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ORIGINAL ARTICLE

Why Cytoskeletal Associated Proteins are Important in Colorectal Cancer Patients: Molecular and Bioinformatic Analysis

Kolorektal Kanser Hastalarında Hücre İskeletiyle İlişkili Proteinler Neden Önemlidir: Moleküler ve Biyoinformatik Analiz

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Abstract

Introduction: It has been aimed to analyze the role of pathogenic effects of mutation and expression anomalies occurring on diaphanous-related formin 1 (DIAPH1), WASP actin nucleation-promoting factor (WASP), myosin heavy chain 9 (MYH9), actinin alpha 1 (ACNT1), filamin A (FLNA), and tubulin beta 1 class VI (TUBB1), which are known as fundamental cellular skeleton proteins, on the development and progression of cancer via bioinformatic tools.

Methods: The genome sequence and expression profiles of 594 Colorectal Cancer (CRC) patients were obtained via bioinformatic tools, which provide data for The Cancer Genome Atlas. The mutation patterns of six genes were determined in detail, and for the prediction of pathogenic properties of identified changes for CRC, Polymorphism Phenotyping v2, Screening for Non-Acceptable Polymorphisms, and the Catalogue Of Somatic Mutations In Cancer were utilized. Apart from the mutation profile, the effects of existing mutations on messenger ribonucleic acid (mRNA) expression and survival were also identified. Moreover, the Search Tool for the Retrieval of Interacting Genes/Proteins network analysis was realized to further comprehend the functional relations of proteins in cellular processes.

Results: There have been 142 distinct point mutations, gene amplification, and deep deletions identified on DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes. ACTN1 and FLNA low mRNA expression levels for DIAPH1 increased, and the mRNA expression level was statistically significant ($p<0.05$). Prognosis-wise, the effect of mRNA expression on survival in the absence of disease was meaningful for FLNA ($p=0.011$).

Discussion and Conclusion: Bioinformatic analysis data in DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes, which are important in CRC pathogenesis revealed in this study, will be a guide for future laboratory studies.

Keywords: Cytoskeleton; Gene Expression; Mutation; Colorectal Cancer

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The impairment or loss of several aspects of normal cellular activity is the common entity of all cancer types. However, the genomic instability engendered using the defects that cause tissue degeneration on cellular morphogenesis, migration, the achieving of the ability of invasion, and anomalies in mitosis also accompany the progression of the disease.[1] The cytoskeleton proteins that are composed of different subcategories of proteins including microtubules, actin, and intermediate filaments are vital for the survival and cellular processes of normal and cancer cells.[2–4] At the same time, the cytoskeleton network at the cellular transformation phase can be reprogrammed to assist the progression of cancer via stimulating survival, growth, and invasion of the tumor cell. Therefore, the tumor cells gain various properties.[3–6] CRC is a heterogeneous disease that causes the colon and rectum mucosa to transform into an invasive cancer that develops because of genetic, epigenetic, and peripheral factors.[7,8] The interactions between the adhesion complexes and cytoskeleton proteins are crucial for the epithelial structure of a normal intestine to survive. The anomalies in the expression and functional activity of the cytoskeleton proteins accompanied by the deterioration of the epithelial homeostasis are known to be the precursors in cancer commencement and progression.[6,7,9] We aim to contribute to the development of potential objectives toward new molecular biomarkers and anticancer medicine by realizing the comprehensive molecular analysis of fundamental cytoskeleton proteins of ACTN1, MYH9, TUBB1, FLNA, DIAPH1, and WASP, whose effects on CRC pathogenesis are not completely discovered, by revealing the potential anomalies on a group of CRC patient group. In this study, we planned the investigation of the effects of the relations, which is influential on CRC malignant cell transformation between the mutations and representation irregularities in their expressions on genomic instability, invasion, and increment of metastasis abilities.

Materials and Methods

Study Group

TCGA CRC (n:526) dataset was downloaded via cBioPortal, and the demographic, clinic, and genetic information was exhibited in Table 1. The data used in our study were obtained from the public database TCGA; therefore, ethical approval was not required.

Analysis of Mutation Profile

Cbio Cancer Genomics Portal is a bioinformatic tool that contains and provides the mutation data, copy number alterations, microarray and RNA sequencing-based mRNA expression alterations, deoxyribonucleic acid (DNA) methylation data, and protein and phosphoprotein levels from the TCGA database.[10] Oncoprint, Mutasyon, and Coexpression tabs were utilized to examine the mutations of DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes on the CRC patients. The type and frequency of the mutations were identified for each gene individually.

Prediction of Pathogenic Mutations

The potential effects of the missense mutations that were identified on DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes were analyzed through two pathogenicity estimators. Polymorphism Phenotyping v2: It is a versatile bioinformatic tool that estimates the potential structural and functional consequences of Mutation/Single Nucleotide polymorphisms on amino acid position. It provides the estimation results between the score interval of 0.0 (benign) and 1.0 (potentially damaging).[11] The Catalogue Of Somatic Mutations In Cancer: It is a comprehensive source for the research of the characteristics of somatic mutations and their effects. The pathogenic effects of the identified mutations are determined through the database using the Functional Analysis through Hidden Markov Models algorithm. This algorithm estimates the functional, molecular, and phenotypical effects of the missense mutations.[12]

Gene Expression Profile and Survival Analyses

GEPIA (Gene Expression Profiling Interactive Analysis) is an interactive bioinformatic tool that was developed to realize the differential expression analysis on tumor or normal tissues, generate profiles with respect to the cancer types or pathological phases, patient survival analysis, analog gene detection, correlation analysis, and other customizable analyses.[13] The effects of mRNA expression levels of DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes and high and low mRNA expression levels on overall and disease-free survival were realized comparatively on the healthy control group and the CRC (n:275) patient group. The analysis of the genes that were predicted by cBioPortal was realized.

Protein–Protein Interaction Analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was applied to evaluate the protein–protein interaction information. The estimated interactions between DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 proteins and the protein net that defines the direct (physical) and indirect (functional) relations among the proteins were generated and analyzed using the STRING database.[14]

Statistical Analyses

All the statistical analyses that were used to evaluate the study data were realized using the GEPIA bioinformatic tool.[13] The survival analysis that we accomplished on the GEPIA database was realized via the exploitation of Kaplan–Meier curves. The log-rank test was applied to investigate the effects of low and high expression groups on survival. The Spearman test was used by the database for correlation studies. In all the studies, $p<0.05$ was determined as the statistically significant value.

Results

Results of Mutation Profile Analysis

There have been 142 different genetic alternations that were identified on DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes among 526 CRC patients. Of these alterations, 117 were missense mutation, three were nonsense mutation, eight were frameshift mutation, 11 were splice region mutation, and one was gene fusion mutation. Moreover, although deep deletion due to homozygote loss was identified on WASP and ACTN1 genes, gene amplification anomalies were detected on WAS, MYH9, FLNA, and TUBB1 genes.

All the genetic alterations given in Appendix 1 are shown with detailed explanations. The probability of hosting a genetic alteration were DIAPH1, 2.9%; WAS, 1.9%; MYH9, 8%; ACTN1, 3%; FLNA, 7%; and TUBB1, 7%. The highest number of alterations occurred on MYH9 (51 mutations), whereas the least number of alterations were detected on TUBB1 (five mutations). It is statistically meaningful that MYH9;-FLNA, DIAPH1;MYH9, and DIAPH1;ACTN1 hosted genetic alterations together ($p=0.016$, $p=0.025$, and $p=0.042$). The localization of mutations detected on the domains of proteins belonging to the study genes is shown in Figure 1 as a representation.

The frameshift mutations that were originated from the shifting of the reading frame that has the capability of causing early termination of polypeptides were detected on DIAPH1, WAS, MYH9, and FLNA genes. The nonsense mutation that gives birth to stop codons was identified on MYH9 and FLNA genes. Because of the splice mutations' presence on the bases of splice region, which is completely preserved during the evolutionary process, the unfunctional transcripts containing the intron information originated and anomalies in the expression occurred. Consequently, a loss of functionality is probable. DIAPH1, ACTN1, MYH9, and FLNA gene mutations were detected on metastatic patients and presented in Appendix 1.

Table 1. Summary of demographic, clinical, and genetic data in CRC patients

Characteristics	Patient data n:594 (%)
Gender	
Male/Female/NA	312/282/2
Diagnosis age, years	
Race category	
White	285 (48)
Black or African American	64 (10.8)
Asian	12 (2.0)
NA	232 (39.1)
American Indian	1 (0.2)
Tumor type	
Colon adenocarcinoma	378 (63.6)
Rectal adenocarcinoma	155 (26.1)
Mucinous adenocarcinoma	61 (10.3)
Sample type	
Primary	594
Overall survival status	
Living	471
Deceased	120
NA	3
Tumor stage code	
T1	20 (3.4)
T2	103 (17.3)
T3	401 (67.5)
T4	30 (5.1)
T4a	27 (4.5)
T4b	10 (1.7)
NA	3 (0.5)
Metastatic stage code	
M0	440 (74.1)
M1	69 (11.6)
MX	62 (10.4)
M1A	11 (1.9)
M1B	3 (0.5)
NA	9 (1.5)
Alteration frequency	**Case (Frequency %)**
DIAPH1 mutation	15 (2.9)
WAS mutation	7 (1.3)
MYH9 mutation	38 (7.2)
ACTN1 mutation	15 (2.9)
FLNA mutation	36 (6.8)
TUBB1 mutation	4 (0.76)
WAS amplification	1 (0.19)
MYH9 amplification	1 (0.19)
FLNA amplification	2 (0.38)
TUBB1 amplification	34 (6.46)
WAS deep deletion	2 (0.38)
ACTN1 deep deletion	1 (0.19)

NA: Not Applicable; MX: Distant Metastasis Cannot Be Assessed; M0: No Distant Metastasis; M1: Distant Metastasis; T: Tumor.

MYH9 Analysis

MYH9 codes a protein addressed as muscle exterior myosin IIA (NMMIIA) of 453 kDa mass in the hexametric form responsible for cell locomotion, megakaryocyte contraction, and the preservation of cytoplasmic matrix. MYH9 gene has four major domains that are called head domain (GHD), neck, coiled-coil tail domain (TD), and non-helical tail domain.[15] In our study, we have identified 20 different mutations that code nonmuscle myosin type IIA (NMMIIA) heavy chain in the region between exons 2 and 19 on the GHD. We identified 39 distinct mutations in the TD region, which are coded by exons 21–40. There was one missense mutation identified in the Neck region, which is coded by exon 20.

TUBB1 Analysis

There are two important domains, namely, Guanosine Triphosphate (GTP) domain and Microtubule-Associated Protein (MAP), that are present in TUBB1 protein, which comprised 451 amino acids.[16] Out of seven mutations that were detected, especially p.V169L and p.R77Q mutations reside on the GTP domain.

FLNA Analysis

FLNA gene integrins interact with transmembrane receptor complexes and secondary messengers to code prevalently expressed filamin A'I of 280 kDA mass actin-binding protein responsible for the rearrangement of the cytoskeleton and realize signal transmission, cell migration, and adhesion. It has four major domains, namely, An N-terminal F-Actin-Binding Area, which comprised two tandem calponin homology (CH) regions (CH1 and CH2), two ROD regions composed of repetitions similar to 23 Ig, and a repetition region on the C-terminal that passes through dimerization before the interaction with the membrane receptors.[17] We have identified 44 distinct mutations on the FLNA gene, and these mutations reside on the domains coded by exons 2–48.

WASP Analysis

WASP gene is responsible for the coding of Wiskott–Aldrich syndrome protein that takes part in the signal transmission from the cell surface receptors to the actin cytoskeleton composed of 502 amino acids. It is composed of N-terminal Ena-VASP homology domain 1 (EVH1), GTPaz bonding domain, poly-proline domain (PPPP), and verproline homology (V) and C-terminal domain composed of central (C) cluster.[18] Out of the seven mutations that were detected, p.A139V and p.R138Q mutations reside on the EVH1 domain; p.X311 splice region mutation resides on the PPPP domain starting point; and p.P353Hfs*92 mutation, which has the potential to change the reading frame, reside on the PPPP domain.

DIAPH1 Analysis

DIAPH1 belongs to the formin family and has two functional regions: formin homology 1 and 2 regions (FH1 and FH2, respectively) and guanosine triphosphatase bonding area and dimerization region and several regulatory domains.[19] Out of 18 mutations we identified, three distinct frameshift mutations were present on FHI and FH2 domains.

ACTN1 Analysis

There is an effective binding domain of two CH regions, four spectrin (SPEC) motives, and two calcium bonding EF hands (EFh), and a calponin-like motive (CaM) is present on the N-terminal of ACTN1 protein. On the C-terminal, two EFh (efhand Ca_insen) are indifferent to degenerate calcium domains residue.[20] The missense mutations of p.F99V and p. R133H15, which are responsible for actin bonding functionality on the CH domain, were of 15 identified mutations. There was also a p.X254_splice mutation identified in the region between the CH domain and SPEC motives.

In Silico Pathogenicity-Estimated Results of Mutations

According to our estimated pathogenetic test, out of the 142 identified mutations on target genes, 117 of them were in the missense mutation character. With respect to the output scores from the two analysis programs, 52 missense mutations were pathogenic. The pathogenicity scores are provided in detail in Appendix 1.

Results of Gene Expression and Survival Analyses

In the light of the analyses results of the high and low mRNA expression levels of DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes of 275 CRC patients, the mRNA expression level of the DIAPH1 gene was higher than the healthy group expression levels of ACTN1 and FLNA, which were detected to be low, which is statistically meaningful (Fig. 2). When a comparison was made on WAS, MYH9, and TUBB1 genes for the healthy group, there was not any discrepancy identified. With respect to the results of our survival analysis, it has been shown that mRNA expression levels have no significant effect on the survival rate. However, when the mRNA expression levels

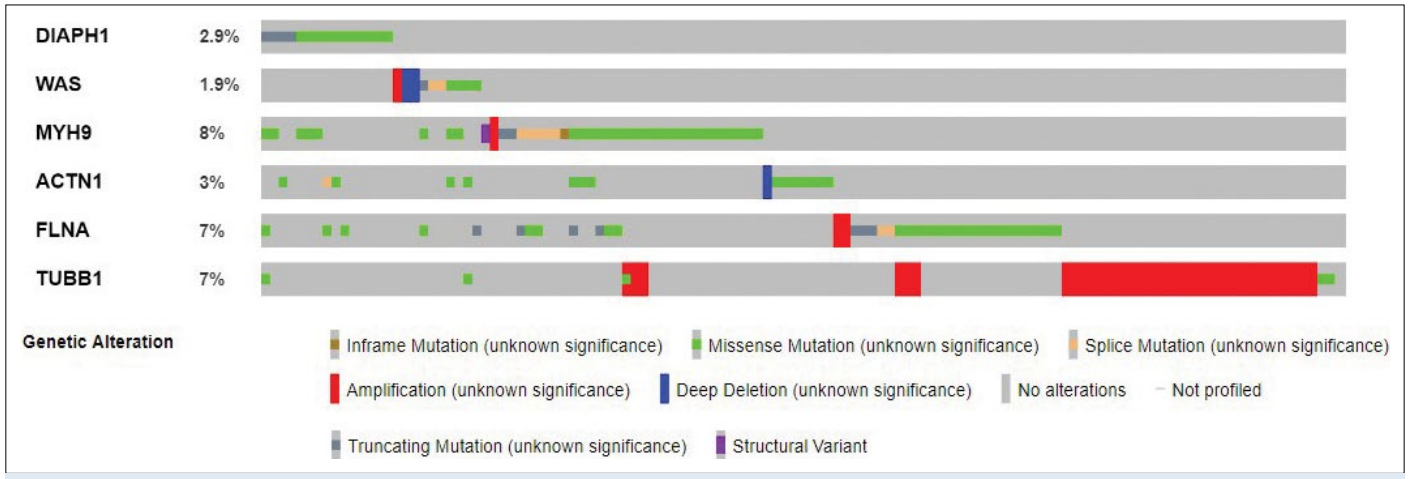

Figure 1. Lollipop diagrams of DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 mutations detected in 594 patients with CRC patients.

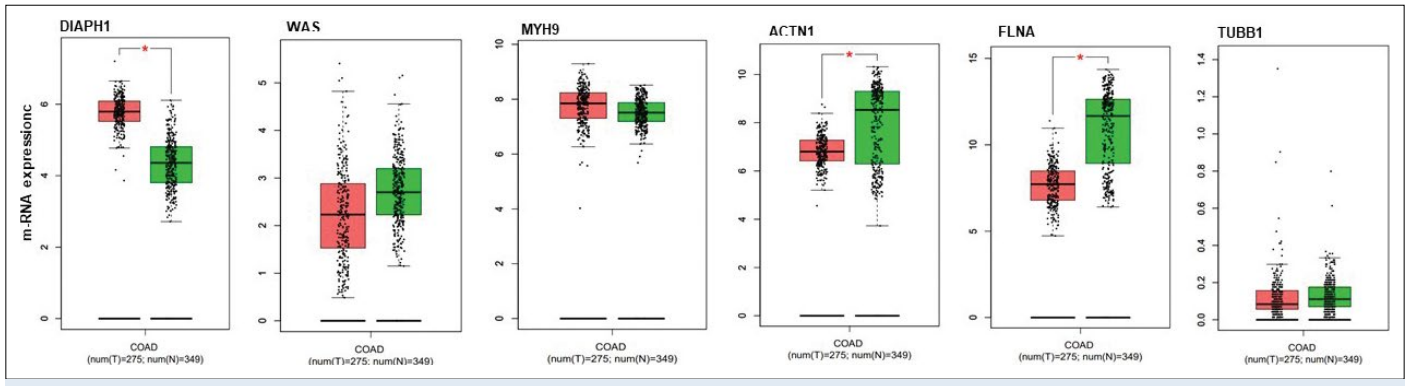

Figure 2. Box plot representation of the comparative mRNA expression level of DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes among CRC patients and healthy tissue samples using GEPIA; the red box indicates cancer tissue, and the green box indicates healthy tissue.

*: Indicates that the results are statistically significant.

were evaluated on the survival rate of disease-free conditions, it has been validated that high mRNA level is advantageous when compared with low mRNA level in terms of the length of the survival without disease, which is statistically significant (p=0.011) (Fig. 3). All the six genes exhibited positive correlation in our correlation analysis with the scope of understanding the comparative analysis of the effects of the association of DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 mutations and APC mutation on mRNA expression level. ACTN1-APC, DIAPH1-APC, FLNA-APC, WASP-APC, and MYH9-APC coexpression analysis showed a positive correlation (p=0.01, p=3.78e−16, p=1.828e−3, p=7.984e−4, and p=1.49e−9) (Fig. 4).

Results of the Protein–Protein Interaction Analysis

STRING analysis was applied to establish the estimated protein interactions net of the DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes. With respect to the analysis, 10 proteins were reported of which all the proteins in relation. It has been seen that mutations exist for every gene that codes the 10 proteins, which is in interaction with DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes that were depicted in Figure 5 in our CRC patient group. In the perspective of proteins that have mutual interaction with our target proteins, CDC5L protein with DIAPH1, TUBB1, and MYH9 proteins have interaction. CDC5 protein is a cell cycle regulator located at the Gap2 (G2)/Mitosis (M) passage, and it is known that it involves in the process of mending DNA damages and mRNA splicing. The CRC patient group has a mutation (2.4%) in their CDC5L gene.

Discussion

Colorectal cancer has the third-highest incidence rate, and it is the culprit behind one-fourth of all cancer deaths worldwide. Although its etiology and pathogenesis are not completely understood, the environmental, ethnic, economical, and genetic factors play a major role in the progression of

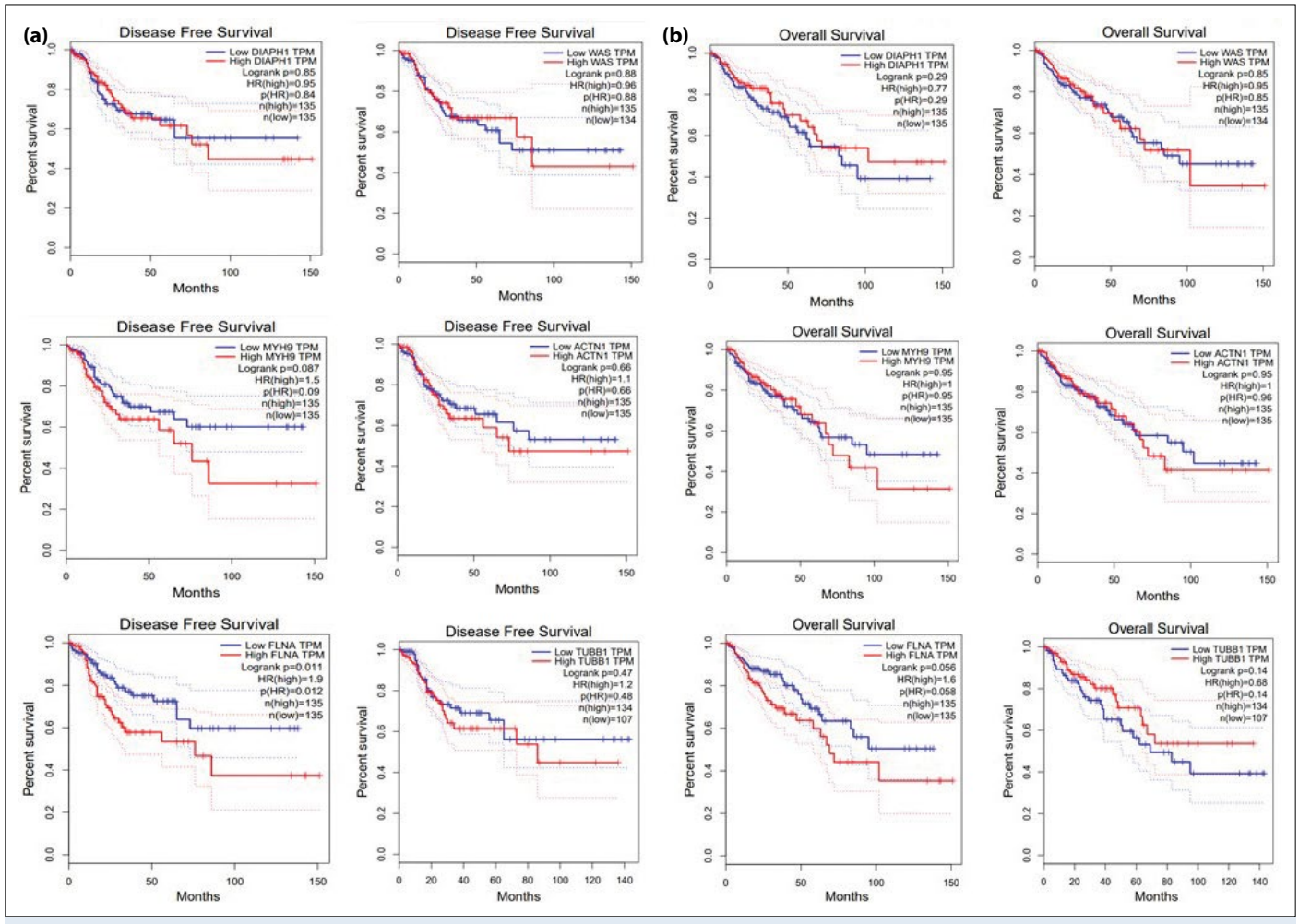

Figure 3. Comparison of the Kaplan–Meier survival curves of the high and low expressions of DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 in CRC patients ($p<0.05$).

CRC.[7,8,21] We have realized comparative and joint comprehensive analyses of mutation and mRNA expression profiles of cytoskeleton proteins DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1, which are known to be effective in the pathogenesis of CRC but not completely enlightened, through various online tools. First, the genome sequences of 594 CRC patients were gathered from the cbiO platform, which provides TGCA datasets. Then, the mutation profiles regarding DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 were analyzed. In our study, we have identified a total of 142 mutations (117 missense mutations, three nonsense mutations, eight frame shift mutations, 11 splice region mutations, one gene fusion, deep deletion, and gene amplification) regarding the six genes in 594 CRC patients. The highest number of mutations were detected on MYH9, whereas the least number of mutations were present on WASP.

MYH9 regulates cancer progression, and it is known to be functional as oncogenic and tumor-suppressing, but its part in CRC is not completely understood yet.[22] Globular head domain is responsible for actin bonding and force generation via MgATPaz activation.[15] On this domain, out of 20 identified mutations 2 (p.G205=,p.X290_splice) of them resided on the splice region. Since these mutations change the p.X290_splice region, which we determined as pathogenic, it will likely result as a passive transcript. It enhances the motion generated by the conformational alterations of the neck domain motor and acts as a lever that operates as a bonding region for tenuous chains using two IQ motives.[15] Because of its pathogenic character, p.K833N mutation exacerbates the function of its domain. Owing to its oncogenic properties, it has been unveiled that it supports tumor formation by regulating MAPK/AKT signalization of MYH9.[15,22] The p.Y9H mutation we detected on the globular domain has shown to be in gain characteristics. Moreover, it is probable that accompanied by the MYH9 gene amplification, it can gain more functionalities. However, we have revealed that mRNA expression level is no different than

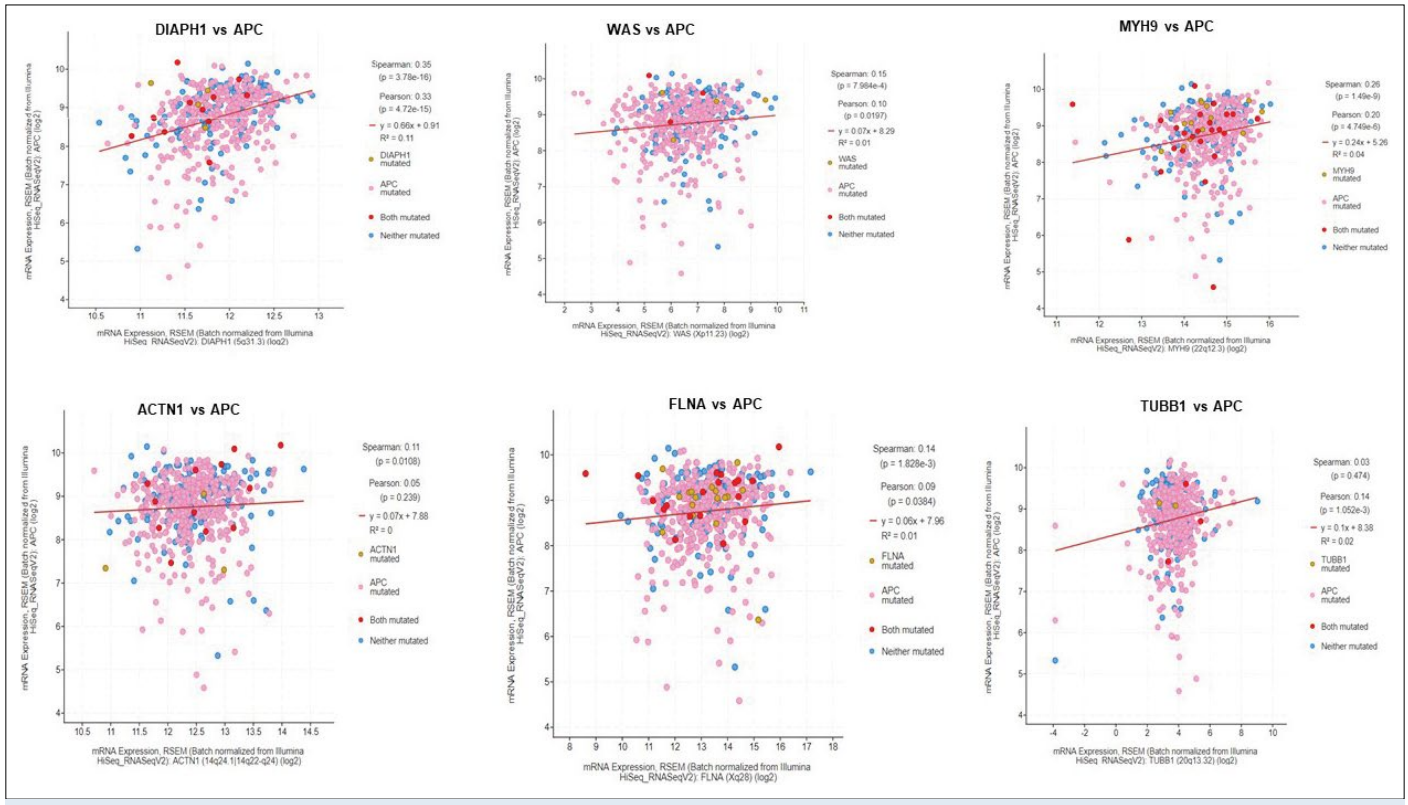

Figure 4. APC mutation and the effect of target gene mutations on normalized gene expression.

that of healthy tissues and it has no power on survival. Microtubules are ubiquitous cytoskeleton units that take place in maintaining the cell morphology, intracell transportation, cell signalization, cell locomotion, and various cellular functionalities including mitosis.[16] It can cause genetic inconsistency and DNA damage accumulation of TUBB1 dysfunction. In our study, this statement has been shown to be conserved. It can cause the chaotic activation of the GTP domain in which p.V169L mutation has rich mRNA expression. Moreover, an association of cell cycle regulator CDC5L p.K487N and with TUBB1 p.R77Q mutations are pathogenic. It has been proven that ACTN1 is related to the negative prognosis in breast cancer, carcinoma with oral squamous, and acute lymphoblastic leukemia; however, there has been no research on the investigation of its role in CRC.[23] In our findings, the ACTN1 mRNA expression levels in cancer tissues are low; still, no significant effects of this case have been found in common and disease-free survival. Nonetheless, because of the presence of p.X254 splice mutation on the splice region, it has been shown that a pathogenic character hinders the production of the functional transcript of 892 amino acid length. The presence of short nonfunctional transcripts might probably be the reason behind the significant scarcity in mRNA expression. It has been shown that AB domain mutations induce increased ACTN1 bonding in the actin filaments. The four SRs domain constitutes the rod area of ACTN1 protein.[20,23] On these SPEC-like repeats 1–4 (SR1–SR4) domains, we have identified nine distinct missense mutations. SR1–SR4 domains are the central region in which actin dimers originate. Four of the missense mutations we detected were identified as pathogenic using the estimation program. Hence, we hypothesize that by impeding the generation of actin dimers, they might cause protein imbalance in the seconder helical structure.

FLNA is interrelated to many cellular functions such as cell signalization, motility, phosphorylation, proteolysis, ion channel regulation, transcription regulation, receptor activation, and muscle development.[17] It has been reported in a few studies that the chemotherapy sensitivity of FLNA can be exploited as a cancer predictor and prognosis biomarker.[17,24] However, the clinical representation of FLNA in CRC and its biological function is controversial. FLNA's abnormal representation in many types of cancers such as breasts cancer, colon cancer, melanoma, and prostate cancer or its mutation has been reported.[17,24] In the previous studies, FLNA's having low expression in CRC and relation to the pathogenesis have been indicated. Parallel to the literature, the mRNA expression level of FLNA in cancer tissues was found to be statistically insignificant in our study, and high FLNA expression has been shown to be effective

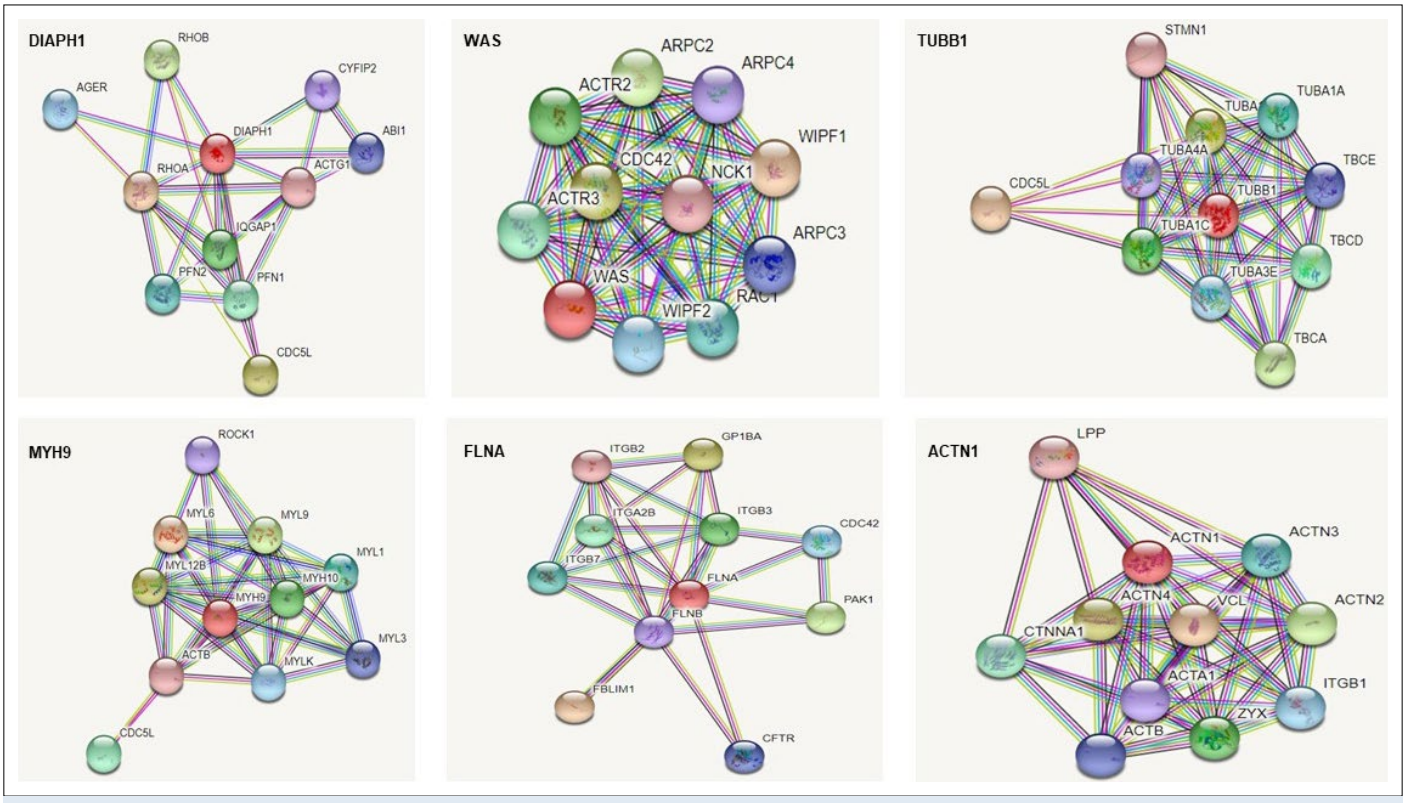

Figure 5. DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 protein–protein interaction networks. The figure was created via the cBioPortal website.

on disease-free survival. p.X1070_splice, p.X713_splice, and p.X1533_splice mutations and p.R2419* nonsense mutation have the potential to cause short, nonfunctional transcripts responsible for the low expression level.

WASP is a key actin regulatory protein accountable for the control of actin polymerization, adhesion, contraction, and preservation of the cell morphology. It generates the membrane protrusions that are seen during the metastasis and invasion of cancer cells.[18] In our study, WASP mutations in patients with metastasis were not determined. However, the p.A139V and p.R138Q mutations we identified are in the WH1 domain. These mutations are known to have pathogenic characteristics. Mutations in this domain have been reported to destabilize the protein's electrostatic force and protein–protein interactions.[18] The presence of p.X259_ splice mutation, which is responsible for the creation of filopodia and CDC-42 affinity on a base with a preserved splice region, might prevent the generation of a transcript. Located on the proline-rich domain, p.P353Hfs*92 frameshift domain responsible for WASP protein and Arp 2/3 complex interaction is in the pathogenic character and can deteriorate the function of the domain.

DIAPH1 is the actin nucleator and is also bonded to the microtubules with high affinity. The frameshift mutations that reside on FH1–FH2 domain with active actin bonding property might reduce the actin bonding capability by generating a stop codon in the early phase of the polypeptide of 1263 amino acid residues.[19,25] Although the high levels of DIAPH1 mRNA are a boosting factor for metastasis progression, the advent of resistance against chemotherapy medicine might lead to a negative prognosis.

Epithelial cells in CRC lose their actin cytoskeleton formation and normal cell-to-cell adhesion properties once they become invasive. Further research must allow the dissolving of new cytoskeleton functionalities and the development of novel therapeutic strategies based on the manipulation of the pertinent molecules. Dynamic actin cytoskeleton characterizes normal epithelial cells, polymerization and depolymerization of actin filaments, and alterations of the cells in the process of migration and mitosis. The interactions between adhesion complexes and cytoskeleton proteins are vital for maintaining the structure of epithelial cell structure and allows for the individual cells to respond to different stimuli and signals in harmony. In our study, as a result of a comprehensive molecular approach, we have identified the target gene mutations and expression abnormalities, which we believe to have a negative impact on the aforementioned epithelial net system. Our results constitute a

preliminary dataset that can be exploited in all the further in vivo and in vitro clinic research aimed at cancer diagnosis and development of new anticancer medicine. Certain restrictive factors are impeding our research in which we investigated the effects of cytoskeleton proteins in CRC pathogenesis. This is because the study was conducted by using bioinformatic tools with a limited experimental setting. Therefore, wet laboratory work is mandatory to reveal the roles of DIAPH1, WASP, MYH9, ACNT1, FLNA, and TUBB1 genes behind CRC pathogenesis.

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https:// http://www.cancer.gov/tcga www.cancer.gov/tcga. We thank the TCGA, GEPIA, cbio Portal and STRING databases for the availability of the data.

Ethical approval and ethical standards: The data used in our study were obtained from public database TCGA, therefore, ethical approval was not required. Availability of data and materials: The data sets generated and analyzed during the current study are available in TGCA database (https://www.cancer.gov/tcga),The cBio cancer genomics portal(http://www.cbioportal.org/).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: DFAB, DTO; Design: DFAB; Supervision: DFAB, DTO; Data Collection or Processing: DFAB; Analysis or Interpretation: DFAB; Literature Search: DFAB, DTO; Writing: DFAB, DTO; Critical Review: DFAB, DTO

Conflict of Interest: None declared.

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Appendix 1. Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
C-1	DIAPH1	c.2108del	COSV53881326	Frame shift deletion	Exon 16	P703Hfs*65	Colon Adenocarcinoma	MX	–	NA
C-2	DIAPH1	c.3542_3543del	COSV53881326	Frame shift deletion	Exon 25	E1181Afs*11	Colon Adenocarcinoma	MX	–	NA
C-3	DIAPH1	c.2505G>T	UNK	Missense mutation	Exon 18	K835N	Rectal Adenocarcinoma	–	Probably Damaging (0.99)	
C-4	DIAPH1	c.1262G>A	COSV53887553	Missense mutation	Exon 12	R421Q	Colon Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (0.99)
C-5	DIAPH1	c.2030del	COSV53879306	Frame shift deletion	Exon 16	P677Hfs*91	Colon Adenocarcinoma	–	–	NA
C-6	DIAPH1	c.1484G>A	COSV99526077	Missense mutation	Exon 15	R495Q	Rectal Adenocarcinoma	–	Benign (0.31)	Pathogenic (0.93)
C-7	DIAPH1	c.3416G>A	UNK	Missense mutation	Exon 24	R1139Q	Colon Adenocarcinoma	–	Probably Damaging (0.95)	
C-8	DIAPH1	c.849G>T	COSV99526083	Missense mutation	Exon 9	M283I	Rectal Adenocarcinoma	–	Benign (0.01)	Pathogenic (0.78)
C-9	DIAPH1	c.3162G>T	UNK	Missense mutation	Exon 23	K1054N	Rectal Adenocarcinoma	–	Probably Damaging (1.00)	
C-10	DIAPH1	c.107C>G	COSV99527098	Missense mutation	Exon 1	S36C	Colon Adenocarcinoma	M1A	Possibly Damaging (0.60)	Neutral (0.33)
C-11	DIAPH1	c.265G>T	COSV99526344	Missense mutation	Exon 1	D89Y	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (0.99)
C-12	DIAPH1	c.1321A>G	COSV99525739	Missense mutation	Exon 3	I441V	Colon Adenocarcinoma	–	Benign (0.04)	Pathogenic (score 0.96)
C-13	DIAPH1	c.505C>T	COSV53881525	Missense mutation	Exon 13	R169C	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.91)
C-14	DIAPH1	c.3566A>C	UNK	Missense mutation	Exon 5	E1189A	Mucinous Adenocarcinoma	–	Possibly Damaging (0.62)	
C-15	DIAPH1	c.3131C>G	UNK	Missense mutation	Exon 22	A1044G	Colon Adenocarcinoma	–	Probably Damaging (0.98)	
C-16	DIAPH1	c.1904G>T	COSV53878373	Missense mutation	Exon 16	G635V	Colon Adenocarcinoma	–	Benign (0.06)	Neutral (score 0.32)
C-17	DIAPH1	c.2008C>T	COSV99525648	Missense	Exon 16	P670S	Colon	–	Probably Damaging	Pathogenic (0.96)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
				mutation			Adenocarcinoma		(1.00)	
C-18	DIAPH1	c.1355T>C	COSV99525952	Missense mutation	Exon 13	F452S	Mucinous Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (1.00)
C-19	WAS	c.926G>A	COSV64996604	Missense mutation	Exon 9	R309H	Rectal Adenocarcinoma	–	Probably Damaging (0.96)	Pathogenic (score 0.92)
C-20	WAS	c.416C>T	COSV100939523	Missense mutation	Exon 4	A139V	Mucinous Adenocarcinoma	–	Benign (0.18)	Neutral (0.04)
C-21	WAS	c.777+1G>A	COSV64997624	Splice region mutation	–	X259_splice	Mucinous Adenocarcinoma	–	–	Pathogenic (score 0.98)
C-22	WAS	c.874G>A	COSV64997001	Missense mutation	Exon 9	D292N	Rectal Adenocarcinoma	–	Possibly Damaging (0.56)	Pathogenic (score 0.88)
C-23	WAS	c.932-3C>T	COSV100939540	Splice region mutation	–	X311_splice	Colon Adenocarcinoma	–	–	NA
C-24	WAS	c.1058del	COSV64996939	Frame shift deletion	Exon 10	P353Hfs*92	Colon Adenocarcinoma	–	–	NA
C-25	WAS	c.413G>A	COSV100939526	Missense mutation	Exon 4	R138Q	Colon Adenocarcinoma	–	Benign (0.05)	Neutral (0.02)
C-26	MYH9	c.3817G>A	COSV53388564	Missense mutation	Exon 28	D1273N	Rectal Adenocarcinoma	–	Benign (0.20)	Pathogenic (0.95)
C-27	MYH9	c.4903G>A	COSV53383317	Missense mutation	Exon 34	E1635K	Rectal Adenocarcinoma	–	Possibly Damaging (0.59)	Pathogenic (score 0.98)
C-28	MYH9	c.25T>C	COSV53389605	Missense mutation	Exon 2	Y9H	Colon Adenocarcinoma	–	Possibly Damaging (0.51)	Pathogenic (score 0.99)
C-29	MYH9	c.2774A>T	COSV53381635	Missense mutation	Exon 22	E925V	Colon Adenocarcinoma	–	Possibly Damaging (0.69)	Pathogenic (score 0.97)
C-30	MYH9	c.5735G>A	COSV53381830	Missense mutation	Exon 40	R1912H	Colon Adenocarcinoma	–	Benign (0.43)	Pathogenic (score 0.96)
C-31	MYH9	c.4975G>A	COSV53390794	Missense mutation	Exon 35	A1659T	Mucinous Adenocarcinoma	–	Benign (0.04)	Neutral (0.07)
C-32	MYH9	c.3110G>A	COSV53384546	Missense mutation	Exon 25	R1037H	Mucinous Adenocarcinoma	–	Possibly Damaging (0.63)	Pathogenic (score 0.93)
C-33	MYH9	c.5089C>T	COSV53386309	Missense mutation	Exon 36	R1697C	Mucinous Adenocarcinoma	–	Probably Damaging (0.95)	Pathogenic (score 0.94)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
C-34	MYH9	c.1178G>A	COSV53393696	Missense mutation	Exon 11	R393H	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.96)
C-35	MYH9	c.4306G>A	COSV53394348	Missense mutation	Exon 31	A1436T	Mucinous Adenocarcinoma	MX	Benign (0.03)	Pathogenic (score 0.96)
C-36	MYH9	c.5494G>A	COSV99337761	Missense mutation	Exon 39	A1832T	Colon Adenocarcinoma	–	Benign (0.63)	Pathogenic (score 0.91)
C-37	MYH9	c.2982G>T	COSV99337762	Missense mutation	Exon 24	K994N	Colon Adenocarcinoma	–	Probably Damaging (0.96)	Pathogenic (score 0.95)
C-38	MYH9	c.1522C>A	UNK	Missense mutation	Exon 13	L508M	Mucinous Adenocarcinoma	–	Possibly Damaging (0.89)	NA
C-39	MYH9	c.4852G>T	UNK	Missense mutation	Exon 34	D1618Y	Rectal Adenocarcinoma	–	Probably Damaging (1.00)	
C-40	MYH9	c.3960G>T	COSV53385658	Missense mutation	Exon 30	E1320D	Rectal Adenocarcinoma	–	Probably Damaging (0.91)	Pathogenic (score 0.98)
C-41	MYH9	c.310C>T	UNK	Missense mutation	Exon 2	R104C	Mucinous Adenocarcinoma	–	Probably Damaging (1.00)	
C-42	MYH9	c.615C>T	COSV53388454	Splice region mutation	Exon 6	G205=	Colon Adenocarcinoma	–		Neutral (score 0.05)
C-43	MYH9	c.869-1G>A	COSV53384961	Missense mutation	–	X290_splice	Colon Adenocarcinoma	–	–	Pathogenic (score 0.97)
C-44	MYH9	c.3214G>A	COSV53394359	Missense mutation	Exon 25	A1072T	Mucinous Adenocarcinoma	MX	Benign (0.06)	Pathogenic (score 0.93)
C-45	MYH9	c.5194G>A	COSV53391190	Missense mutation	Exon 37	A1732T	Mucinous Adenocarcinoma	–	Benign (0.09)	Pathogenic (score 0.97)
C-46	MYH9	c.2116C>T	COSV53382079	Missense mutation	Exon 17	Q706*	Colon Adenocarcinoma	MX	–	Pathogenic (score 0.98)
C-47	MYH9	c.2789G>A	UNK	Missense mutation	Exon 22	R930H	Rectal Adenocarcinoma	–	Possibly Damaging (0.86)	–
C-48	MYH9	c.5314C>A	COSV99339263	Missense mutation	Exon 38	H1772N	Colon Adenocarcinoma	–	Benign (0.00)	Pathogenic (score 0.70)
C-49	MYH9	c.5860G>A	COSV53384222	Missense mutation	Exon 41	A1954T	Colon Adenocarcinoma	–	Benign (0.01)	Pathogenic (score 0.91)
C-50	MYH9	c.4592A>T	COSV53384229	Missense	Exon 33	E1531V	Colon	–	Probably Damaging	Pathogenic (score 0.96)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
				mutation			Adenocarcinoma		(0.99)	
C-51	MYH9	c.1475A>G	COSV99338074	Missense mutation	Exon 13	Y492C	Colon Adenocarcinoma	–	Probably Damaging (0.95)	Pathogenic (score 0.96)
C-52	MYH9	c.176T>C	COSV53384237	Missense mutation	Exon 2	V59A	Colon Adenocarcinoma	–	Benign (0.00)	–
C-53	MYH9	c.3619C>T	COSV53387907	Missense mutation	Exon 27	Q1207*	Colon Adenocarcinoma	–	–	Pathogenic (score 0.98)
C-54	MYH9	c.2680G>A	COSV53386704	Missense mutation	Exon 22	E894K	Colon Adenocarcinoma	–	Possibly Damaging (0.69)	Pathogenic (score 0.96)
C-55	MYH9	c.5174G>A	COSV53382234	Missense mutation	Exon 37	R1725Q	Mucinous Adenocarcinoma	MX	Possibly Damaging (0.84)	Pathogenic (score 0.97)
C-56	MYH9	c.1280G>A	COSV99338405	Missense mutation	Exon 12	R427H	Mucinous Adenocarcinoma	MX	Possibly Damaging (0.49)	Pathogenic (score 0.94)
C-57	MYH9	c.2499G>T	COSV99338829	Missense mutation	Exon 20	K833N	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.96)
C-58	MYH9	c.2157G>A	COSV99337910	Missense mutation	Exon 17	Q719=	Mucinous Adenocarcinoma	–	–	Pathogenic (score 0.88)
C-59	MYH9	c.889T>C	UNK	Missense mutation	Exon 9	Y297H		–	Probably Damaging (0.92)	
C-60	MYH9	c.683A>G	UNK	Missense mutation	Exon 6	K228R	Colon Adenocarcinoma	–	Probably Damaging (0.79)	
C-61	MYH9	c.4220C>T	COSV53386467	Missense mutation	Exon 31	A1407V	Colon Adenocarcinoma	–	Benign (0.15)	Pathogenic (score 0.98)
C-62	MYH9	c.2305G>A	COSV53386477	Missense mutation	Exon 19	A769T	Colon Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (score 0.95)
C-63	MYH9	c.272G>A	COSV53394072	Missense mutation	Exon 2	C91Y	Colon Adenocarcinoma	–	Benign (0.01)	Pathogenic (score 0.93)
C-64	MYH9	c.1166T>A	COSV99338035	Missense mutation	Exon 11	I389N	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.97)
C-65	MYH9	c.1153T>G	COSV99338036	Missense mutation	Exon 11	F385V	Colon Adenocarcinoma	–	Probably Damaging (0.94)	Pathogenic (score 0.97)
C-66	MYH9	c.3881G>A	COSV53390783	Missense mutation	Exon 29	S1294N	Colon Adenocarcinoma	MX	Benign (0.01)	Pathogenic (score 0.87)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
C-67	MYH9	c.5573C>T	COSV99337619	Missense mutation	Exon 39	A1858V	Colon Adenocarcinoma	MX	Benign (0.06)	Pathogenic (score 0.96)
C-68	MYH9	c.3797G>A	COSV53383072	Missense mutation	Exon 28	R1266H	Colon Adenocarcinoma	–	Probably Damaging (0.93)	Pathogenic (score 0.95)
C-69	MYH9	c.2159+2T>C	COSV99339755	Splice region mutation	–	X720_splice	Colon Adenocarcinoma	–	–	Pathogenic (score 0.98)
C-70	MYH9	c.5546T>C	COSV53394175	Missense mutation	Exon 39	L1849P	Colon Adenocarcinoma	–	Possibly Damaging (0.72)	Pathogenic (score 0.96)
C-71	MYH9	c.3914C>T	COSV53394185	Missense mutation	Exon 29	A1305V	Colon Adenocarcinoma	–	Benign (0.05)	Pathogenic (score 0.93)
C-72	MYH9	c.2819_2821dup	COSV53383979	Frame shift insersion	Exon 22	K940dup	Colon Adenocarcinoma	–	–	NA
C-73	MYH9	c.584C>T	COSV53390806	Missense mutation	Exon 5	A195V	Mucinous Adenocarcinoma	–	Possibly Damaging (0.68)	Pathogenic (score 0.96)
C-74	MYH9	c.902G>A	COSV99338639	Missense mutation	Exon 9	R301H	Colon Adenocarcinoma	–	Benign (0.32)	Pathogenic (score 0.95)
C-75	MYH9	c.5595C>T	COSV53386713	Splice region mutation	Exon 40	A1865=	Colon Adenocarcinoma	–	–	Neutral (score 0.09)
C-76	MYH9	c.705+1G>A	COSV53383218	Splice region mutation	–	X235_splice	Colon Adenocarcinoma	–	–	Pathogenic (score 0.98)
C-77	MYH9		UNK	Fusion	–	MYH9-SORCS1	Rectal Adenocarcinoma	–	–	
C-78	ACTN1	c.2140C>T	COSV51991205	Missense mutation	Exon 18	R714C	Colon Adenocarcinoma	–	Probably Damaging (0.98)	Pathogenic (score 0.96)
C-79	ACTN1	c.1495C>T	COSV51988432	Missense mutation	Exon 14	R499W	Colon Adenocarcinoma	–	Probably Damaging (0.93)	Pathogenic (score 0.96)
C-80	ACTN1	c.2278C>T	UNK	Missense mutation	Exon 18	R760W	Mucinous Adenocarcinoma	–	Possibly Damaging (0.53)	NA
C-81	ACTN1	c.1501G>A	UNK	Missense mutation	Exon 14	E501K	Rectal Adenocarcinoma	–	Benign (0.31)	NA
C-82	ACTN1	c.1219G>A	COSV99516943	Missense mutation	Exon 11	E407K	Rectal Adenocarcinoma	–	Possibly Damaging (0.73)	Pathogenic (score 0.97)
C-83	ACTN1	c.2548C>T	COSV51988400	Missense	Exon 21	R850C	Colon	–	Probably Damaging	Pathogenic (score 0.99)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
				mutation			Adenocarcinoma		(0.95)	
C-84	ACTN1	c.295T>G	COSV99518578	Missense mutation	Exon 3	F99V	Rectal Adenocarcinoma	–	Possibly Damaging (0.66)	Pathogenic (score 0.99
C-85	ACTN1	c.1667C>T	COSV51991746	Missense mutation	Exon 15	A556V	Rectal Adenocarcinoma	–	Benign (0.15)	Pathogenic (score 0.99)
C-86	ACTN1	c.398G>A	COSV51991475	Missense mutation	Exon 4	R133H	Rectal Adenocarcinoma	–	Possibly Damaging (0.86)	Pathogenic (score 0.77)
C-87	ACTN1	c.2546G>A	COSV51987977	Missense mutation	Exon 21	R849H	Mucinous Adenocarcinoma	MX	Probably Damaging (0.92)	Pathogenic (score 0.93)
C-88	ACTN1	c.2173G>A	COSV51994635	Missense mutation	Exon 18	A725T	Colon Adenocarcinoma	–	Benign (0.12)	Pathogenic (score 0.96)
C-89	ACTN1	c.2171T>G	COSV51991923	Missense mutation	Exon 14	I724S	Colon Adenocarcinoma	–	Possibly Damaging (0.85)	Pathogenic (score 0.97)
C-90	ACTN1	c.1504A>C	COSV51997078	Missense mutation	Exon 14	K502Q	Mucinous Adenocarcinoma	–	Benign (0.00)	Pathogenic (score 0.99)
C-91	ACTN1	c.2342A>G	COSV51992840	Missense mutation	Exon 19	D781G	Colon Adenocarcinoma	M1	Benign (0.01)	Pathogenic (score 0.99)
C-92	ACTN1	c.762+3A>G	COSV99517365	Splice region mutation	–	X254_splice	Mucinous Adenocarcinoma	MX	–	Pathogenic (score 0.99)
C-93	FLNA	c.2651G>A	COSV61043136	Missense mutation	Exon 18	R884H	Rectal Adenocarcinoma	–	Probably Damaging (0.97)	Neutral (score 0.34)
C-94	FLNA	c.5146del	COSV61041499	Missense mutation	Exon 31		Colon Adenocarcinoma	–	–	–
C-95	FLNA	c.1771G>A	COSV61038114	Missense mutation	Exon 12	V591I	Rectal Adenocarcinoma	MX	Probably Damaging (0.95)	–
C-96	FLNA	c.2033G>A	COSV61038176	Missense mutation	Exon 14	R678H	Colon Adenocarcinoma	–	Benign (0.00)	Neutral (score 0.07)
C-97	FLNA	c.7558del	UNK	Missense mutation	Exon 47	R2520Vfs*16	Colon Adenocarcinoma	–	–	–
C-98	FLNA	c.3094C>T	UNK	Missense mutation	Exon 21	R1032C	Colon Adenocarcinoma	MX	Possibly Damaging (0.77)	–
C-99	FLNA	c.4060G>A	COSV61050253	Missense mutation	Exon 24	D1354N	Colon Adenocarcinoma	–	Possibly Damaging (0.54)	Neutral (score 0.43)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
C-100	FLNA	c.4495G>A	COSV100775409	Missense mutation	Exon 27	V1499M	Mucinous Adenocarcinoma	–	Benign (0.16)	Pathogenic (score 0.83)
C-101	FLNA	c.1882G>A	COSV100774671	Missense mutation	Exon 13	D628N	Colon Adenocarcinoma	–	Benign (0.41)	Pathogenic (score 0.89)
C-102	FLNA	c.6602G>A	COSV61041891	Missense mutation	Exon 41	R2201H	Mucinous Adenocarcinoma	–	Benign (0.22)	Pathogenic (score 0.94)
C-103	FLNA	c.1931C>T	COSV100774735	Missense mutation	Exon 13	A644V	Mucinous Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (score 0.92)
C-104	FLNA	c.7144G>T	UNK	Missense mutation	Exon 44	E2382*	Colon Adenocarcinoma	–	–	–
C-105	FLNA	c.1873G>A	COSV61044608	Missense mutation	Exon 13	D625N	Mucinous Adenocarcinoma	–	Probably Damaging (0.95)	Pathogenic (score 0.90)
C-106	FLNA	c.6863G>A	COSV100774292	Missense mutation	Exon 44	R2288H	Colon Adenocarcinoma	–	Benign (0.26)	Pathogenic (score 0.75)
C-107	FLNA	c.1310G>A	COSV61051859	Missense mutation	Exon 9	R437Q	Colon Adenocarcinoma	MX	Benign (0.05)	Neutral (score 0.39)
C-108	FLNA	c.7825G>A	COSV61039008	Missense mutation	Exon 48	V2609M	Colon Adenocarcinoma	–	Benign (0.03)	Neutral (score 0.14)
C-109	FLNA	c.206C>T	COSV61045030	Missense mutation	Exon 2	T69M	Rectal Adenocarcinoma	–	Possibly Damaging (0.56)	Pathogenic (score 0.82)
C-110	FLNA	c.3699C>G	COSV61045599	Missense mutation	Exon 22	I1233M	Rectal Adenocarcinoma	–	Probably Damaging (0.94)	Pathogenic (score 0.80)
C-111	FLNA	c.3208-2A>G	COSV100774874	Splice region mutation		X1070_splice	Colon Adenocarcinoma	M1A	–	Pathogenic (score 0.91)
C-112	FLNA	c.7354G>A	COSV100774869	Missense mutation	Exon 46	V2452M	Colon Adenocarcinoma	–	Probably Damaging (0.98)	Pathogenic (score 0.83)
C-113	FLNA	c.3353G>T	COSV61040689	Missense mutation	Exon 22	G1118V	Colon Adenocarcinoma	–	Possibly Damaging (0.46)	Pathogenic (score 0.95)
C-114	FLNA	c.220G>A	COSV100774207	Missense mutation	Exon 2	G74R	Colon Adenocarcinoma	–	Benign (0.31)	Pathogenic (score 0.99)
C-115	FLNA	c.5917A>T	COSV61041588	Missense mutation	Exon 37	I1973F	Colon Adenocarcinoma	–	Benign (0.18)	Pathogenic (score 0.80)
C-116	FLNA	c.2252G>A	COSV61041602	Missense	Exon 15	G751D	Colon	–	Benign (0.17)	Pathogenic (score 0.77)

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
				mutation			Adenocarcinoma			
C-118	FLNA	c.4084G>A	COSV100774284	Missense mutation	Exon 24	G1362R	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.95)
C-119	FLNA	c.7042A>G	COSV100774667	Missense mutation	Exon 44	N2348D	Colon Adenocarcinoma	–	Benign (0.10)	Pathogenic (score 0.93)
C-120	FLNA	c.3215C>T	COSV100774670	Missense mutation	Exon 22	A1072V	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.87)
C-121	FLNA	c.4475G>A	UNK	Missense mutation	Exon 27	G1492D	Colon Adenocarcinoma	–	Probably Damaging (0.99)	–
C-122	FLNA	c.4062C>G	COSV100774231	Missense mutation	Exon 24	D1354E	Colon Adenocarcinoma	–	Probably Damaging (0.97)	Pathogenic (score 0.83)
C-123	FLNA	c.4903C>T	COSV61040067	Missense mutation	Exon 29	R1635C	Mucinous Adenocarcinoma	MX	Possibly Damaging (0.84)	Pathogenic (score 0.96)
C-124	FLNA	c.1897G>A	COSV61044313	Missense mutation	Exon 13	V633M	Colon Adenocarcinoma	–	Probably Damaging (0.98)	Pathogenic (score 0.89)
C-125	FLNA	c.7208A>G	UNK	Missense mutation	Exon 45	D2403G	Colon Adenocarcinoma	MX	Benign (0.16)	–
C-126	FLNA	c.3377A>G	COSV61041615	Missense mutation	Exon 22	Y1126C	Colon Adenocarcinoma	–	Benign (0.43)	Pathogenic (score 0.94)
C-127	FLNA	c.2488G>T	COSV61041891	Missense mutation	Exon 17	D830Y	Colon Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (score 0.94)
C-128	FLNA	c.7799C>T	COSV61051415	Missense mutation	Exon 48	P2600L	Mucinous Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (score 0.97)
C-129	FLNA	c.7088C>T	COSV61046152	Missense mutation	Exon 44	A2363V	Mucinous Adenocarcinoma	–	Benign (0.01)	Neutral (score 0.11)
C-130	FLNA	c.5069C>T	COSV61036621	Missense mutation	Exon 31	T1690M	Colon Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (score 0.95)
C-131	FLNA	c.2137-2A>G	COSV100775285	Splice region mutation	–	X713_splice	Colon Adenocarcinoma	–	–	Pathogenic (score 0.96)
C-132	FLNA	c.4599-1G>A	COSV100775425	Splice region mutation	–	X1533_splice	Colon Adenocarcinoma	–	–	Pathogenic (score 0.95)
C-133	FLNA	c.3076del	COSV61039186	Frame shift deletion	Exon 21	A1026Lfs*20	Colon Adenocarcinoma	–	–	NA

Appendix 1 (cont.). Characteristics of detected mutations in DIAPH1, WAS, MYH9, ACNT1, FLNA, and TUBB1 genes

No	Gene	Nt alteration	Rs number	Alteration type	Localization	AA position	Previously determined disease/ browser	Metastasis status	Clinical significance	
									Poly-Phen2 (score)	COSMIC prediction (score)
C-134	FLNA	c.4196A>G	COSV100775042	Missense mutation	Exon 25	K1399R	Colon Adenocarcinoma	–	Possibly Damaging (0.86)	Pathogenic (score 0.89)
C-135	FLNA	c.3337G>A	COSV61043746	Missense mutation	Exon 22	E1113K	Colon Adenocarcinoma	–	–	Pathogenic (score 0.83)
C-136	FLNA	c.7255C>T	UNK	Nonsense mutation	Exon 45	R2419*	Mucinous Adenocarcinoma	MX	–	
C-137	FLNA	c.6710del	COSV61045578	Frame shift deletion	–	G2237Efs*109	Colon Adenocarcinoma	–	–	–
C-138	TUBB1	c.895A>G	COSV53886308	Missense mutation	Exon 4	T299A	Colon Adenocarcinoma	–	Benign (0.00)	Pathogenic (0.89)
C-139	TUBB1	c.230G>A	COSV53886673	Missense mutation	Exon 3	R77Q	Rectal Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (0.98)
C-140	TUBB1	c.1118C>T	COSV99414046	Missense mutation	Exon 4	A373V	Colon Adenocarcinoma	–	Probably Damaging (1.00)	Pathogenic (0.97)
C-141	TUBB1	c.761C>T	COSV53886041	Missense mutation	Exon 4	A254V	Rectal Adenocarcinoma	–	Probably Damaging (0.99)	Pathogenic (0.96)
C-142	TUBB1	c.505G>C	COSV53888460	Missense mutation	Exon 4	V169L	Colon Adenocarcinoma	–	Benign (0.18)	Pathogenic (0.95)

C: Change; Nt: Nucleotid; Missense Mutation; AA: Amino Acid; c: Complementary DNA; dup: Duplication; del: Deletion; MX: Distant metastasis cannot be assessed; M0: No distant metastasis; M1: Distant metastasis; fs: Frameshift; Abbreviations of amino acids are used. UNK: Unknown; DIAPH1: Diaphanous Related Formin 1; WASP: Actin Nucleation Promoting Factor; MYH9: Myosin Heavy Chain 9; ACNT1: Actinin Alpha 1; FLNA: Filamin A; TUBB1: Tubulin Beta 1 Class VI.