NEB mutation is associated with high mutational burden and worse colorectal-cancer survival

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Research

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Abstract

Background: Nebulin (NEB) as a protein-coding gene have been well demonstrated its vital roles in skeletal muscles but no study for association between NEB mutation and cancer.

Methods: Here, we systematically analyzed mutation spectrum of colorectal cancer (CRC) samples and associations between NEB somatic mutation and CRC patients' tumor mutation burden (TMB) and prognosis by using TCGA-COAD project mutation dataset.

Results: Mutational signatures detected from CRC samples indicated pivotal roles of defective DNA damage repair process and polymerase activity in CRC initiation. NEB somatic mutation was found in about 16% CRC patients. Besides, NEB mutation was identified as an independent factor for high CRC TMB and inferior prognosis. Significantly mutated gene (SMG) analysis identified several genes including RNF43, SETD1B, MBD6 and so on, whose mutation states were profoundly influenced by NEB mutation and might be tumor driver genes in CRC initiation and progression.

Conclusions: In conclusion, NEB might be a novel CRC-related genes that could affect patients' prognosis by perturbing genome instability.

Introduction

According to the statistics report in 2018, colorectal cancer (CRC) is the 4th common cancer in China (1). It is estimated that the burden of CRC will increase by 60% new cases by 2030 (2). Risk factors, including obesity, unhealthy diet, and smoking, were responsible for approximately 46% of the incidence and mortality of CRC in China (3), and these environmental influences were interacted with dynamic molecular events consisting of germ-line factors and accumulated somatic changes, in the colorectal epithelium that drive the initiation and promotion of CRC (4). Persistent genetic instability is critical for the development of CRC (5). 16% of the CRC cases are found to be hypermutated, and one quarter of them are composed of somatic mutations (6).

Nebulin is a giant protein component of the cytoskeletal matrix expressed in different tissues (7). Nebulin regulates the actin filament architecture through stabilizing filamentous actin and preventing actin depolymerization (8). Moreover, it also affects contractility directly by promoting the interaction of myosin and actin and regulating calcium handling (9, 10). The NEB gene encodes nebulin, which mutation has been associated with multiple congenital diseases, such as fetal akinesia deformation sequence (FADS)/arthrogryposis multiplex congenita (AMC) (11), lethal multiple pterygium syndrome (12), and fetal akinesia/arthrogryposis multiplex congenita (11). Recent studies have shown that, the overexpression of members of Nebulin family, including nebulette (NEBL) and LIM and SH3 protein 2 (LASP2), promoted the migration of some cancer cells (13, 14), and was involved in the lymph node metastasis in CRC (15). But so far, the perception of nebulin function in tumor is still insufficient.

In this study, we analyzed the overall mutational spectrum of 399 samples obtained from The Cancer Genome Atlas (TCGA) database. Subsequently, we examined the association between the gene with the highest mutation rate among the imprinted gene family and the tumor mutational burden (TMB) by Wilcoxon rank-sum test. Survival analysis was performed to investigate the effects of the target gene mutation on CRC carcinogenesis and progression. Thus, our research would be helpful for the investigation of CRC development and its treatment.

Materials And Methods

2.1 Study population

All the population in this study was obtained from the Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/). A total of 399 CRC patient tumor samples were included in the TCGA-COAD project mutation dataset and 378 of which were annotated with complete information about overall survival and retained for the prognosis analysis.

2.2 Mutational signature analysis

Mutational signature (MS) represents the combination of mutation-causal related processes such as defective DNA damage repair, exposure to reagent or radiation and so on, which could be summarized by 96 mutation types of triplet-bases. In this study, we extracted MSs for the 378 CRC samples based on their single nucleotide variation via Signature Analyzer software (https://software.broadinstitute.org/cancer/cga/msp) which employs Bayesian nonnegative matrix decomposition method. Besides, cosine similarities between MSs of CRC and those well-known ones deposited in Catalogue of Somatic Mutations in Cancer (COSMIC, https://cancer.sanger.ac.uk/cosmic) were also calculated to infer the potential underlying inducement of CRC initiation and progression.

2.3 Significantly mutated gene analysis

Driver and passenger mutations co-exist in tumor cells and tumor driver mutations, which were also called significantly mutated genes (SMG), play the leading role in cancinogenesis. MutSigCV (https://software.broadinstitute.org/cancer/cga/mutsig) method was used in this study to identify SMGs in CRC samples with and without NEB mutations

2.4 Tumor mutation burden

Tumor mutation burden (TMB) represents the number of somatic mutations occurred in one million bases which was recently demonstrated to be closely associated with tumorigenesis and immunotherapy sensitivity. In this study, each CRC patient's TMB was calculated through dividing the total mutation number by 30, i.e. exon length in megabyte, and compared between CRC samples with and without NEB mutation via wilcoxon test.

2.5 Prognosis analysis

Kaplan-Meier analysis was used to estimate CRC patients' overall survival (OS) probability. Significance of OS difference stratified by NEB mutated state was determined by log-rank test. P value < 0.05 was used as the significant threshold.

Results

3.1 Mutation spectrum of CRC patients from TCGA

Mutation spectrum of CRC patients from TCGA was first explored which uncovered the predominance of missense mutation variant class and C to T transition as shown in Figure S1. Mutational signature (MS) is a combination of multiple mutational processes which could reflect to some extent the incentives through which those mutational processes occurred, such as defective DNA repair, mutagen exposures and so on. In this study, we summarized a total of eight MSs which named W1-8 as shown in Figure S2A. Cosine similarities between our MSs and those deposited in COSMIC database were calculated and provided as a heatmap in Figure S2B which shows high similarities between our MSs and signature1, signature6, signature10, signature28 and signature30. Signature1 might be a cell division/mitotic clock and correlated with individual age. Signature6 is associated with defective DNA mismatch repair and microsatellite instability which could contribute genome instability. Percentages of mutations attributed to each signature at individual and whole level were shown in Figure S3A and Figure S3B, respectively.

Mutations located in the top 30 most frequently mutated genes could cover all the 399 CRC samples and TTN represented the most frequently one whose mutations were found in about 74% samples (Figure 1A). Genes belong to NEB gene family that with mutation rate greater than 1% along with several well-known DNA damage repair (DDR)-related genes including BRCA1, BRCA2, POLE and MLH3 were analyzed for their mutation state across CRC samples. As a result, NEB was mutated in 16% CRC samples that was distinctly higher than PEG3, the second frequently mutated genes in Figure 1B which mutated in 9% CRC samples. Besides, CRC samples harbored NEB mutations also tend to have mutations located in BRCA1, BRCA2, POLE and MLH3 and shows higher mutation number than those without NEB mutation, which should indicate potential roles of NEB in genome stability.

3.2 NEB mutation is closely associated with CRC patients' tumor mutation burden

Tumor mutation burden (TMB) that represents somatic mutation number within a million bases is closely related with genome integrity and immunotherapy sensitivity of malignant tumor patients. In this study, TMB of CRC patients with at least one NEB mutation was significantly higher than those without NEB mutation (Figure 2A). What's more, NEB mutation was recognized as an independent high CRC TMB signature after excluded influences of confounding factors including patients' age, gender, tumor stage and mutation state of BRCA1, BRCA2 and POLE as shown in Figure 2B.

3.3 NEB mutation is closely associated with CRC patients' overall survival

Kaplan-Meier curves of samples stratified by NEB mutation state were plotted and log-rank test indicated that NEB mutation was significantly associated with inferior CRC overall survival (Figure 3A). Association between NEB mutation and CRC overall survival was still significant after controlling for confounding factors including patients' age, gender, tumor stage and mutation states of BRCA1, BRCA2 and POLE (Figure 3B).

3.4 Significantly mutated genes

We analyzed the SMGs in samples with and without NEB mutations, and Figure 4A and Figure 4B illustrated the mutation spectrum of SMGs NEB mutated and wild-type CRC samples, respectively. APC, TP53 and FBXW7 were identified simultaneously as SMGs in the two sample groups. Mutation frequency of SMGs in the two groups of samples were detailed shown in Figure 5, and several genes such as SETD1B, RNF43, MBD6 and etc were only significantly mutated in NEB mutated samples, whose mutation state might be profoundly influenced by NEB.

Discussion

Tumor genomic instability is positively correlated with immunotherapy response, however in CRC the clinical application largely focuses on a subset of high microsatellite instable (MSI) patients (16). Recently, TMB was identified as a promising biomarker in this field (17). A study including 462 CRC samples reported that high TMB was found in 11.4% of the CRC samples, and the alternate DNA repair pathways were potentially dysregulated, including the nucleotide excision pathway, DNA double strand break repair, and the previously described proofreading pathway (18).

In this study, Mutational signatures detected from CRC samples indicated pivotal roles of defective DNA damage repair process and polymerase activity in CRC initiation. A complex DNA repair machinery has evolved to protect genomic integrity in the face of a myriad of DNA damage sources. As DNA repair failure can lead to carcinogenesis and tumor genomic instability, which is considered as portend particular vulnerabilities of the cancer that can be exploited therapeutically (19).

We found that NEB mutation was mutated in 16% of the CRC samples, and the CRC samples harbored NEB mutation also tend to have mutations located in BRCA1, BRCA2, POLE and MLH3, indicating its potential role in genome stability. As it has been reported that, CRC cases carrying one of the specific mutations (such as BRCA1/2 and POLE) exhibited increased mutational burden (20), we further performed multivariate analysis using Wilcoxon rank-sum test. The results suggested NEB mutation associated with high CRC TMB independently. Significantly mutated gene (SMG) analysis identified several genes including SETD1B, RNF43, MBD6 and so on, whose mutation states were profoundly influenced by NEB mutation. SETD1B mutation was associated with intellectual disability, epilepsy and autism (21), and was also found to be mutated in primary hepatic neuroendocrine (22), hepatocellular (23), gastric and colorectal tumors (24). RNF43 is a tumour suppressor gene that frequently mutated in colorectal and endometrial cancers (25,26). MBD6 is a gene encoding a protein with a methyl-CpG

binding domain, and its mutational and expressional alterations were also found in CRC (27). Therefore, we suspected that NEB mutation might be tumor driver genes in CRC initiation and progression.

Moreover, NEB mutation was significantly associated with inferior CRC overall survival. Goodman AM et al. suggested that higher TMB was able to predict a favorable outcome to PD-1/PD-L1 blockade across diverse tumors including CRC (28). Moreover, model animal study showed that, the mutation of NEB could affect the skeletal muscle phenotype (29). Low muscle mass is a sign of cachexia syndrome, and is present in nearly 40% of CRC patients (30). Moreover, the muscle area was found to decrease significantly during chemotherapy (31). The severe postoperative complication rate was significantly higher in patients with low skeletal muscle and density, which was associated with short-term outcome in patients undergoing CRC surgery (32). Williams JP et al. Found that, CRC patients displayed reduced postprandial muscle protein synthesis and a trend toward increased muscle protein breakdown (33). Combined with our results, we speculate that this feature may be related to NEB mutation, which would be which will be confirmed in future experiments.

Conclusions

In this study, we systematically analyzed mutation spectrum of 399 CRC samples, and evaluated the associations between NEB somatic mutation with TMB and prognosis. The results showed that, the process of defective DNA damage repair process and polymerase activity played pivotal roles in CRC initiation. NEB somatic mutation seemed to be tumor driver genes in CRC initiation and progression, and was identified as an independent factor for high CRC TMB and inferior prognosis. Our findings may provide clues for further researches on NEB family genes and functional implications of NEB mutations in cancers.

Abbreviations

CRC	colorectal cancer
FADS	fetal akinesia deformation sequence
AMC	arthrogryposis multiplex congenita
TCGA	The Cancer Genome Atlas
TMB	tumor mutational burden
MS	Mutational Signature
COSMIC	Catalogue of Somatic Mutations in Cancer
SMG	significantly mutated genes
OS	overall survival
DDR	DNA Damage Repair
MSI	microsatellite instable
NEB	Nebulin
CRC	colorectal cancer

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets analysed during the current study are available in the [TCGA] repository, [https://portal.gdc.cancer.gov/].

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Mingyue Xu, Lijun Yuan, Yan Wang and Xipeng Zhang put forward the ideas of this article, written this article and analysed the data. Shuo Chen and Lin Zhang helped for acquisition of data and analysis and interpretation of data. Mingyue Xu, Lijun Yuan, Yan Wang and Xipeng Zhang helped for revising the manuscript. All authors read and approved the final manuscript.

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Figures

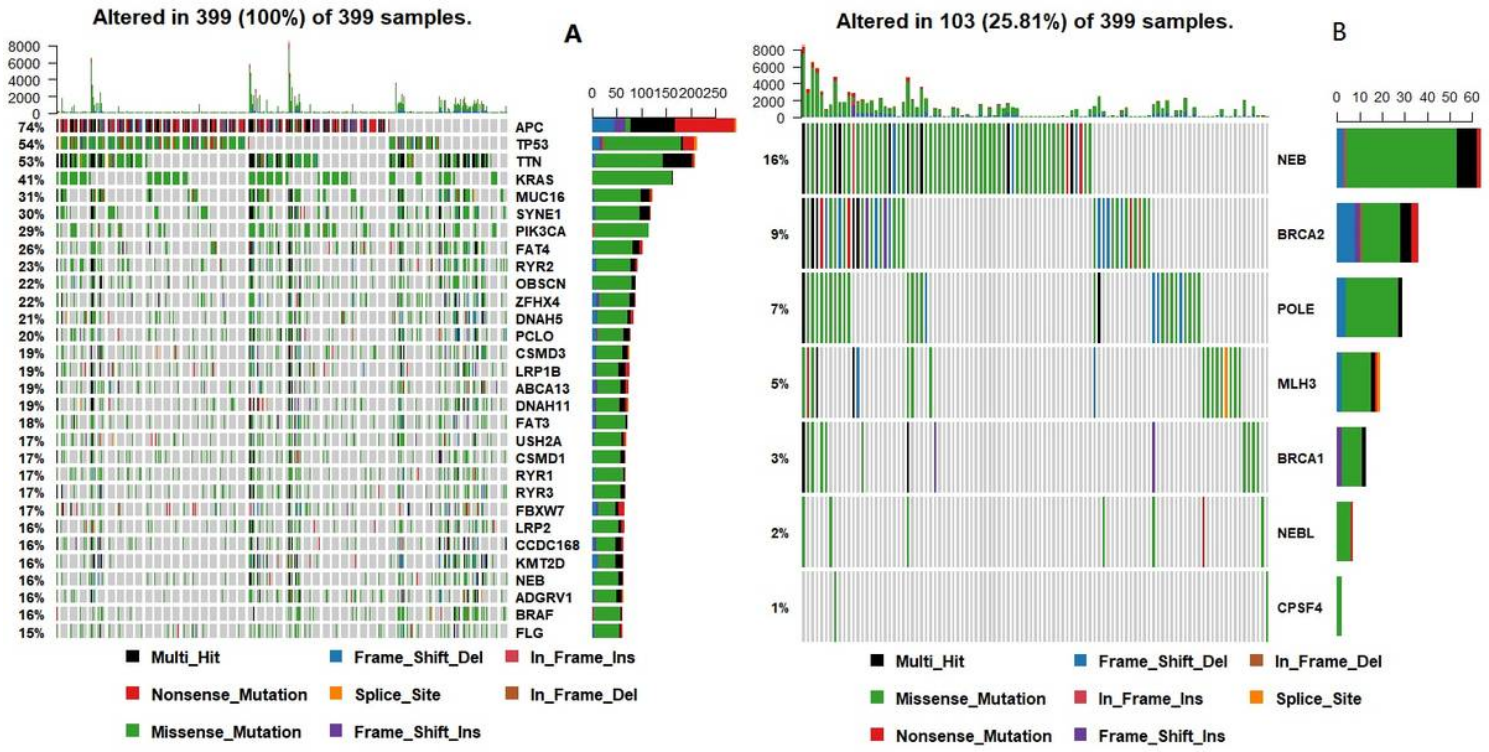

Figure 1

Somatic mutation spectrum of the 399 CRC patients from TCGA. (A) Mutation spectrum of the top 30 most frequently mutated genes across all the CRC patients. (B) Mutation spectrum of genes belong to NEB gene family that with mutation rates > 1% along with BRCA1, BRCA2, POLE and MLH3. Number of mutations occurred in each patient and distribution of mutation types across the CRC patients of each gene were provided on the top and right panel, respectively. Mutation types were specified by different colors.

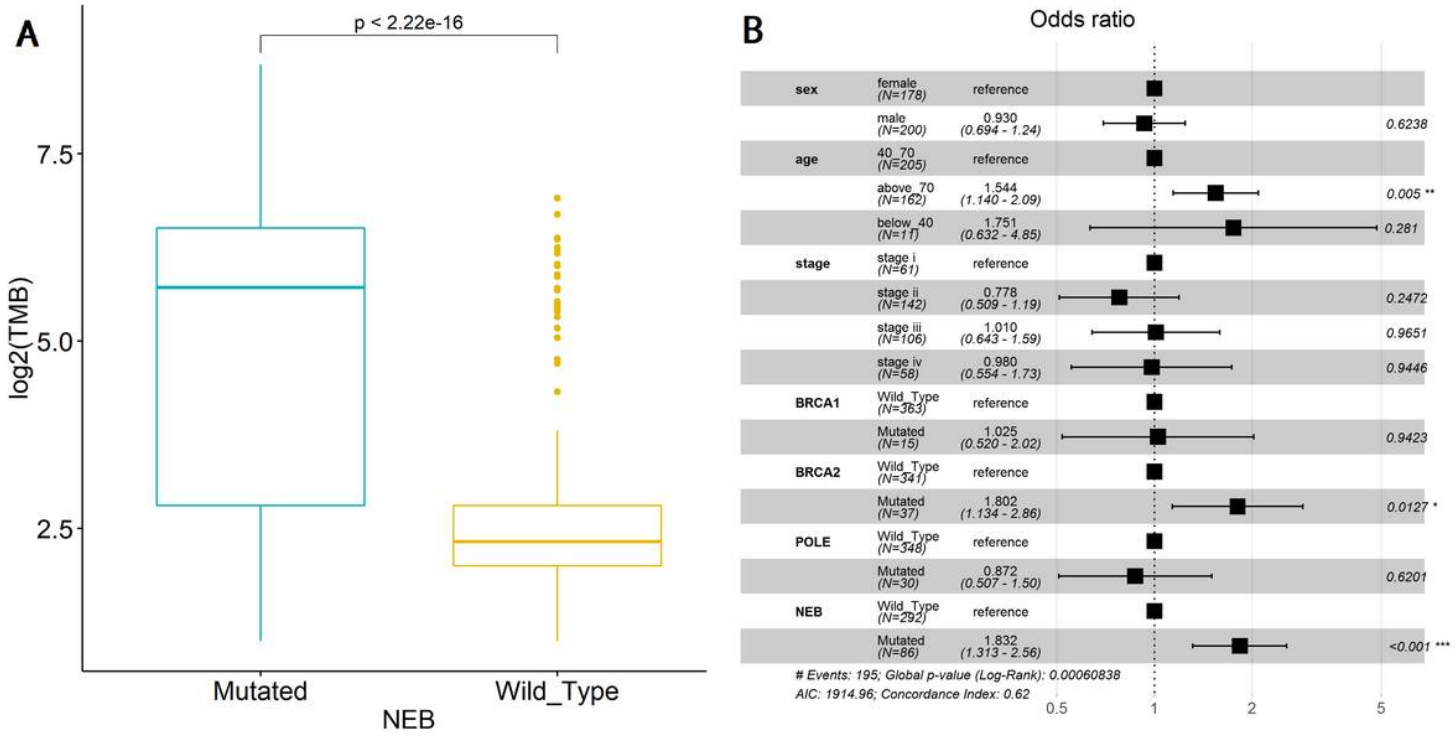

Figure 2

Association of NEB mutation and CRC patients' TMB. (A) Boxplot shows TMB distribution across patients with and without NEB mutation along with p value on the top of boxes which illustrates the significance of differences between the two TMB groups. (B) Forest plot shows NEB as an independent factor that could influence CRC patients' TMB after fit out patients' age, gender, tumor stage, and mutated status of other DNA damage repair related genes including BRCA1, BRCA2 and POLE.

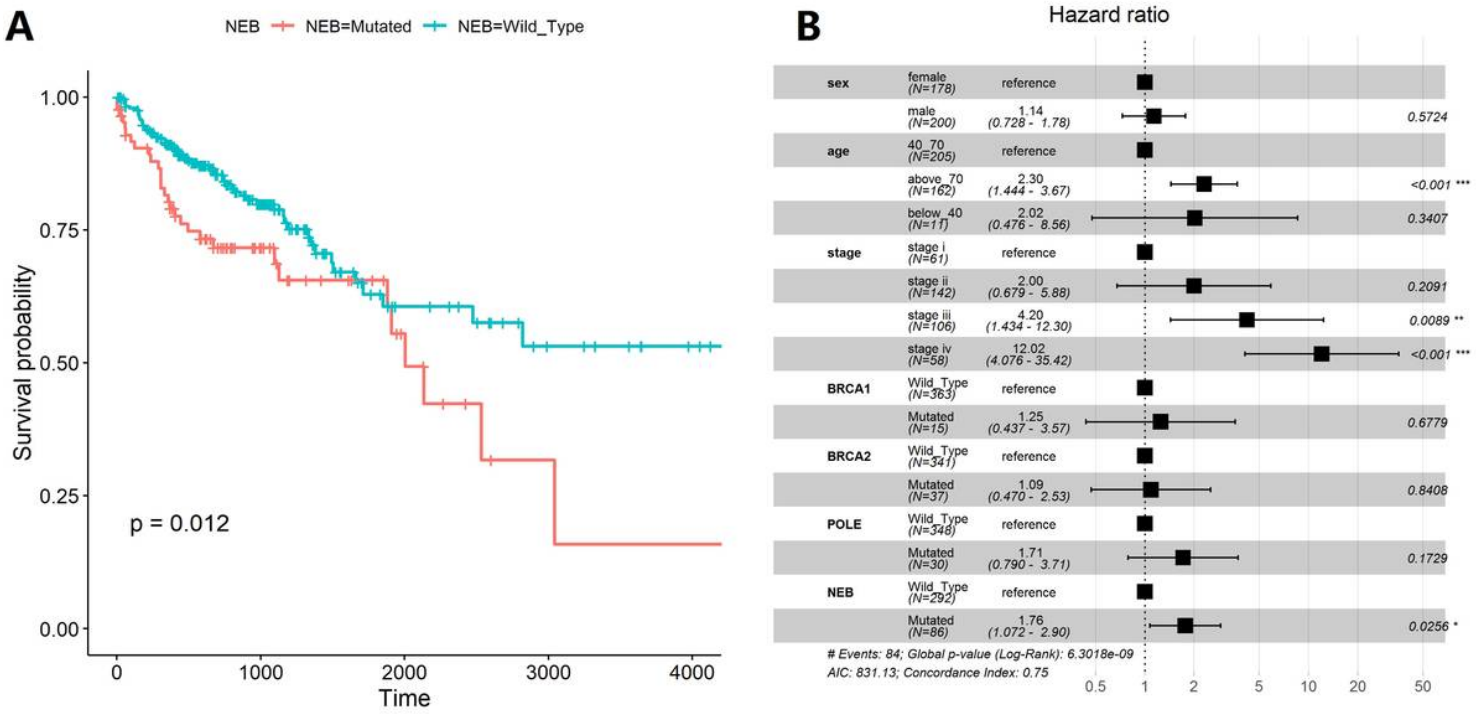

Figure 3

Association of NEB mutation and CRC patients' overall survival. (A) Kaplan-Meier curves of CRC patients stratified by NEB mutated status, i.e. mutated and wild-type, with the log-rank p value provided. (B) Forest plot shows NEB as an independent factor that could influence CRC patients' overall survival after fit out patients' age, gender, tumor stage, and mutated status of other DNA damage repair related genes including BRCA1, BRCA2 and POLE.

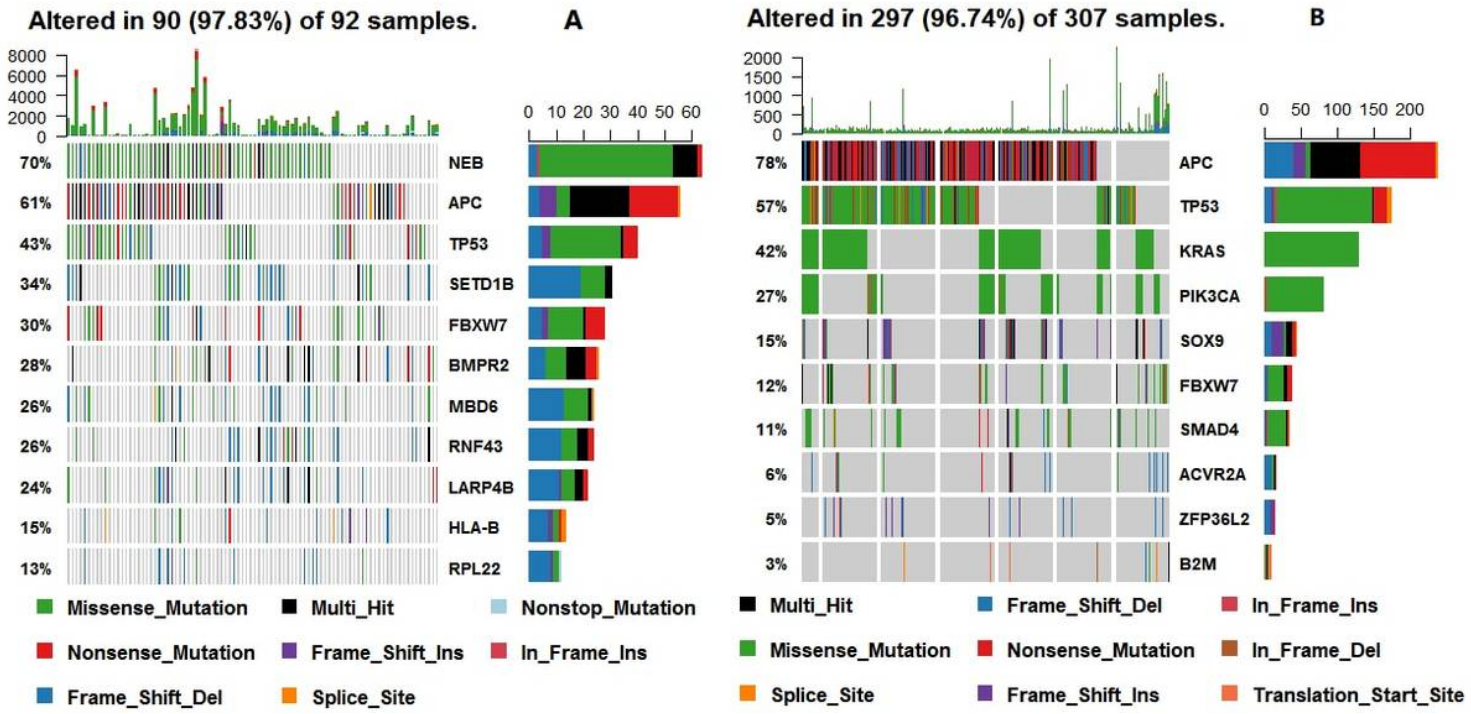

Figure 4

Mutation spectrum of significantly mutated genes across NEB mutated (A) and wild-type (B) CRC samples. Number of mutations occurred in each patient and distribution of mutation types across the corresponding CRC patients of each gene were provided on the top and right panel, respectively. Mutation types were specified by different colors.

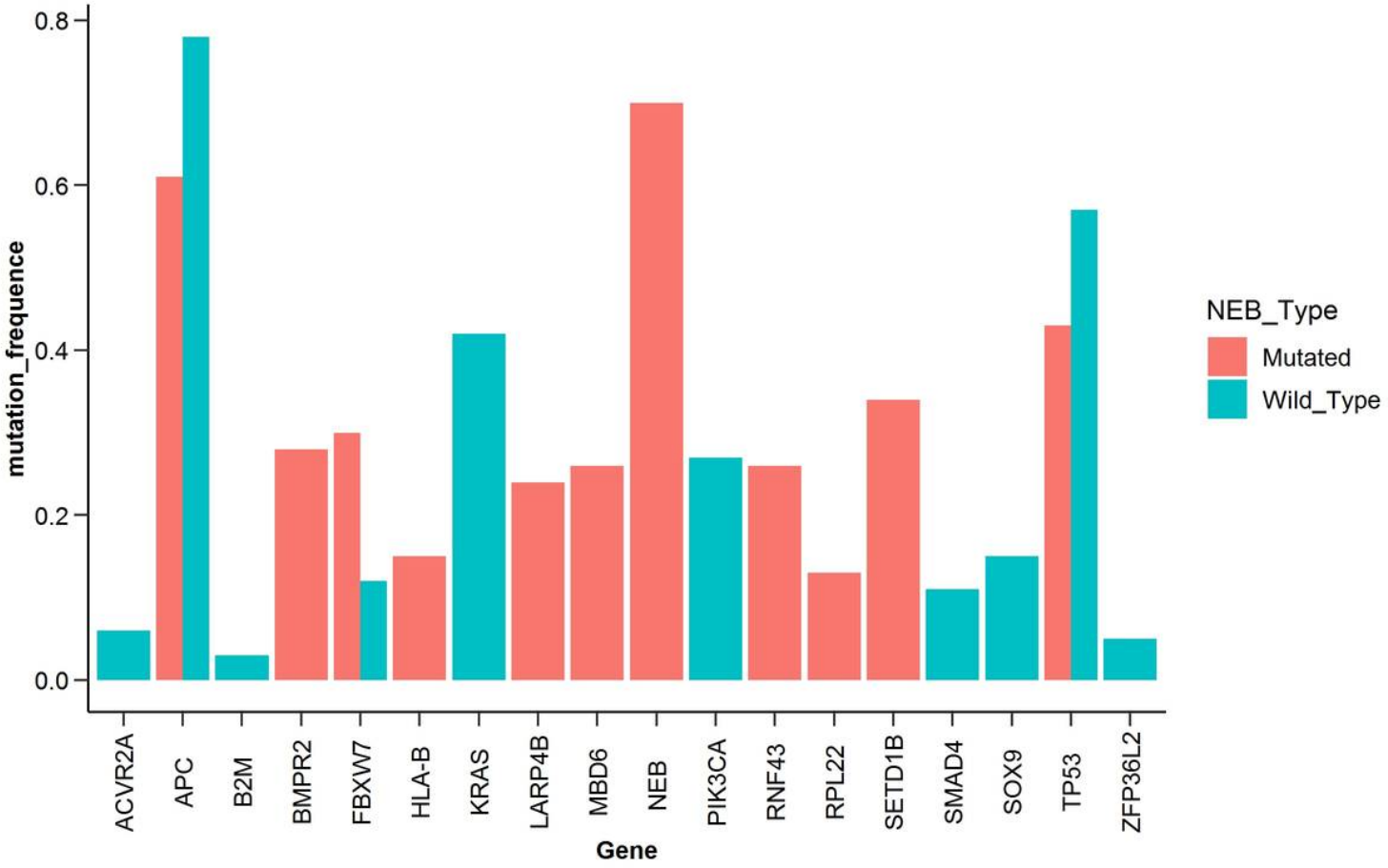

Figure 5

Histogram illustrates mutation frequency of significantly mutated genes in NEB mutated and wild-type CRC samples which specified by red and blue color, respectively.

Supplementary Files

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