Indonesian Genomic Landscape of Pathogenic Mutation of APC, KRAS, TP53, PIK3CA, and MLH1 in Colorectal Cancer

Vania Myralda Giamour Marbun (✉ vania.myralda@gmail.com)

Rumah Sakit Umum Pusat Nasional Dr. Cipto Mangunkusumo

Toar Jean Maurice Lalisang

Rumah Sakit Umum Pusat Nasional Dr. Cipto Mangunkusumo

Linda Erlina

Indonesian Medical Education and Research Institution (IMERI), Universitas Indonesia

Research Article

Keywords: colorectal cancer, genetic mutation, pathogenic mutation, next-generation sequencing, APC, TP53, PIK3CA, KRAS, MLH1

Posted Date: December 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-900277/v2>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background : Knowing colorectal cancer's heterogeneity and dynamic features, recognizing its biological behaviour requires detailed identification of mutated genes involved. Colorectal cancer (CRC) requires several mutated genes to occur and those are dissimilar in each person hence essential to be discovered in specific population. Until recently, there is no known study describing genomic landscape of CRC in Indonesian population. This study aims to describe profile of pathogenic mutation of *APC*, *TP53*, *PIK3CA*, *KRAS*, and *MLH1* in CRC patients treated at 3 different hospitals in Jakarta.

Methods : This is a descriptive study conducted on CRC patients who underwent neoadjuvant, surgical, and adjuvant therapy at RSCM, RSKJ, and MRCCC in 2017-2018. DNA analysis was performed using next-generation sequencing and aligned against GRCh38. Pathogenic variant was identified using ACMG classification and FATHMM score. Data related to behaviour and survival were collected from medical records.

Results : There were total 22 subjects in which *APC*, *TP53*, and *PIK3CA* were mutated. *KRAS* mutation occurred in 64%, while *MLH1* in 45%. Five types of mutation were identified, including nonsense, missense, frameshift, splice-site, and silent mutation. There are 4 groups of co-occurring mutations, which are *APC*, *TP53*, *PIK3CA* (triple mutation/TM) alone; TM+*KRAS*; TM+*MLH1*; and TM+*KRAS*+*MLH1*, presenting different nature and survival.

Conclusion : Indonesia having various ethnicities with diverse diet and lifestyle has distinct profile of pathogenic mutation presenting mostly with locally-advanced stage with various outcome and survival rate.

Introduction

Colorectal cancer has been known as one of the most well-studied malignancy. Its dynamic and heterogeneity are characterized by many interconnecting molecular etiopathogenesis exhibiting different behavior inter and intratumor.¹⁻⁵ Based on recent biomolecular study, genetic and epigenetic analysis can evaluate the nature of the tumor, hence able to predict heredity, progressivity, recurrency, response to therapy, and even survival rate. Those variables cannot be estimated by AJCC staging system alone. For this reason, precision medicine rooting to genomic profile of each individual, are starting to advance.

Colorectal malignancy, which involves at least three or four genetic mutation, are feasible for next-generation sequencing methods.^{2,6} Two of the three most common carcinogenic pathways are chromosomal and microsatellite instability.⁷⁻¹⁰ Five genes which frequently involved are *APC*, *TP53*, *KRAS*, *PIK3CA*, and *MLH1*. Different group of age, gender, and geographic location actually have different variations of mutation and genes involved so that study on specific population is important in advancing precision medicine.¹¹ Until recently, there is no publication providing genomic landscape of colorectal cancer in Indonesian population. This study aims to analyze genomic profile of colorectal cancer in Indonesia.

Methods

This is a descriptive study in patients with colorectal malignancies who underwent surgery and/or chemoradiation and/or chemotherapy at RSCM, RSKJ, and MRCCC in 2017-2018 whose tumor tissue specimens were still properly stored in the form of formalin-fixed paraffin-embedded (FFPE). This study has been reported in line with STROCSS criteria.¹²

Sample Preparation

All sequencing preparation was performed by Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia at Bioinformatics Core Facility of Indonesia Medical Education and Research Institute (IMERI).

DNA extraction was performed using QIAamp DNA FFPE Tissue Kit. The quality of extracted DNA was evaluated using an absorbance ratio of 260 nm to 280 nm (A_{260}/A_{280}) and 260 nm to 230 nm (A_{260}/A_{230}). Purity criterion for samples with the A_{260}/A_{280} ratio is within the range of 1.8–2.0 and A_{260}/A_{230} ratio is within the range of 2.0–2.2. After purity criterion fulfilled, sequencing was done utilizing *AmpliSeq Cancer HotSpot Panel v2 for Illumina*. Results in FASTQ format were quality-checked with FASTQC (v.0.9.5; <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and aligned against *Genome Reference Consortium Human Reference 38* (GRCh38). Variant calling was done using LoFreq and annotated with SNPEFF and filtered with SNPSift. Annotation results were stored in variant call format (VCF) file.

Inclusion criteria, exclusion criteria, and identification of pathogenic mutation

- a. Retrieval of VFC files fullfiling inclusion and exclusion criteria
 - Inclusion criteria: FFPE samples fulfilling DNA purity criterion and showing PASS status in FASTQC
 - Exclusion criteria: none
- b. Data filtering based on estimation of putative impact or deleteriousness showing HIGH.
- c. Identification of single nucleotide variant (SNV) and synchronization with 3 databases
 - ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>)
 - COSMIC (<https://cancer.sanger.ac.uk/cosmic>)
 - The Ensembl project (<https://asia.ensembl.org/info/index.html>)
- d. Identification of somatic effect based on American College of Medical Genetics (ACMG) classification and Functional Analysis through Hidden Markov Models (FATHMM) score.
- e. Selection of SNVs meeting pathogenic criteria (ACMG pathogenic variant and/or FATHMM score 0,7.
- f. Identification of SNVs of APC, TP53, PIK3CA, KRAS, and MLH1
- g. Matching samples’ numbers and medical record data

Results

Patients characteristics

Twenty two samples were collected in accordance with sample preparation procedures mentioned above. Among these samples, 41% (9/22) were diagnosed with stage 3b of which 7 were elective cases. Fifty-nine percent (13/22) had lymphovascular invasion, of which 1 was diagnosed with stage 2A, and 12 were in stage 3B-4C.

Table 1 Patients characteristics

	Variables	Numbers	%
Age	< 50 y.o.	9	41
	≥50 y.o.	13	59
Gender	Male	13	59
	Female	9	41
Death	Yes	11	50
	No	11	50
Grade	Well	13	59
	Moderate	6	27
	Poor	3	14
Stage	1	2	9,1
	2A/B/C	5/1/-	27,3
	3A/B/C	-/9/-	40,9
	4A/B/C	2/1/2	22,7
Lymphovascular invasion	Yes	13	59
	No	9	41
Tumor location	Group 1	3	14
	Group 2	4	18
	Group 3	15	68
Perioperative management	None	13	59
	Neoadjuvant chemoradiation	-	-
	Adjuvant chemotherapy	7	32
	Neoadjuvant chemoradiation + adjuvant chemotherapy	2	9
Family history of cancer	Colon cancer	1	9
	Breast cancer	1	
Average interval from diagnosed to death		259 hari (3-882)	

Group 1: Caecum to two-third proximal of transverse colon; Group 2: One third distal of transverse colon to sigmoid;
Group 3: Rectum to anus.

Pathogenic mutation mapping in whole chromosomes

There were pathogenic mutation in almost all somatic chromosomes except 6, 9, 14, 16, 21, and 22 which involved 25 genes and 641 SNV (Table 2). Three types of mutation were identified i.e. synonymous (silent mutation); non-synonymous (nonsense, missense, and frameshift); and splice-site mutation.

Table 2 Pathogenic mutation mapping

Chr	1	2	3		4	5	7	8	10	11	12	13	15	17	18	19	20	Total Genes	Total SNVs														
Gene	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y								
Pts																																	
1(1)					2	2		1	1	2								2	1			4	5	1				10	21				
2(3)					1	1	1	1	3	1					1	1	1				4	1	10	1			1	1	15	29			
3(6)	1					2		1	2	3	1	1								1			7					9	19				
4(14)	1	1					1		1	3											1			3			1				8	12	
5(15)	1	2		1		1		1	2		1					1			1			4	2					11	17				
6(16)					1		2		2		1					1			1			4	1					8	13				
7(19+19b)			2				2	2	1	4	2	1					1	2	4	1	14	2	1	1				15	40				
8(20)					1	2	1	1	3	2											1	5	1	9	2	1				12	29		
9(22)	1					1		3		2	1													9	1				1	8	19		
10(23)					1		2	2	1		1											4			4	1			1	9	17		
11(29)				1				1	1	3		2	1				1			1			4			1	1	12	18				
12(34)	1	1			2	1	2			6	1	2	1	1			1	1			3			10	1	1			1	17	36		
13(3737)	1	1					1	2	2		5	2	2				2			1	3			10	4			1	1	15	38		
14(9)	1					1		1		3		1	1						1	1	3			7	1	2				12	23		
15(11)	1	1		1		1	3			6	1	2			1	1			2	3			9	4	1				15	37			
16(12)	1	2	1	3		2	2	1	1	7	5	2	1				1	1	4	6			9	4			1				19	55	
17(13)				1		2		4		1	3	3	1				2	1			1	4			17	4	1	1				14	46
18(14)			1	1	2		2		3		3	1				1	1			1	4	1			6	5			1	1	17	35	
19(16)	1	1		1	1		2		1		3	1	1	1						2	3	1	14	2	2				16	37			
20(17)					2		1		1		1	1										1	4			13	6				9	30	
21(18)					2		3		1		4	2	1				1			1	4			12			1	1				13	34
22(19a)				1		1		2		1	2	1	1	2	1			2			1	6			10	3	1	1				16	36
Total Patients	10	5	9	2	17	10	22	3	15	22	16	15	4	5	9	4	6	14	21	5	22	18	12	10	5			641					

Information:

- a. NRAS
- b. ALK
- c. IDH1
- d. ERBB4
- e. VHL
- f. MLH1
- g. PIK3CA
- h. CTNNB1
- i. KIT
- j. APC
- k. BRAF
- l. EGFR
- m. FGFR1
- n. RET
- o. PTEN
- p. FGFR2

- q. ATM
- r. KRAS
- s. RB1
- t. IDH2
- u. TP53
- v. SMAD4
- w. STK11
- x. SRC
- y. GNAS

Pathogenic mutation mapping of APC, TP53, PIK3CA, KRAS, and MLH1

Two types of APC pathogenic mutation occurred concurrently (nonsense and missense) in 1 patient. TP53 mutation also have 5 concurrent mutations in 1 patient (nonsense, missense, frameshift, silent, and splice-site) and only 3 of 22 patients having singular missense mutation. Only 1 type of pathogenic mutation occurring in MLH1 (nonsense) and PIK3CA (missense). Singular KRAS mutation occurred in 10 patients (8 missense and 2 silent) and multiple mutations occurred in 4 patients (Table 3).

Table 3 Pathogenic mutation mapping of 5 genes

Gene	n = 22																					
MLH1																						
PIK3CA																						
APC																						
KRAS																						
TP53																						

- Nonsense
- Missense
- Frameshift
- Splice-site
- Silent
- None

Co-occurring mutation in more than 3 genes were presented in all subjects. Combination of triple mutation (APC, TP53, PIKCA) occurred in 4 of 22 patients. Combination of quintuple mutation (APC, TP53, PIKCA, KRAS, MLH1) occurred in 6 of 22 patients. (Table 4)

Table 4 Subjects with co-occurring mutation

Co-occurring mutation	Number of subjects
APC + TP53 + PIK3CA + KRAS + MLH1	6
APC + TP53 + PIK3CA + KRAS	8
APC + TP53 + PIK3CA + MLH1	4
APC + TP53 + PIK3CA	4
Total	22

APC mutation

Gene mutation occurred in 100% subjects with 17 SNVs (16 missense and 1 nonsense). Mutation cluster region (MCR) were located in exon 14-17. Median of SNV frequency was 4 (range 1-10). Most frequently occurred SNV was Q879* (Table 5).

Table 5 APC Mutation

Nonsense mutation SNV (n=16)			Missense mutation SNV (n=1)		
Nucleotide change	Codon	Number (n=22)	Nucleotide change	Codon	Number (n=22)
C>T	Q879*	10	C>T	T1493M	1
	Q1123*	8			
	R876*	6			
	R1114*	6			
	Q1367*	6			
	Q1517*	5			
	Q1095*	4			
	Q1303*	4			
	Q1096*	4			
	Q1378*	4			
	Q1291*	2			
	Q1294*	2			
	Q1429*	2			
	Q1444*	1			
	R1450*	1			
	Q1469*	1			

KRAS mutation

In this study, KRAS mutation occurred in 14 of 22 patients (63,6%). Nine SNVs were identified in 3 types of mutations i.e. missense, nonsense, and silent. Nonsense mutation cause termination of codon 22, missenses occurred in 6 codons, and silent in 2 codons. Most frequently occurred SNVs are T20= in 4 subjects, A146T and P34L in 3 subjects (Table 6).

Table 6 KRAS mutation

Nonsense mutation SNV (n=1)			Missense mutation SNV (n=6)			Silent mutation SNV (n=2)		
Nucleotide change	Codon	Number (n=14)	Nucleotide change	Codon	Number (n=14)	Nucleotide change	Codon	Number (n=14)
C>T	Q22*	1	G>A	A146T	3	G>A	T20=	4
				V14I	2	C>T	G13=	3
				G13S	2			
				A59T	1			
			C>T	P34L	3			
				T58I	2			

TP53 mutation

TP53 mutation also occurred in 100% subjects in with 65 SNVs categorized into 5 types of mutations i.e. (1) missense, (2) nonsense; (3) frameshift; (4) silent; (5) splice-site. In missense mutation, 2 most frequent SNVs are M237I and C238Y. In nonsense mutation, 2 most frequent SNVs are R342* and R213* (6 of 22 patients) (Table 7-8).

Table 7 TP53 mutation

Missense mutation SNV (n=49)					
Nucleotide change	Codon	Number (n=22)	Nucleotide change	Codon	Number (n=22)
G>A	M237I	8	A>G	M237V	2
	C238Y	7		H214R	2
	R248Q	6		K132E	1
	C277Y	6		Q192R	1
	G245S	6		N235D	1
	G245D	5		Y236C	1
	G244D	4	C>T	S127F	6
	V197M	4		R248W	6
	R213Q	4		R282W	5
	R175H	4		T256I	4
	E258K	4		A138V	4
	R273H	4		P152L	4
	R196Q	3		L194F	4
	C135Y	3		P250L	3
	G154S	3		R273C	3
	R280K	3		P152S	2
	R267Q	2		T155I	2
	E285K	2		P278L	2
	E286K	1		R175C	1
	R290H	1	G>C	V272L	1
	C275Y	1		L755S	1
	G266E	1	T>C	F134L	1
	R249K	1		C238R	1
	R156H	1		L252P	1
	R158H	1			

Table 8 TP53 mutation (cont.)

Nonsense mutation SNV (n=6)			Frameshift mutation SNV (n=1)			<i>Splice-site mutation</i> SNV (n=6)			<i>Mutasi silent</i> SNV (n=3)		
Nucleotide change	Codon	Number (n=22)	Nucleotide change	Codon	Number (n=22)	Nucleotide change	Codon	Number (n=22)	Nucleotide change	Codon	Number (n=22)
C>T	R342*	6	G>A	M1?	4	c.919+1G>A	p.?	4	C>T	G244=	1
	R213*	6				c.673-1G>A	p.?	3	G>A	V272=	1
	R196*	5				c.560-1G>A	p.?	3	C>A	R213=	1
	Q136*	4				c.994-1G>A	p.?	2			
	Q165*	1				c.559+2T>C	p.?	1			
G>A	W91*	2				c.376-2A>G	p.?	1			

PIK3CA mutation

Mutation of PIK3CA occurred in exon 2, 5, 7, 8, 10, 19, and 21. In this study PIK3CA missense mutation were identified in all subjects. Median of SNV frequency was 4 (range 1-16). Most frequently occurred SNV was G914R (Table 9).

Table 9 PIK3CA mutation

Substitusi - Mutasi <i>missense</i> SNV (n=9)		
Nucleotide change	Codon	Number (n=22)
G>A	G914R	16
	V71I	7
	R88Q	5
	G1049S	4
	R398H	2
	E542K	1
C>T	H1047Y	3
A>G	H1047R	2
T>A	N345K	1

MLH1 Mutation

MLH1 mutation occurred in 10 of 22 (45,45%) subjects. Nonsense mutation occurred in exon 9-13 causing termination in 4 codons. Most frequently occurred SNV was Q391* (Table 10).

Table 10 MLH1 mutation

Nonsense mutation SNV (n=4)		
Nucleotide change	Codon	Number (n=10)
C>T	Q391*	6
	Q382*	5
	Q409*	2
	Q398*	1

Biological behavior of malignancy with co-occurring mutation (Table 6)

Co-occurring mutation of APC, TP53, PIK3CA, and KRAS were identified in 8 patients with average age of 48,5 years old, with locally-advanced stage (n=5), located in rectum (n=6), well-differentiated (n=6), and positive lymphovascular invasion (n=5).

Co-occurring mutation of APC, TP53, PIK3CA, and MLH1 were identified in 4 patients, with average age of 52,3 years old, with locally advanced stage (n=3), located in rectum (n=3), without lymphovascular invasion.

Quintuple mutation were identified in 6 patients, dominated by older age, locally-advanced stage, well-differentiated, positive lymphovascular invasion, and located in rectum or left colon.

Table 11. Clinical Manifestation of each combination of co-occurring mutations

	<i>APC + TP53 + PIK3CA + KRAS</i> (Cluster 1) n = 8	<i>APC + TP53 + PIK3CA + MLH1</i> (Cluster 2) n = 4	<i>APC + TP53 + PIK3CA + KRAS + MLH1</i> (Cluster 3) n = 6	<i>APC + TP53 + PIK3CA</i> (Cluster 4) n = 4
Age (mean) (range)	Mean 48,516 (27-75)	Mean 52,319 (27-67)	Mean 58,713 (40-74)	Mean 56,322 (39-87)
50 y.o.	4	3	4	2
50 y.o.	4	1	2	2
Stage				
Early	1	0	1	0
Locally-advanced	5	3	4	3
Advanced	2	1	1	1
Lymphovascular invasion				
Yes	5	0	4	4
No	3	4	2	0
Tumor location				
Group 1	1	1	0	1
Group 2	1	0	3	0
Group 3	6	3	3	3
Grade				
Well	6	2	5	0
Moderate	1	0	0	2
Poor	1	2	1	2
Mortality				
Yes	4	1	3	3
No	4	3	3	1

Survival

Patients with co-occurring mutation of APC, TP53, PIK3CA, and MLH1 (cluster 3) had the longest median life expectancy (1197 days) compared to cluster 2 with shortest median life expectancy (577 days) (Table 4, Figure 1).

Fifty percent of subjects of cluster 1 and 2 were deceased in less than 6 months after therapy, in cluster 4, 50% of subjects were deceased before month 15. All patients of cluster 3 can survive up to 30 months after therapy and only 1 patient deceased afterwards. Cluster 2 and 4 shows highest mortality rate with highest number of deceased patients in shortest period compared to other clusters (Figure 2).

Other findings

Early recurrence (<5 years) occurred in 2 patients of cluster 4, of which 1 patient underwent neoadjuvant chemoradiation and adjuvant chemotherapy (MFOLFOX6) and another was given XELOX after surgery. Both patients have disease-free interval of 15 months.

One patient was given anti-EGFR therapy (cetuximab) + MFOLFOX6. Patient's PCR result for KRAS was wild-type. There is no data of therapeutic response due to patient's death during midcycle (127 days after surgery). This patient was included in cluster 2 (with KRAS mutation) and also had EGFR mutation (rs121913467).

One patient was given anti-VEGF therapy (bevacizumab) + MFOLFOX6 after diagnosed with local recurrence after 1-year of oral capecitabine. This patient was included in cluster 3 with noted BRAF mutation as well (rs121913353). Patients was known to have complete response to bevacizumab.

Two of 22 patients had family history of malignancy (Table 1). In patient with family history of colon cancer, there was 1 germline mutation identified in STK11, meanwhile in patient with family history of breast cancer, 2 germline TP53 mutations were identified.

Discussion

Colorectal cancer (CRC) patients in Indonesia are dominated by male (59%), more than 50 years old (59%), with well-differentiated (59%), stage 3B (40,9%), located in rectum (68%). Recently, incidence of CRC in young adults increased by 1,4% per year influenced by obesity and sedentary life style.¹³ High percentage of locally-advanced stage on hospital admission can be caused by low educational level about CRC risk factors and importance of screening especially in individuals with family history of malignancy. Intricate system of national health insurance also has role in slacking patients with unspecific complaints to see doctors before having clear disorder and getting worse. These are several of many reasons that cause delay in diagnosis and management of CRC.

The heterogeneous and dynamic nature of the CRC are related with its overlapping pathways of carcinogenesis. There are 4 principles of neoplasia in CRC, (1) colorectal tumors arise due to the activation of proto-oncogene mutations into oncogenes and inactivation of tumor suppressor genes¹⁴; (2) at least mutations in 4-5 genes are required for malignant formation; (3) accumulation of numbers is more important than sequence of mutations in determining tumor biologic behavior; (4) the mutated tumor suppressor gene continues to express the phenotype without loss of heterozygosity.²

The theory of colorectal neoplasia, namely adenoma-carcinoma sequence (ACS) states that the formation of colorectal carcinoma must be preceded by the presence of an adenoma.^{1,2} Changes in the normal intestinal mucosal epithelium to adenoma are triggered by mutations in the tumor suppressor gene, that is APC. APC can be detected in the aberrant crypt foci (ACF), which is a precursor lesion that occurred early in the beginning of the formation of adenomatous polyps and can only appear in dysplastic lesions.¹⁵

All subjects (100%) in this study had nonsynonymous mutations in APC. Only 2 patients had adenomas on colonoscopy. One of those had tubulous adenomas with mild dysplasia on colonoscopy and first-degree relative with CRC. Nonsense mutated APC was found at codons 879, 1095, 1123, which completely stopped glutamine production (Q). Meanwhile, in another patient with villous adenomas and well-differentiated adenocarcinoma, nonsense mutations were found at codons 876, 879, 1096, 1291, 1294 and 1517 that stopped the production of the amino acids glutamine (Q) and arginine (R). Mutations in APC are known to have high-penetrance that can reach 100% for FAP and CRC.¹⁶⁻¹⁹ In contrast to the Japanese population, whose APC mutations scattered at codons 142 – 1513, subjects in this study had APC mutations occur at codons 876 – 1517 with mutation cluster region (MCR) in exons 14 – 17.^{20,21}

After the normal mucosal epithelium turned into an early adenoma, KRAS mutation occurred subsequently triggering the change of early into intermediate adenoma. However, in contrast to APC, KRAS can act on nondysplastic ACF precursor lesions.¹⁵

In this study, mutations in the KRAS gene occurred in 14 of 22 samples (63.6%) at 9 codons and were most commonly found in older age group, locally-advanced stage, well-differentiated/low grade, with positive lymphovascular invasion, and located at the rectum. There were differences of codon location in missense mutation between Jakarta (Indonesia) and United States population, i.e. codons 13, 14, 34, 58, 59, 146 VS 12, 13, 61, 146.²² In addition, nonsense mutations were also found at codon 22 which only occurred in 1 patient. This patient diagnosed with stage 2A (pT3N0M0) undergoing elective curative resection and was given 8 cycles of capecitabine adjuvant chemotherapy with complete response. Mutation located in codon 12 has more aggressive behavior than codon 13, because patients were commonly presented in advanced stage.²² Nevertheless, number of cases with metastases involving KRAS mutation in this study was found in 3 of 5 samples without involvement of codon 12.

KRAS mutation can occur concomitantly with APC mutation leading to increased accumulation of b-catenin in the cytoplasm, by destroying its binding to E-cadherin, which has actually been increased due to loss of mutated APC degradation function. This causes the Wnt signal to become more active so that motility and cell invasion are more aggressive.^{15,18,21,23-26} In CRC, the combination of APC and KRAS mutations (co-occurring mutations) can occur up to 80%, whereas in this study only occurred in 63.6% of subjects.²⁷

In this study, patients with APC, TP53 and KRAS mutations were predominantly 50 years old, with locally-advanced stage and positive lymphovascular invasion. Two shortest median life expectancy were found in patients with KRAS mutation (Figure 1), in addition 50% of patients died within 6 months after therapy (Figure 2).

Before turning into carcinoma, intermediate adenomas differentiate into late adenomas triggered by mutations in the SMAD4, CDC4, and DCC genes.^{2,7} In this study, we found SMAD4 nonsense and missense mutations in 18 of 22 patients (82%).

In ACS theory, late adenomas who developed into carcinomas have mutations in TP53, TGFBR2, BAX, and IGF2R. Mutated TP53 was found in all subjects in this study in the form of nonsense, missense, frameshift, splice-site, and silent mutation. Five most frequently occurred codon locations in this study were 237, 238, 127, G245S, and R248Q. Those are different compared to world database in The Cancer Genome Atlas Program (TCGA) portal which stated that the five codon positions with the highest frequency were 175, 282, 248, R273H, and R273C.²⁸

In contrast to the UK population, in 64% (14 out of 22) subjects, TP53 and KRAS mutations co-occurred.^{18,21} In Indian population, these two combinations were only found in 13 of 112 cases, whereas the study by Timar can occur in up to ~40%.^{27,29} TP53 and KRAS activate different carcinogenesis pathways so that they rarely coexist.³⁰

Similar to APC and TP53, PIK3CA mutations were found in all subjects (100%) with 9 SNVs. PIK3CA has no role in the aggressive behavior of CRC.^{31,32} Even so, when it occurs concurrently with KRAS mutations, it will show evident aggressive behavior, especially when occurs in exons 9 and 20. While in this study mutations occurred in exons 2, 3, and 4, aggressive behavior presenting as locally-advanced stage and positive lymphovascular invasion can be found.

Mutations in MLH1 can also occur in non-hereditary/sporadic CRC. The existence of microsatellite instability due to mutations in genes that play roles in the MMR system such as MLH1 actually provides a good prognosis with a higher survival rate.³³ In this study, the group of cases with MLH1 mutations alone, had the highest median life expectancy and had a 30-month survival rate of up to 100%.

Referring to the colorectal neoplasia principle mentioned above, all subjects in this study indeed involved activation of oncogenes (PIK3CA and KRAS) and inactivation of tumor suppressor genes (APC, TP53 and MLH1) and also involved a range of 8-19 mutated genes per person. In this study, mutated APC and KRAS, which supposed to be occurred on early sequence of ACS, supports what Fearon stated about unimportance of mutational sequence in determining tumor biologic behavior.^{1,2}

We are strongly aware of our study's limitation. Limited number of samples is the lack of this study. Further research is truly required to accomplish complete mapping of Indonesian profile, especially in investigating our unique findings in each of genes described.

Nevertheless, this is the first study fully describes the profile of pathogenic mutations of CRC in Indonesian population with its unique characteristics, compiled of various ethnicities with diverse diet and lifestyle which may have roles in contributing natures of Indonesian version of CRC presenting in locally-advanced stage with large tumor size and moderate-severe malnutrition status. This study is also the first in the world to examine the co-occurring mutations of APC, TP53, PIK3CA, KRAS, and MLH1.

Conclusions

1. Different profile of pathogenic mutation in colorectal cancer patients is found in Indonesia's population
2. Mutated APC, TP53, and PIK3CA occurred in 100% subjects, while KRAS and MLH1 occurred in 63,6% and 45,4% subjects
3. The longest median life expectancy occurred in the group of patients with mutations APC, TP53, PIK3CA, and MLH1 with a 30-month postoperative survival of 100%.
4. The shortest median life expectancy occurred in the group of patients with APC, TP53, PIK3CA, and KRAS mutations with a 50% life expectancy <6 months post-treatment.

Abbreviations

ACF : aberrant crypt foci

ACMG : American College of Medical Genetics

ACS : adenoma-carcinoma sequence

AJCC : American Joint Committee on Cancer

APC : Adenomatous Polyposis Coli

BAX : Bcl-2 Associated X protein

CDC4 : Cell division control protein 4

CRC : colorectal cancer

DCC : deleted in colorectal cancer

DNA : deoxyribonucleic acid

EGFR : Epidermal Growth Factor Receptor

FAP : Familial Adenomatous Polyposis

FATHMM : Functional Analysis through Hidden Markov Models

FFPE : Formalin-fixed Paraffin-Embedded

GRCh38 : *Genome Reference Consortium Human Reference 38*

IGF2R : Insulin Like Growth Factor 2 Receptor

KRAS : Kirsten rat sarcoma viral oncogene

MFOLFOX6 : modified Folinic Acid + 5-Fluorouracil + Oxaliplatin

MLH1 : MutL Homolog 1

MMR	: mismatch repair
PIK3CA	: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
SMAD4	: SMAD family member 4, Mothers against decapentaplegic homolog 4
SNPEFF	: SNP Effect
SNV	: Single Nucleotide Variant
TGFB2	: Transforming Growth Factor Beta Receptor 2
TP53	: Tumor Protein 53
VCF	: Variant Call Format
VEGF	: Vascular Endothelial Growth Factor
Wnt	: Wingless-related integration site

Declarations

Ethical Approval

The Ethics Committee of the Faculty of Medicine, Universitas Indonesia – RSUPN Cipto Mangunkusumo with regards of the Protection of human rights and welfare in medical research, has carefully reviewed the research with registry number: KET-445/UN2.F1/ETIK/PPM.00.02/2021. All procedures of Ethical Approval are performed in accordance with ICH-GCP standard procedure.

Declarations

Authors declare no conflict of interest. Provenance and peer review. Not commissioned, externally peer-reviewed.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to presence of individuals' personal details but are available from the corresponding author on reasonable request.

Authors' Contribution

VMGM analysed and interpreted data related to colorectal cancer, gathered information in medical records, and synchronized patients' data with VCF data files, and was a major contributor in writing the manuscript.

LE carried out the molecular genetics study and participated in sequence alignment.

TJML participated in its design and coordination and helped to draft the manuscript.

All authors read and approved the final manuscript.

Consent for publication

Consent was not required in this study because no details on individuals reported within the manuscript. This was already reviewed by the Ethics Committee mentioned above.

Acknowledgment

Not applicable

Competing Interest

Not applicable

Funding

No funding was obtained in this study.

References

1. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC. Genetic Alterations During Colorectal-Tumor Development. *N Engl J Med*. 1988;319(9). doi:10.1097/00043764-198910000-00001
2. Fearon ER, Vogelstein B. A Genetic Model for Colorectal Tumorigenesis. *Cell*. 1990;61:759-767.
3. Sagaert X, Vanstapel A, Verbeek S. Tumor Heterogeneity in Colorectal Cancer: What Do We Know So Far? *Pathobiology*. 2018;85(1-2):72-84. doi:10.1159/000486721
4. Molinari C, Marisi G, Passardi A, Matteucci L, De Maio G, Ulivi P. Heterogeneity in colorectal cancer: A challenge for personalized medicine? *Int J Mol Sci*. 2018;19(12). doi:10.3390/ijms19123733
5. Hiley CT, Swanton C. Spatial and temporal cancer evolution: causes and consequences of tumour diversity. *Clin Med*. 2014;14(6):s33-s37. doi:10.7861/clinmedicine.14-6-s33
6. Del Vecchio F, Mastroiaco V, Di Marco A, et al. Next-generation sequencing: Recent applications to the analysis of colorectal cancer. *J Transl Med*. 2017;15(1):1-19. doi:10.1186/s12967-017-1353-y
7. Nguyen HT, Duong HQ. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy (review). *Oncol Lett*. 2018;16(1):9-18. doi:10.3892/ol.2018.8679
8. Tariq K, Ghias K. Colorectal cancer carcinogenesis: a review of mechanisms. *Cancer Biol Med*. 2016;13(1):120-135. doi:10.28092/j.issn.2095-3941.2015.0103
9. Kang S, Na Y, Joung SY, Lee S II, Oh SC, Min BW. The significance of microsatellite instability in colorectal cancer after controlling for clinicopathological factors. *Med (United States)*. 2018;97(9):1-6. doi:10.1097/MD.00000000000010019
10. Harada S, Morlote D. Molecular Pathology of Colorectal Cancer. *Adv Anat Pathol*. 2020;27(1):20-26. doi:10.1097/PAP.0000000000000247
11. Nagahashi M, Wakai T, Shimada Y, et al. Genomic landscape of colorectal cancer in Japan: Clinical implications of comprehensive genomic sequencing for precision medicine. *Genome Med*. 2016;8(1):1-13. doi:10.1186/s13073-016-0387-8
12. Agha R, Abdall-Razak A, Crossley E, Dowlut N, Iosifidis C, Mathew G. STROCSS 2019 Guideline: Strengthening the reporting of cohort studies in surgery. *Int J Surg*. 2019;72(October):156-165. doi:10.1016/j.ijsu.2019.11.002
13. Venugopal A, Stoffel EM. Colorectal cancer in young adults. In: *Current Treatment Options in Gastroenterology*. Vol 17. Current Treatment Options in Gastroenterology; 2019:89-98. doi:10.1007/s11938-019-00219-4
14. Bunz F. *Principles of Cancer Genetics*; 2008. doi:10.1007/978-1-4020-6784-6
15. Janssen KP, Alberici P, Fsihi H, et al. APC and Oncogenic KRAS Are Synergistic in Enhancing Wnt Signaling in Intestinal Tumor Formation and Progression. *Gastroenterology*. 2006;131(4):1096-1109. doi:10.1053/j.gastro.2006.08.011

16. Jasperson KW, Patel SG, Ahnen DJ. APC -Associated Polyposis Conditions Summary Clinical characteristics Diagnosis Suggestive Findings. 2019:1-38.
17. Fearnhead NS, Britton MP, Bodmer WF. The ABC of APC. *Hum Mol Genet.* 2001;10(7):721-733. doi:10.1093/hmg/10.7.721
18. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJC. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut.* 2005;54(9):1283-1286. doi:10.1136/gut.2005.066514
19. Win AK, Jenkins MA, Dowty JG, et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol Biomarkers Prev.* 2017;26(3):404-412. doi:10.1158/1055-9965.EPI-16-0693
20. Miyoshi Y, Nagase H, Ando H, et al. Somatic mutations of the APC gene in colorectal tumors: Mutation cluster region in the APC gene. *Hum Mol Genet.* 1992;1(4):229-233. doi:10.1093/hmg/1.4.229
21. Smith G, Carey FA, Beattie J, et al. Mutations in APC, Kirsten-ras, and p53—alternative genetic pathways to colorectal cancer. *Mutat Res - Fundam Mol Mech Mutagen.* 2002;99(14):9433-9438. doi:10.1016/j.mrfmmm.2009.06.011
22. He K, Wang Y, Zhong Y, Pan X, Si L, Lu J. Kras codon 12 mutation is associated with more aggressive invasiveness in synchronous metastatic colorectal cancer (Mcr): Retrospective research. *Onco Targets Ther.* 2020;13:12601-12613. doi:10.2147/OTT.S279312
23. Tian X, Liu Z, Niu B, et al. E-Cadherin/ β -catenin complex and the epithelial barrier. *J Biomed Biotechnol.* 2011;2011. doi:10.1155/2011/567305
24. Harington KJ, Syrigos KN. The role of E-cadherin-catenin complex: More than an intercellular glue? *Ann Surg Oncol.* 2000;7(10):783-788. doi:10.1007/s10434-000-0783-5
25. Ilyas M, Tomlinson IPM. The interactions of APC, E-cadherin and β -catenin in tumour development and progression. *J Pathol.* 1997;182(2):128-137. doi:10.1002/(SICI)1096-9896(199706)182:2<128::AID-PATH839>3.0.CO;2-Q
26. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene.* 2017;36(11):1461-1473. doi:10.1038/onc.2016.304
27. Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. *Cancer Metastasis Rev.* 2020;39(4):1029-1038. doi:10.1007/s10555-020-09915-5
28. Grossman RL, Heath AP, Ferretti V, et al. Toward a Shared Vision for Cancer Genomic Data. *N Engl J Med.* 2016;375(12):1109-1112. doi:10.1056/NEJMp1607591.Toward
29. Jauhri M, Bhatnagar A, Gupta S, et al. Prevalence and coexistence of KRAS, BRAF, PIK3CA, NRAS, TP53, and APC mutations in Indian colorectal cancer patients: Next-generation sequencing–based cohort study. *Tumor Biol.* 2017;39(2). doi:10.1177/1010428317692265
30. Pino MS, Chung DC. THE CHROMOSOMAL INSTABILITY PATHWAY IN COLON. *Gastroenterol* 138(6) 2059–2072. 2010;138(6):2059-2072. doi:10.1053/j.gastro.2009.12.065.THE
31. Jin J, Shi Y, Zhang S, Yang S. PIK3CA mutation and clinicopathological features of colorectal cancer: a systematic review and Meta-Analysis. *Acta Oncol (Madr).* 2020;59(1):66-74. doi:10.1080/0284186X.2019.1664764
32. Jang S, Hong M, Shin MK, et al. KRAS and PIK3CA mutations in colorectal adenocarcinomas correlate with aggressive histological features and behavior. *Hum Pathol.* 2017;65:21-30. doi:10.1016/j.humpath.2017.01.010
33. Wang SM, Jiang B, Deng Y, Huang SL, Fang MZ, Wang Y. Clinical significance of MLH1/MSH2 for stage II/III sporadic colorectal cancer. *World J Gastrointest Oncol.* 2019;11(11):1065-1080. doi:10.4251/wjgo.v11.i11.1065

Figures

Figure 1

Median life expectancy in days

Figure 2

Survival rate based on co-occurring mutation