	*SYBL1/ VAMP7*	del	

Table 3
Genomic imbalances found in tumor tissue of patients operated on colorectal cancer comparing the results from MLPA and SNP array.

Patient	MLPA				Array			
	Duplications		Deletions		Duplications		Deletions	
	Genes*	Genomic Position	Genes	Genomic Position	Genes	Genomic Position	Genes	Genomic Position
13	*TNFRSF18*	1p36.33					*FBXO25*	8p
	ADAP1	7p22.3					*NKX3-1*	8p
	UNC84	7p22.3					*NRG1*	8p
	BET1L	11p15.5					*DOCK8*	9p24
	PSPC1	13q12.11					*DMRT1*	9p24
	CDC16	13q34					*TP53*	17p13.3p13.1
	RICB-8	14q23.2						
	DECR2	16p13.3						
	SOX12	20p13						
	ZCCHC3	20p13						
	OPRL1	20q13.33						
	UCKL1	20q13.33						
14	*SH3BP5L*	1q44	*CD2-LCR*	1p13.1	*TNFRSF18*	1p11.2-q44	*CT66*	7q11.22
	DEK	6p22.3	*TNFRSF18*	1p36.33	*EPS15*	1p11.2-q44	*GALNT17*	7q11.22
	NCAPD3	11q25	*CASP7*	10q25.3	*TP73*	1p11.2-q44	*AUTS2*	7q11.23
	ZNF10	12q24.33	*RBFA*	18q23	*OPRL1*	20q11.21-q13.33		
	PSPC1	13q12.11	*SOX12*	20p13	*UCKL1*	20q11.21-q13.33		
	ADPRTL2 - PARP2	14q11.2	*RBM11*	21q11.2				
	MAPRE	17q21.2						
	TPX2	20q11.21						
	ZNF217	20q13.2						
	SYBL1	Xq28, Yq12						
23	*TNFRSF18*	1p36.33	*DECR2*	16p13.3	*CIDEC*	3p25.3	*RUNX3*	1p36.11

* http://www.ncbi.nlm.nih.gov/clinvar/

					XRCC1	19q13.31	*STMN1*	1p36.11
					CADM4	19q13.31	*RARB*	3p24.2
					PVR	19q13.31	*ATPSCKMT*	5p15.2
26	*LTBP1*	2p22.3	*FRG1*	4q35.2	*TXK/ NFXL1/ UCHL1*	4p13-p12	*TNFRSF14*	1p36.32
	ADAP1	7p22.3	*CDCA*	5p15.33	*EGF*	4q25	*TP73*	1p36.32
	PSPC1	13q12.11	*CDCA2*	8p21.2	*CDC16*	6p25.3-q27	*RUNX3*	1p36.11
	ICAM1	19p13.2	*LPL*	8p21.3	*IRF4*	6p25.3-q27	*LCK*	1p35.2
	CDC34	19p13.3	*ZMYND11*	10p15.3	*RB1*	6p25.3-q27	*EPS15*	1p32.3
	PPAP2C	19p13.3	*DIP2C*	10p15.3	*ADAP1*	7p22.3-p21.3	*ORC1*	1p32.3
	UCKL1	20q13.33	*PMP22*	17p12	*RB1*	7p21.3-p11.2	*F3*	1p21.3
	OPRL1	20q13.33	*THOC1*	18p11.32	*EIF3E*	8q21.3-q24.3	*VCAM1*	1p21.2
			RBFA	18q23	*RECQL4*	8q21.3-q24.3	*VCL*	10q22.2
			RBM11	21q11.2	*TBP/ WISP1*	8q21.3-q24.3	*GHITM*	10q23.1
			S100B	21q22.3	*ZNF367*	9q22.32-q22.33	*NRG3*	10q23.1
			PRMT2	21q22.3	*KLF4*	9q31.2	*STIM1*	11p15.4
			SYBL1	Xq28, Yq12	*ZNF367*	9q32-q34.3	*ILK*	11p15.4
					EGR2	10q21.3	*CDKN1C*	11p15.4
					CDC16/ MIR17	13q22.3-q34	*ATM*	11q22.3
					ADRM1/ TPX2	20q11.21-q13.33	*NPAT*	11q22.3
					OPRL1	20q11.21-q13.33	*DDX10*	11q22.3
					ZNF217/ UCKL1	20q11.21-q13.33	*TNFRSF13B*	17p11.2
					RB1/ WISP2	20q11.21-q13.33		

* http://www.ncbi.nlm.nih.gov/clinvar/

Table 4
Genomic imbalances in surrounding nonneoplastic tissue of patients operated on colorectal cancer comparing MLPA and SNP array.

Patient ID	MLPA				SNP Array			
	Duplications		Deletions		Duplications		Deletions	
	Genes*	Genomic Position	Genes	Genomic Position	Genes	Genomic Position	Genes	Genomic Position
13	*PPAP2C*	19p13.3					*PPAP2C*	19p13.3
	BC2 - CHMP2A	19q13.43					*BC2 - CHMP2A*	19q13.43
	ZCCHC3	20p13					*ZCCHC3*	20p13
14	*WISP1 - CCN4*	8q24.22	*ZNF10*	12q24.33	*NLGN1*	3q26.31		
	TNFRSF6B	20q13.33	*EIF3E*	8q23.1	*TNFSF10*	3q26.31		
23	*FRG1*	4q35.2	*GABRB3*	15q12	*SH3BP5L*	1q44	*RUNX3*	1p36.11
	RECQL4	8q24.3	*ARSA*	22q13.33			*STMN1*	1p36.11
	CDX2	13q12.2	*CDC34*	19p13.3			*RARB*	3p24.2
	CD2-LCR	1p13.1	*PSPC1*	13q12.11			*ATPSCKMT*	5p15.2
	ADRM1	20q13.33	*BCL2L1*	20q11.21				
	IL17RA	22q11.1						
26					*MORF4L2*	Xq22.2	*CDC73*	1q31.2
							LYST	1q42.3
							ADM	11p15.4
							EIF2S1	14q23.3
							ZBTB1	14q23.3
							ZNF	19p12
							KIR3DL1	19q13.42
							KIR2DL3	19q13.42
							KIR2DL1	19q13.42

* http://www.ncbi.nlm.nih.gov/clinvar/

Figures

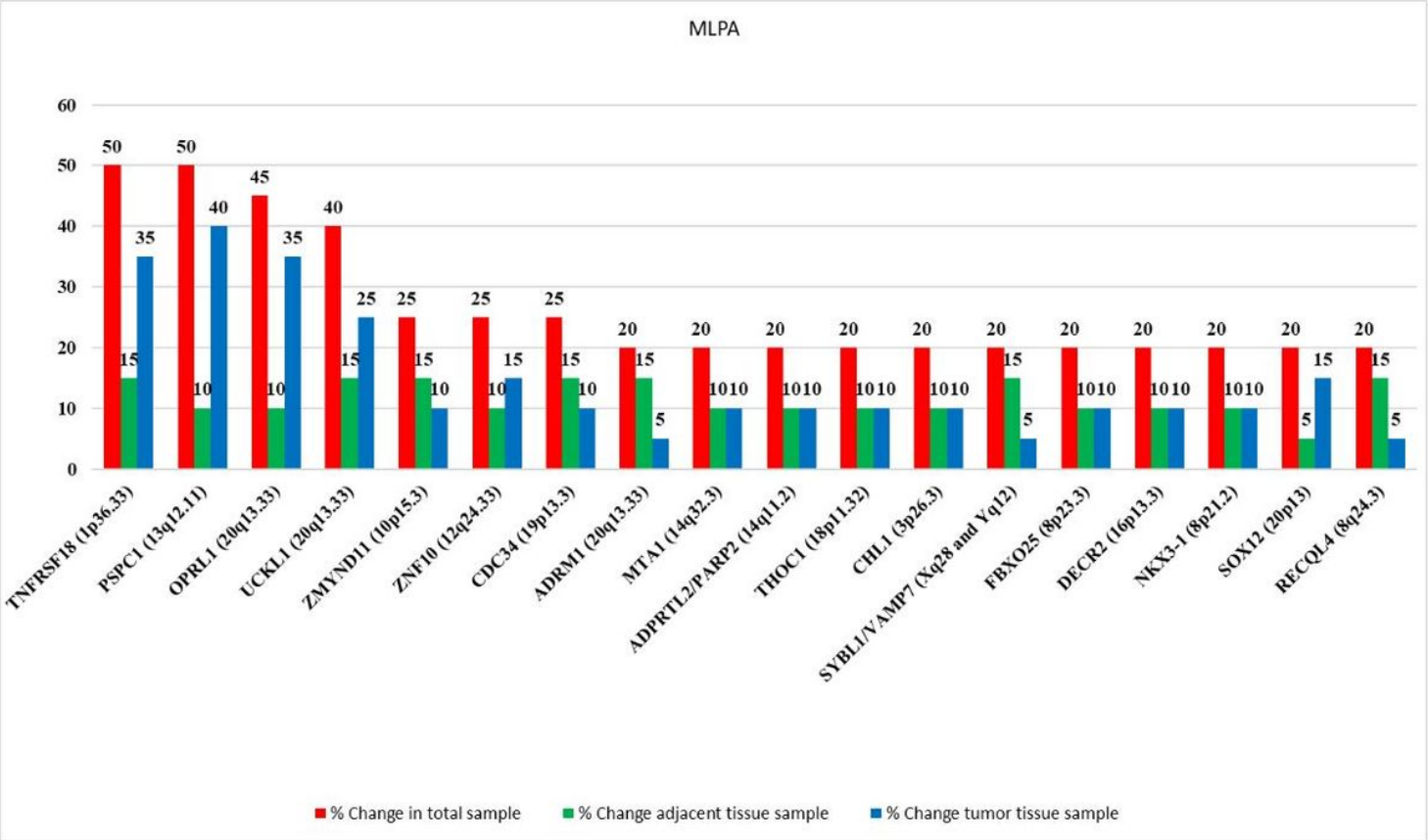

Figure 1

Most relevant genomic changes found using MLPA among all colorectal cancer patients.

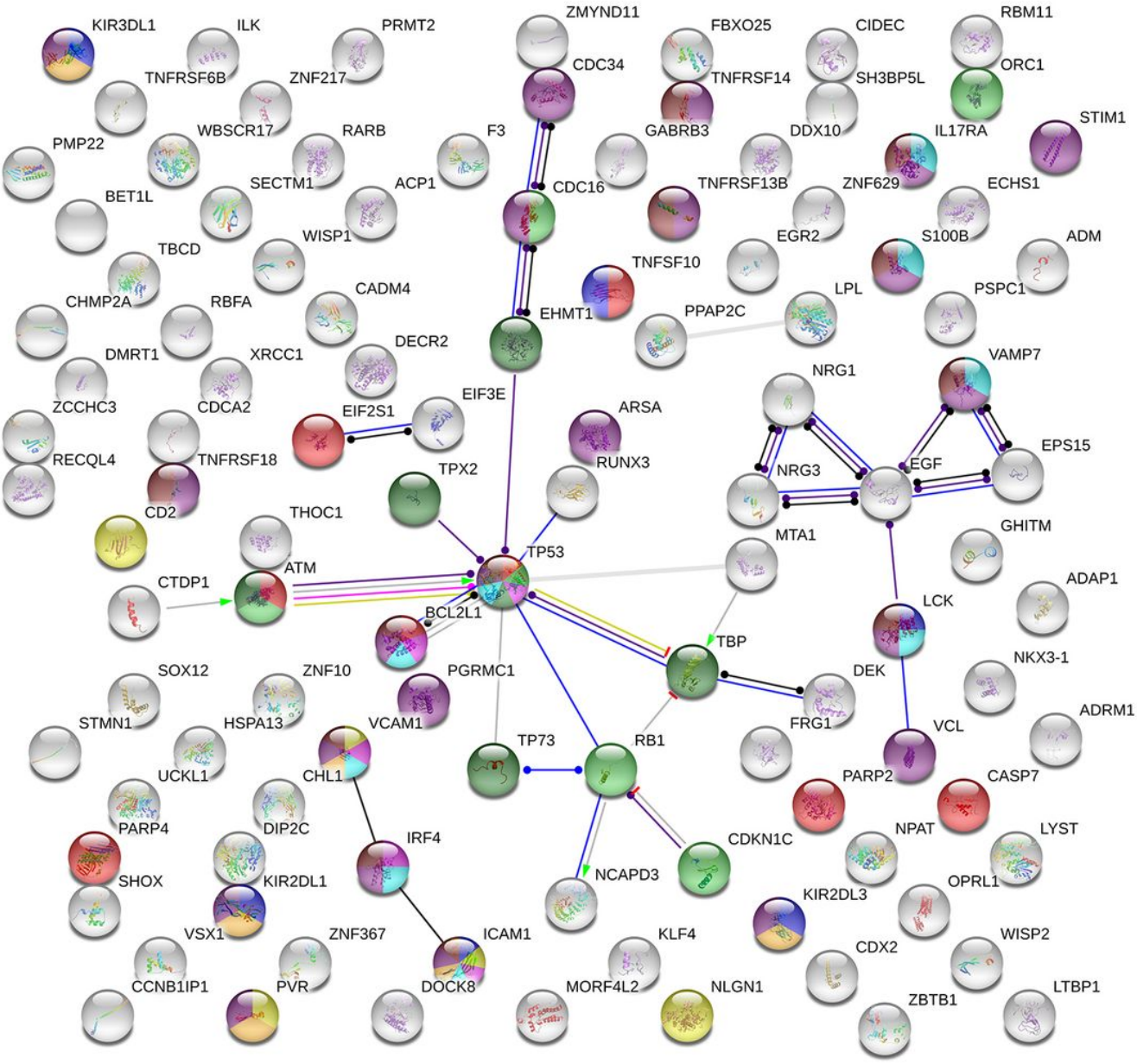

Figure 2

Graphical representation of protein networks associated with the relevant genes identified in our study using the STRING database and the Genetic Ontology classification (confidence score > 0.9). The graph shows the main cellular pathways: cellular apoptosis (red node), natural killer (NK)-cell-mediated cytotoxicity pathway (blue node), cell cycle pathway (green node), cell adhesion molecules (CAMs) (yellow node), interleukin-4 and interleukin-13 signaling (rose node), regulation of TP53 activity pathways (dark green node), signaling by interleukins (light blue node), immunoregulatory interactions between a lymphoid and a nonlymphoid cell (dark yellow node), immune system (purple node), and cytokine signaling in the immune system (dark red node).

Supplementary Files

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