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STK11 p.G270W: A Novel Mutation Detected in a Case of MSI High Mixed Medullary–Mucinous Carcinoma of the Transverse Colon

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Abstract. *Background/Aim: To report a case of mixed medullary/mucinous adenocarcinoma with unusual mutational gene profile. Patients and Methods: A 79-year-old female was diagnosed with a colorectal carcinoma of the transverse colon. The diagnostic work-up of this case included thorough clinicopathological evaluation, immunohistochemistry and next generation Sequencing. Results: The clinicopathological evaluation showed a tumor with morphological features of both medullary and mucinous colorectal carcinoma. Immunohistochemistry revealed the loss of DNA mismatch repair proteins. NGS showed that the medullary component of this tumor had a novel STK11 p.G270W mutation, which was not present in the mucinous component. Both the medullary and mucinous components also had BRAF V600E and AKT1 (pE17K) mutations. Conclusion: We report a novel mutation STK11 (p.G270W), in medullary carcinoma of the colon with an associated mucinous component.*

Medullary carcinoma and mucinous adenocarcinoma are two variants of colon carcinoma associated with microsatellite instability (MSI) and occur mostly in the right side of the colon (1). Histologically, medullary carcinoma is characterized by a tumor with a pushing infiltrating border and is composed of pleomorphic cells with a syncytial growth pattern and infiltration by lymphocytes. The mucinous tumors are usually well differentiated and are characterized by lakes of mucin containing floating clusters of neoplastic colonic epithelium (2, 3). These tumors usually have a Crohn's like lymphocytic reaction (4). Detection of MSI in colorectal cancer is important not only for detecting hereditary non polyposis colorectal cancer (HNPCC) but also for the prognosis and treatment decisions (5). MSI high (MSI-H) colon adenocarcinoma (CA) has a better overall prognosis compared to microsatellite stable (MSS) CA and is less responsive to 5 flourouracil-based chemotherapy regimen in most of the studies (6). MSI tumors are a heterogeneous group of cancers with a distinct oncogenic pathway. Proteins within the DNA mismatch repair (MMR) system include: MLH1, PMS2, MSH2, MSH6, MLH3, MSH3, PMS1, and Exo1. MSI-H CA are further divided into four categories: i) sporadic, ii) HNPCC or Lynch syndrome, iii) unexplained defect in MMR CA and iv) constitutional MMR deficiency syndrome. The most common and relevant heterodimers in colorectal carcinogenesis are MLH1/PMS2 and MSH2/MSH6 (7). The incidence of *RAS/RAF/PI3KA* and *TP53* gene mutations is well established in CA of the left side; however, less information is available on the other, less frequently mutated, genes in CA. Some of the less frequently mutated genes include: *FBXW7, PTEN, SMAD4, EGFR, CTNNB1, AKT1, STK11, ERBB2, ERBB4, ALK, MAP2K1* and *NOTCH*, which may serve as prognostic and predictive markers (8). *Serine/threonine protein kinase 11* (*STK11*) tumor suppressor mutations/ loss of heterozygosity have been associated primarily with left sided colon carcinomas and germ line mutations of this gene are classically detected in individuals with Peutz-Jegher syndrome (9).

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Key Words: Colon cancer, MSI, medullary, mucinous, STK11, mutation.

Table I. *Mutated genes and type of mutations in each tumor component.*

Genes mutated in CRC	Type of mutation in medullary component	Type of mutation in mucinous component
BRAF	V600E	V600E
CDH1	A617T	A617T
SMAD4	R361H	R361H
SMAD4	V354Wfs*30	V354Wfs*30
PTEN	F206V	F206V
STK11	G270W	Not detected
AKT1	E17K	E17K
MSH6	F989Lfs*5	F989Lfs*5

CRC: Colorectal cancer.

Here we report a case of transverse colon carcinoma exhibiting a mixed medullary-mucinous morphology. Molecular testing of this tumor revealed a novel mutation in *STK11* in the medullary component. Both mucinous and medullary components contained *BRAF* (V600E) and *AKT1* (pE17K) mutations.

Patients and Methods

Case report. We report a case of a transverse colon carcinoma in a 79-year-old female. She initially presented with iron deficiency anemia, 6-month history of abdominal pain and 40 lb weight loss over 1 year. Computed tomography (CT) of the abdomen showed a 6.2×4.2 cm mass in middle transverse colon with several enlarged nodes in small bowel mesentery and omentum. Colonoscopy showed a large obstructing mass of 60 cm. Biopsy showed invasive adenocarcinoma. Positron emission tomography (PET) scan revealed a large hypermetabolic transverse colon mass with several hypermetabolic omental nodules as well as a hypermetabolic adrenal nodule. The patient underwent exploratory laparoscopy and partial colectomy that showed a T3N1b medullary carcinoma with 4/20 positive lymph nodes.

Immunohistochemistry and mutation analysis. The pathology report and the H&E slides were reviewed by 2 pathologists (DC and MS) to confirm the diagnosis. A formalin-fixed paraffin embedded block, most representative of the lesion, was selected. Each component was micro-dissected and tested separately using immunohistochemistry (IHC) and Next Generation Sequencing (NGS). Four micron-thick sections from this block were used for immunohistochemistry. Immunostaining for MLH1, PMS2, MSH2, MSH6, CAM 5.2, CK20, Calretinin, Chromogranin, Synaptophysin, CD56, CK5/6, Ki-67, CK7, p63, and p40 was performed using the automated Dako (Agilent Technologies, Santa Clara, CA, USA) and Ventana (Ventana Medical System Inc, Tucson, AZ, USA) immunostainers. The tumor was assessed for somatic mutations using the NGS TruSight Tumor Panel (Illumina, Inc.; www.illumina.com/company/legal.html). The panel enables the comprehensive evaluation of mutations across 26 therapeutically relevant genes selected from the College of American Pathologists (CAP) and National Comprehensive Cancer Network (NCCN) guidelines. The procedure was optimized for the sequencing of genomic DNA extracted from formalin-fixed, paraffin-embedded tissues and produced highly accurate mutation profiling. The DNA library was prepared and sequenced according to manufacturer's recommendations using the MiSeq system (lllumina, Inc.). Data analysis and reporting were performed using the Clinical Genomics Workspace (CGW) software package (PierianDx, Inc; St. Louis, MO, USA) for NGS. For the data analysis, Perian DX uses the Integrative Genomics Viewer, a high-performance visualization tool that allows interactive exploration of large integrated genomic datasets.

Results

Review of the H&E slides confirmed that the adenocarcinoma exhibited 2 different histological components (Figure 1). The first component was characterized by a well-circumscribed medullary carcinoma composed of pleomorphic cells with a syncytial growth pattern, intermixed lymphocytes and focal areas of necrosis (Figure 1A). The second component was represented by a well-differentiated mucinous adenocarcinoma (Figure 1B). Using immunohistochemistry the tumor was positive for CAM5.2 and CK20, and negative for CK7, Calretinin, p63, chromogranin synaptophysin, CK5/6, p40 and CD56. CK20 was positive in the mucinous area and negative in the medullary area. The Ki-67-positive immunostaining decorated 100% of the tumor. Immunostaining for DNA MMR proteins showed loss of MLH1 and PMS2, consistent with typical MSI-H (Figure 2). Targeted NGS sequencing revealed several uncommon mutations in both components (Table I and Figure 3). Both components of the tumor revealed a *BRAF* (V600E) mutation, *AKT1* (p.E17K) mutation, as well as mutations in *CDH1* (A617T), *SMAD4* (R361H and *V354Wfs*30*, *pTEN* (F206V) and *MSH6* (F989Lfs). Unexpectedly, a novel mutation in *STK11* (p. G270W) (Figure 3) and a mutation in *AKT1* (p. E17K) were also detected. While the *AKT1* (pE17K) mutation was present in both tumor components, the *STK11* (p. G270W) mutation was only found in the medullary component. A PubMed and cosmic database search did not show any matching results for the *STK11* (p. G270W) mutation in medullary colorectal carcinoma.

Discussion

Colorectal cancer (CA) is the fourth most commonly diagnosed cancer in adults in the United States and is the second leading cause of cancer death, leading to over 50,000 deaths annually (10). The pathogenesis of Ca is very complex and diverse and is also influenced by multiple factors, some of which are related to diet and lifestyle, while others are related to genetic predisposition (11). CA evolves through a stepwise accumulation of genetic and epigenetic alterations, leading to the transformation of normal colonic mucosa into invasive cancer (12). CA molecular pathways are characterized by distinct models of genetic instability, subsequent clinical manifestations, and pathological behavior

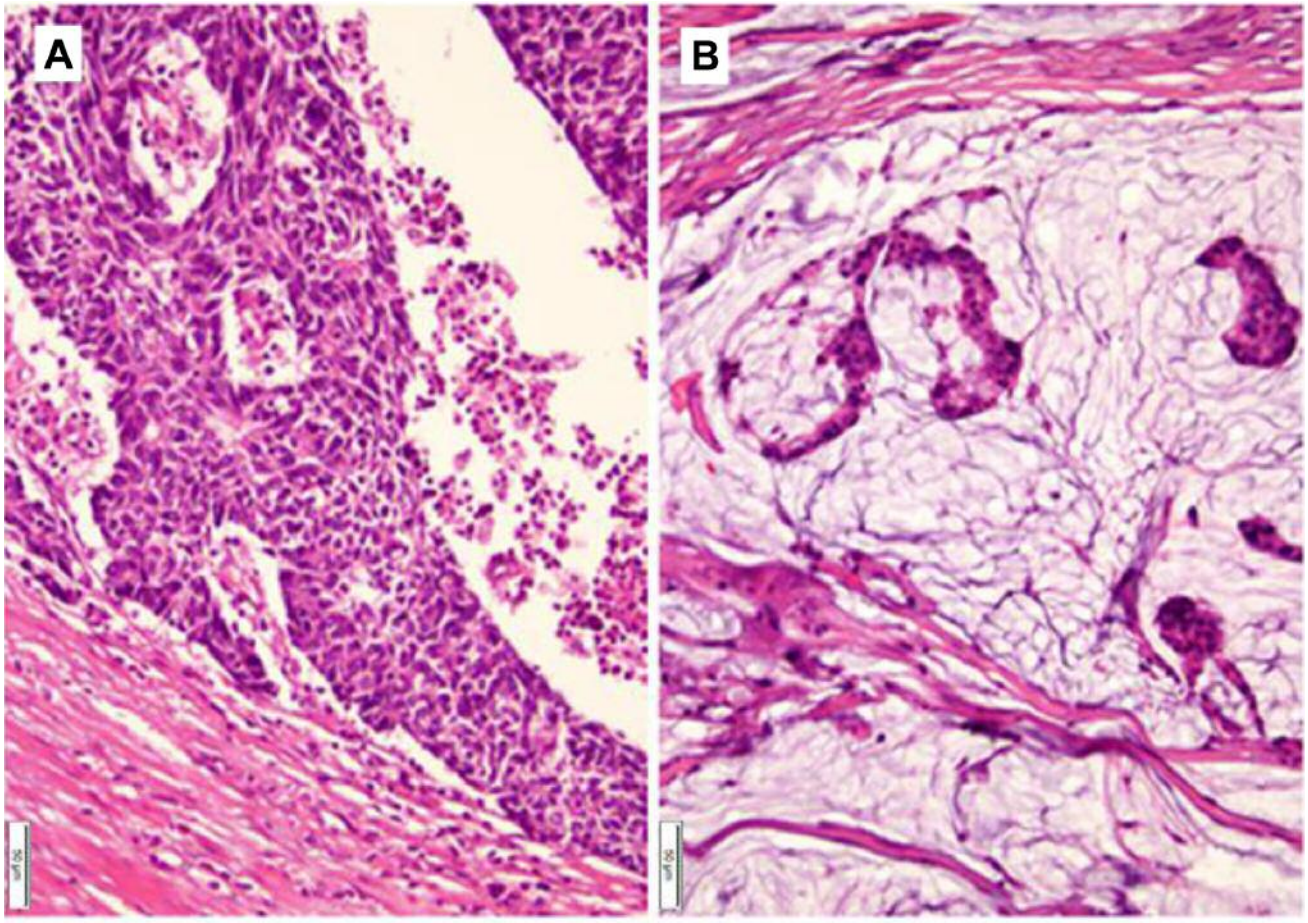

Figure 1. *The two histological components of the adenocarcinoma. (A) A well-circumscribed medullary component with a syncytial growth pattern (arrow), intermixed lymphocytes (LY) and areas of necrosis (asterisk). (B) A well-differentiated mucinous component (arrow) with groups of tumor cells floating within the mucin (asterisk).*

characteristics. Most CA follow the chromosomal instability (CIN) pathway, characterized by a widespread loss of heterozygosity and gross chromosomal abnormalities. The second pathway involves approximately 15% of CA and is due to derangement of the DNA Mismatch Repair (MMR) system and consequent MSI (11). The type of CA that develops through the MSI pathway presents peculiar clinical features: it is more often located in the proximal colon and has a poorly differentiated, and presents a mucinous or medullary histotype, and often presents intense peritumoral and intratumoral lymphocytic infiltrations. The prognosis and survival of patients affected by MSI-high CA is better and longer compared to patients with chromosomal instability, despite the fact that MSI-high CA do not respond to 5-fluorouracyl-based chemotherapies (11). Medullary and mucinous histologies are a well-defined characteristic of MSI CA; however, mixed morphology is seldom described in the literature. Medullary carcinoma is a morphological variant of colon cancer similar to undifferentiated adenocarcinoma that tends to display a distinct clinical behavior. It is typically more common in older females, less likely to present with nodal involvement and generally has a better prognosis and strong association with MSI, present in at least 60% of cases (13). Mucinous adenocarcinoma is a histological subtype of colorectal cancer with a substantial amount of mucin (>50% of the tumor) retained within the tumor. Mucinous adenocarcinoma has a worse prognosis, occurs at a younger age, is usually of larger size, and it represents one of the tumor types more commonly present among right-sided MSI-H CA (14). Our case showed mixed medullary and mucinous morphology with MSI-H. Several mutations were detected in both components of this particular case. The *BRAF* (p.V600E) and *AKT1* (p. E17K) mutations were present in both components of the tumor while the novel *STK11* (p. G270W) mutation was present only in the medullary component of this tumor. This finding

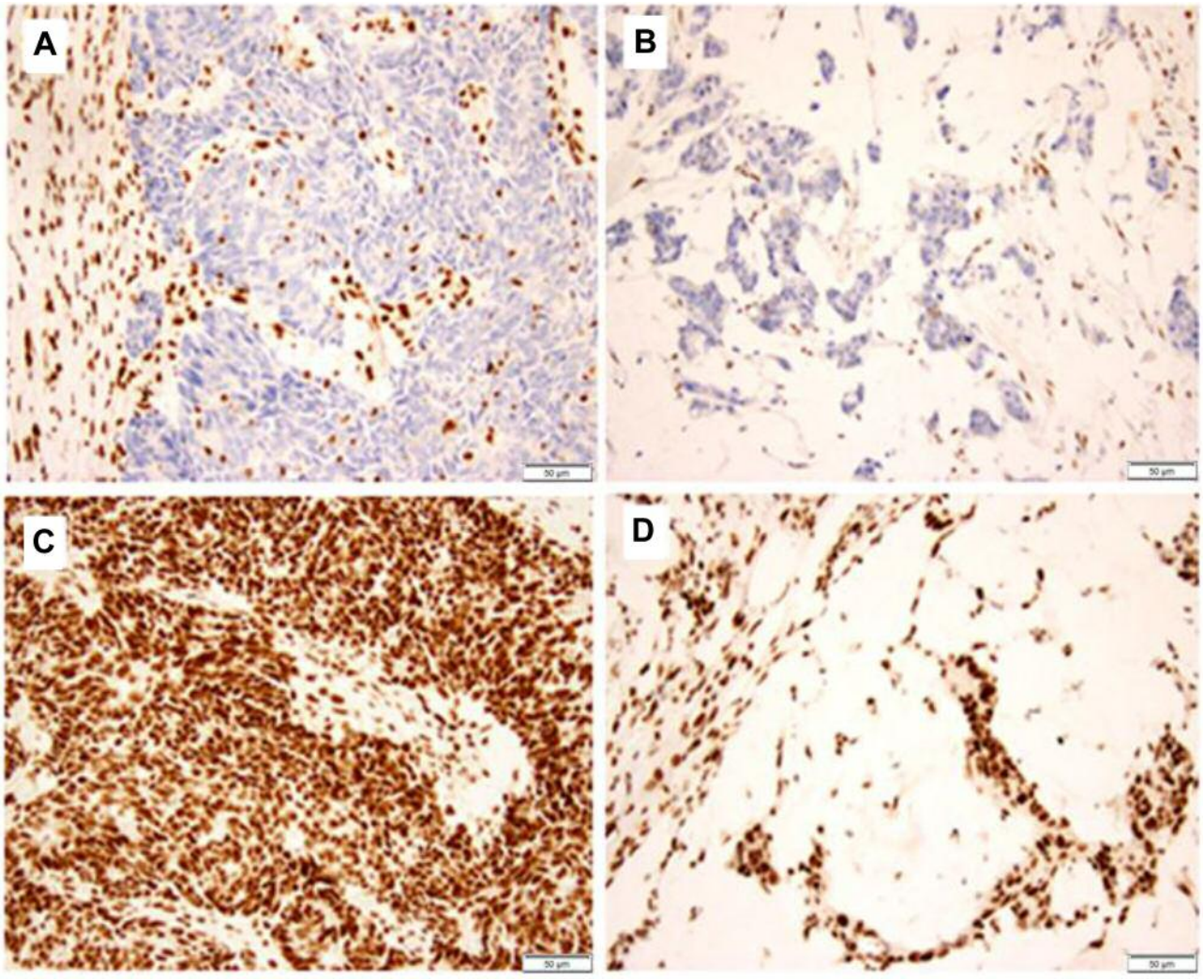

Figure 2. *Immunohistochemical analysis of mismatched repair proteins. Loss of MLH1 nuclear protein expression in both the medullary (A, brown) and the mucinous component (B, brown). MSH2 nuclear protein expression is retained in both components (C and D, brown).*

raises the possibility that the medullary and mucinous components of this tumor may have arose from the same ancestor tumor cell/clone, which differentiated later into morphologically distinct components.

It is possible that the tumors medullary component acquired this new *STK11* mutation while tumor's cells underwent morphological differentiation. If this is true, the *STK11* mutation is not an initial driver mutation for this cancer but a later event, which may provide a therapeutic target for treatment. Alternatively, the tumor ancestor cell may have acquired the *STK11* mutation as an initial event and, a portion of the offspring tumor cells, may have subsequently lost this mutation while differentiating into the mucinous type. We believe this to be less likely true since the patient did not undergo *STK11* molecular targeting therapy, which is usually the driving force behind tumor clone selection. The *STK11* gene regulates cell polarity and functions as a tumor suppressor (15).

To our knowledge this is the first report of a *STK11* (p. G270W) mutation in medullary colorectal carcinoma. Our findings suggest that STK11 may play an important role in the carcinogenesis of medullary colon carcinoma.

The *AKT1* (p.E17K) mutation has been previously shown to have significant association with the mucinous morphology of colorectal carcinoma and with the concurrent *BRAF* (V600E) mutation (16). The detection of *AKT1* (p.E17K) mutation in our case was not, however, limited to the mucinous component of the tumor. We report a novel mutation *STK11* (p.G270W) in a medullary carcinoma of the colon with an associated mucinous component. Interestingly

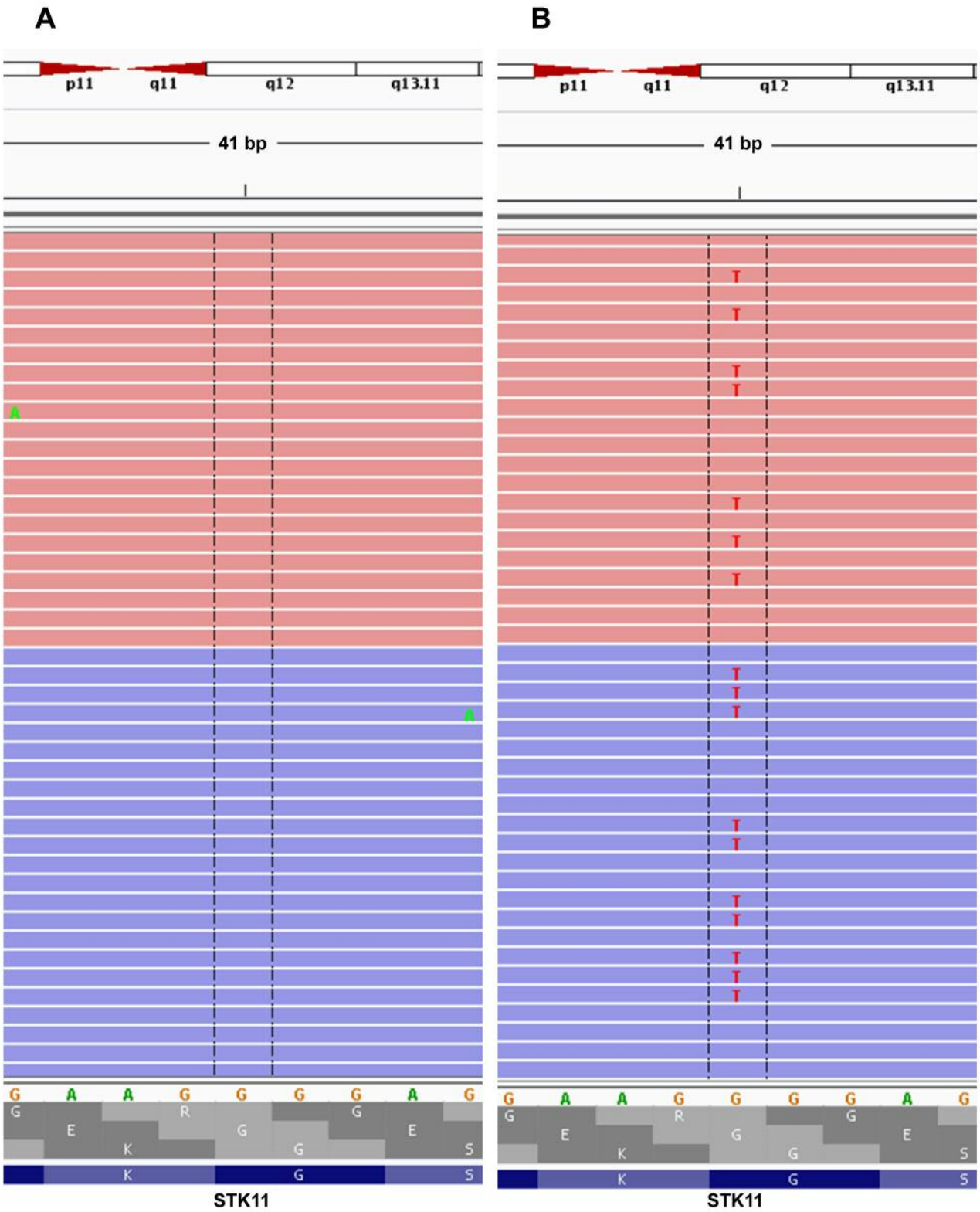

Figure 3. *The Integrative Genomic Viewer view of STK11 next generation sequencing. (A) Sequencing results from the mucinous component, showing no STK11 mutation at codon 270. (B) Sequencing results from the medullary component, showing STK11 G270W mutation with both forward and reverse sequencing. The sequencing depth at this codon is 12108x. The mutant allele frequency is 38%.*

the same tumor also harbored a. *AKT1* (p.E17K) that is typically seen in mucinous MSS CA. Our data demonstrate that the heterogeneity of this patient's colon cancer is also reflected at the molecular level.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

MS and DW evaluated the slides, collected the data and drafted the manuscript. ER collected and evaluated the molecular data. DQ and AM evaluated the histological and molecular data and reviewed the manuscript. DC evaluated the histological and molecular data and finalized the manuscript. JS helped in revising the figures and reviewing the manuscript.

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