Review Article

Molecular pathology of colorectal cancer

The Saudi situation in perspective

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ABSTRACT

سرطان القولون والمستقيم (CRC) أحد أكثر أنواع السرطان شيوعًا في جميع أنحاء العالم، وهوأكثر أسباب الوفيات الناجمة عن السرطان شيوعًا. في الآونة الأخيرة، ظهر تقدم كبير في توضيح التغيرات الجزيئية للمرض، وكانت النتائج تحسين فهم بيولوجيا CRC، واكتشاف المؤشرات الحيوية ذات الأهمية التشخيصية والإنذارية والعلاجية. في هذا التقرير، أجرينا تقييم لبحوث علم الأمراض الجزيئي لـ CRC المنبثقة من المملكة العربية السعودية. الحكم هو أن البيانات المتعلقة بالتغيرات الجزيئية في CRC من المرضى السعوديين متواضعة في أحسن الأحوال. تنعكس هذه الندرة في بيانات علم الأمراض الجزيئي بشكل مناسب في ندرة الواسمات الجزيئية الموصى بها للاختبار بواسطة إرشادات المركز الوطني السعودي للسرطان لعلاج CRC. يلزم إجراء دراسات متعددة المؤسسات ومتعددة الأقاليم على نطاق واسع لتوليد البيانات الجزيئية التي من شأنها أن تقدم إرشادات للتشخيص والتنبؤ والتقسيم الطبقي للمخاطر لمرضى CRC السعوديين.

Colorectal cancer (CRC) is one of the most common cancers worldwide, and one of the most common causes of cancer deaths. In recent times, significant advancements have been made in elucidating the molecular alterations of the disease, and the results have been an improved understanding of CRC biology, as well as the discovery of biomarkers of diagnostic, prognostic, and therapeutic significance. In this review, an evaluation is carried out of the molecular pathology research of CRC emanating from Saudi Arabia. The verdict is that the data on the molecular alterations in CRC from Saudi patients is at best modest. This dearth of molecular pathology data is aptly reflected in the paucity of molecular markers recommended for testing by the Saudi National Cancer Centre guidelines for CRC management. Large scale multi-institutional and multiregional translational studies are required to generate molecular data that would inform diagnostic, prognostic, and risk-stratification guidelines for Saudi CRC patients.

Keywords: colorectal cancer, Saudi Arabia, molecular pathology, genetic alterations

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Colorectal cancer (CRC) is the 3rd most common cancer worldwide after breast and lung cancers, with reportedly more than 1.9 million new cases (9.8% of all cancers) diagnosed in 2020.[1,2] It is also the 2nd most common cause of cancer deaths with 915,880 deaths (9.2% of all cancers) in the same year.[1,2] In Saudi Arabia, CRC is the second most common cancer after breast cancer, with an age-standardised ratio (ASR) incidence rate of 13.9/100,000 population (14.4% relative frequency ratio) in both genders in the period under review. Colorectal cancer also has the highest number of new cancer cases (4007 cases) in the 2020 period. Colorectal cancer is the most common cancer in the male gender (ASR incidence rate of 16.1/100.000 and relative frequency ratio of 19.3%); and the 3rd most common cancer in the female gender (ASR of 10.9/100,000 or relative frequency ratio of 9.2%) after breast and thyroid cancers.[1,2] Accordingly, a 2020 systematic review by Alqahtani et al,[3] classed CRC as the second most common cancer after breast cancer, with an incidence rate of 14.6% and a mortality rate of 1.5%, making it the 16th most common cause of cancer deaths in Saudi Arabia. But perhaps more importantly, the rate of CRC in Saudi Arabia has been increasing over the years, especially among the young.[3-6]

The mortality rate patterns for CRC worldwide largely paralleled the incidence rate patterns, as the highest mortality rates of 8.2-11.8/100,000 were noted in countries with the highest incidence rates, while the lowest mortality rates of 3.2-7.2/100,000 were found in countries with the lowest rates.[1,2] The mortality rate for CRC in Saudi Arabia (7.3/100,000 population) falls in the upper limits of the mortality rates found in regions with the lowest mortality rates.[1,2]

The molecular pathology of cancer has improved our understanding of carcinogenesis, and has enabled the discovery of diagnostic, prognostic, and predictive molecular markers of clinical utility.[7,8] The aim of this review is to explore the molecular pathology research that has been carried out on CRC from Saudi patients, with a view to understanding the prevalence and clinicopathological correlates of clinically and biologically relevant genetic mutations in Saudi CRC patients.

Molecular pathology of CRC. The development of CRC involves a step-by-step accumulation of mutations and epigenetic changes in oncogenes and tumour suppressor genes.[9-11] Three molecular pathogenetic patterns of presentation have been documented for CRCs: I) hereditary CRC syndromes (up to 5% of CRC cases); II) familial, or, non-syndromic CRC (20-30% of cases); and III) sporadic CRC (approximately 70-75%).[9-13] However, most molecular pathology studies of CRC from Saudi Arabia have been hospital-based researches (**Tables 1-3**). As a result, the data emanating from the molecular pathology studies of Saudi CRC has been modest.

Hereditary CRC syndromes. The 2 most common hereditary CRC syndromes are Lynch (hereditary non-polyposis colon cancer [HNPCC]) and the familial adenomatous polyposis (FAP) syndromes.[10,14] Lynch syndrome (LS) variants include Turcot, Muir-Torre, and constitutional mismatch repair deficiency (CMMRD) syndromes.[10] Familial adenomatous polyposis syndrome variants include attenuated adenomatous polyposis coli (AAPC, also known as hereditary flat adenoma), Gardner, and Turcot syndromes.[10,13] The lesser-known inherited CRC syndromes include NTHL1-associated polyposis (NAP) and MUTYH-associated polyposis (MAP) syndromes.[10,13] Other syndromes with frequent CRC presentation are Cowden, Juvenile Polyposis, Peutz-Jeghers, Bannayan-Riley-Ruvalcada, Cronkite-Canada, and Proteus and Proteus-like syndromes.[10]

Lynch syndrome is the most common CRC syndrome with a frequency of 2-5% of all CRCs.[10] It is an autosomal dominant condition resulting from mutations in the DNA mismatch repair (MMR) genes MLH1, PMS2, MSH2, and MSH6.[10,15] The MMR genes repair DNA mismatches which amass during normal DNA replication.[10,15] Mutations of the MMR genes confer a mutator phenotype on the DNA of LS patients, with resultant genome-wide accumulations of short insertions and deletions (indels) and other mutations. Indels are most notable in microsatellite sequences of the genome, and cause microsatellite instability (MSI).[10] Microsatellite instability induces frameshift mutations in growth-signalling genes, thereby providing a background for tumour development.[10] The lifetime risk of CRC for LS patients is in the range of 52-82%.[16,17] Lynch syndrome is associated with familial clustering of tumours of the colon, rectum, endometrium, stomach, small bowel, ovary, the hepatobiliary tract, pancreas, ureters, skin, and brain.[10,15-17] Lynch syndrome patients have an earlier onset of CRC relative to patients with sporadic CRCs.[10] Due to the high frequency of LS and the risks of metachronous tumours, it has been recommended that all CRC patients undergo screening for LS.[15,18] The benefits of LS screening programme are the better chances of early tumour detection at metachronous sites, as well as the early tumour detection in the LS patients' relatives.[15] Despite the importance of the LS diagnosis, only 3 groups have published research on the CRC syndrome in Saudi Arabia.[19-21] The frequency of LS based on the 3 published articles from 2 of the 3 groups was between 0.99-7.2%. Siraj et al,[19] who have so far found a LS prevalence of 2.2% (up from 0.99%), used a combination of MMR immunohistochemistry, microsatellite analysis by PCR, BRAF mutation testing, and deep sequencing to investigate mutations in the MMR genes, and found that 26 of the 1207 CRC cases harboured various germline mutations in MLH1, MSH2, PMS2, MSH6, and EPCAM.[19,20] These mutations varied in their lifetime risks of CRC by the age of 50 years: MLH1 of 14%, MSH2 of 35%, MSH6 of 20%, PMS2 of 26%, and EPCAM of 7%. Interestingly, 3 founder mutations were responsible for 11/26 cases (42.3%) of LS cases in the Siraj et al[19] series. Of note, the PMS2 c.1376C>G; p.S459X variant was identified in 6/11 cases with founder mutations.[19] This variant have been previously described in a European family with LS.[22,23] Alqahtani et al,[21] on the other hand found an extrapolated estimate of 7.2% of LS in CRC Saudi patients who are less than 60 years. In that study the authors described germline mutations in 8 of 13 CRC cases with molecular (MSI and BRAF mutations) and clinical features sugestive of LS. MLH1 and MSH2 each had 4 germline mutations that were classified as pathogenic by prediction tools.[21] Rasool et al[24] analysed germline DNA from 12 presumed LS patients (based on the revised Bethesda and Amsterdam criteria) and identified structural losses in 2p21-p16.3 (MSH2,

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Table 1 - Molecular pathology of colorectal cancer syndromes from Saudi patients.

Syndromes	Germline alterations	Molecular methodology	Sample size	References
Lynch syndrome	*PMS2* c.1376C>G, p.S459X, *MLH1* c.1652A>C, p. N551T *MSH2* c.2262-2267 delTTCTAC, p.T756-Y757 del *MSH2* c.1226_1227delAG *MSH2* c.1964.delT, p. V655fs *MSH2* c.289C>T, Q97* *MSH6* c.3475insT, p. Y1159fs *MSH6* c.3475insT	MMR protein by IHC, MSI, and BRAF mutation testing and deep sequencing	2.2% of all CRC (N=1207)	19,20
Lynch syndrome	*MSH2* c.737A>T, p.K246I *MSH2* c. 2262_2267del, p.S755_T756del *MSH2* c.367-? __1276+? Del *MSH2* c.2089T>C, p.C697R *MSH2* c. 2038C>T, p.R680* *MLH1* c.62C>T, p.A21V *MLH1* c.1961C>T, p.P654L *MLH1* c.677G>A, p.R226Q *MLH1* c.454-?_545+?del	MMR protein by IHC, MSI, BRAF mutation testing, MLPA, deep sequencing, and Sanger sequencing	7.2% of all CRC (N=284)	21
Lynch syndrome	Deletion of chromosomal regions: 2p21-2p16.3 (*MSH2, MSH6*, and *EPCAM*) 3p23-3p14.2 (*MLH1*) 7p22.1 (*PMS2*) 1p34.1-1p33 (*MUTYH*)	Whole genome comparative genomic hybridization array	N=12 (LS patients)	24
FAP syndrome	*PMS2* c.1376C>G, p.S459X	WES	One case report	19

FAP: familial adenomatous polyposis, MMR: mismatch repair, IHC: immunohistochemistry, MSI: microsatellite instability, BRAF: B-Raf, MLPA: multiplex ligation probe amplification, WES: whole-exome sequencing, CRC: colorectal cancer, LS: Lynch syndrome

MSH6, and EPCAM) in 8, 3p23-p14.2 (MLH1) in 5, 7p22.1 (PMS2) in 2, and 1p34.1-p33 (MUTYH) in one LS cases. However, the study did not explore the rate of LS in CRC population. Therefore, whilst the prevalence of LS in Saudi Arabia, as found by Siraj et al[19] and Alqahtani[24] studies, may be comparable to that found in Europe and elsewhere, the patterns of LS-causing mutations differ. PMS2 and MUTYH mutations may be more common causes of LS in Saudi Arabia's CRC population than in other populations (Table 1).

Familial adenomatous polyposis is an autosomal dominant syndrome with characteristic early onset of hundreds to thousands of adenomatous polyps in the large bowel.[10,13] Transmitted germline mutations in APC, account for 75-80% of FAP. De novo germline mutations of APC in a parent of the affected individual accounts for a further 20-30%.[10,14] Classical FAP patients presents in the 16-19 years age group, and by the 35-40 years of age develop CRC, if untreated by prophylactic colectomy.[10,13,14] Other features of classical FAP syndrome include gastric fundic polyp, duodenal adenomas, gastric adenomas, peri-ampullary adenomas, congenital hypertrophy of retinal pigment epithelium and cribriform-morular papillary thyroid carcinoma.[10,13,14] Attenuated FAP, Turcot and Gardner syndromes, are also caused by APC mutations.[10,13,14] Adenomatous polyposis coli is a 15 exon-long, tumour suppressor gene which maps to 5q21-22.[10,13,14] Over a thousand different mutations, which include point mutations, short deletions, and large rearrangement, have been found in classical FAP and its variants.[10] The severe forms of FAP are caused by APC mutations that cluster in the codons 1250-1464 hotspot of exon 15.[10,13,14] On the other hand, the milder forms (fewer polyps and later onset of disease) of FAP are caused by the mutations scattered across exons 1-14.[10,13] Adenomatous polyposis coli stabilizes microtubules via its functions in the alignment of metaphase chromosome. In the WNT signalling pathway it prevents the overactivation of β-catenin by binding and sequestering it in the cell membrane. Adenomatous polyposis coli mutations cause disinhibition of β-catenin and its translocation to the nucleus where it effects the upregulation of cell cycle regulators CCND1, C-MYC, and PPARD.[10,13,14] Subsequently, there is unrestrained cell proliferation, a condition which provides the background for the accumulation of mutations in cell cycle regulators such as KRAS and TP53, in the progression to malignant transformation.[10,13,14] Therefore, FAP-associated

Table 2 - Genetic risks for colorectal cancer in Saudi patients.

Genetic loci	Study approach	Number of CRC cases	Molecular methodologies	Risk patterns	References
ABCC1 C218T	Case-control	N=51, 65 controls	PCR-RFLP and DNA sequencing (Sanger)	Increased CRC risk, OR=3.4	40
ADIPOQ G276T	Case-control	N=60, 60 controls	PCR-RFLP	Increased CRC risk, OR=2.64	41
CYP1A1wt/*2A	Case-control	N=92, 79 controls	PCR-RFLP	Increased CRC risk, OR=3.65	42
GSTM1	Case-control	N=80, 78 controls	Diplex PCR	Increased CRC risk, OR=3.7	43
TP53 rs1042522	Case-control	N=80, 78 controls	TaqMan real-time PCR assays	Increased CRC risk, OR=1.6	43
KIR 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1	Case-control	N=70, 70 controls	PCR	Increased CRC risk, OR=8.6-fold, 3-fold, 2.5-fold, 4.5-fold, and 16.25-fold	44
IL17A rs2275913: GA and AA genotypes	Case-control	N=117, 100 controls	TaqMan allelic discrimination assay (PCR)	Increased CRC risk, OR=2-fold and 2.8-fold	45
NOTCH3 rs1043994: G>A	Case-control	N=134, 139 controls	TaqMan allelic discrimination assay (PCR)	Increased CRC risk in males, OR=1.971	46
PARP1 K933N and K945N	Case-control	N=50, 50 controls	PCR and Sanger sequencing	Increased CRC risk OR=3.1429 for K933N G>T heterozygote; OR=2.5714 for K945N G>T heterozygote	47
PRNCR1 rs1456315: CC genotype	Case-control	N=144, 130 controls	TaqMan assays	Increased CRC risk, OR=2.09	48
RETN rs1862513 and rs375367	Case-control	N=60, 60 controls	PCR-RFLP	Increased CRC risk, OR=2.48 (rs1862513); OR=6.5 (rs375367)	49
TDG rs4135113	Case-control	N=100, 192 controls	TaqMan genotyping	Increased CRC risk, OR>3.6; OR=5 in those >57 years	50
TLR9 rs352139	Case-control	N=115, 102 controls	TaqMan allelic discrimination assay (PCR)	Increased CRC risk for rectal cancers, OR=3.552 and 1.809 for GG genotype and G allele	51
TNFA rs361525 (G238A)	Case-control	N=100, 100 controls	TaqMan allelic discrimination assay	Increased CRC risk, OR=14.663 for AA genotype and 7.647 for the A allele	52
TSLP rs10043985	Case-control	N=112, 108 controls	TaqMan genotyping assay	Increased CRC risk, OR=16.52 for AC genotype and 10.837 for the C allele	53
VDR1 ApaI rs797232	Case-control	N=100, 100 controls	PCR amplification, followed by Sanger sequencing	Increased CRC risk, OR=1.778 in patients >57 years (rs797232 and C allele)	54
VDR1 TaqI rs731236	Case-control	N=132, 124 controls	PCR-RFLP	Increased CRC risk, OR=6.18	55
XRCC1 A399G	Case-control	N=100, 100 controls	PCR-RFLP and PCR-CTPP	Increased CRC risk, OR=2.1	56
ABCB1 G2677T	Case-control	N=62, 100 controls	PCR-RFLP	Reduced CRC risk, OR=0.004 for heterozygotes GT and 0.005 homozygotes TT	57
ADIPOQ T45G	Case-control	N=60, 60 controls	PCR-RFLP	Reduced CRC risk, OR=0.41 for the G allele	41
CTNNB1 rs4135385	Case-control	N=122, 110 controls	TaqMan assays	Reduced CRC risk, OR=0.092 for GG genotype	58
LRP6 rs2284396	Case-control	N=122, 110 controls	TaqMan assays	Reduced CRC risk, OR=0.250 for the CC genotype in cases >57 years; OR=0.561 for the C allele	58
SFRP3 rs7775	Case-control	N=122, 110 controls	TaqMan assays	Reduced CRC risk, OR=0.397 for the Gly allele in female gender	58
CYP19A1 rs4774585 rs4775936	Case-control	N=100, 100 controls	TaqMan genotyping by real-time PCR	Reduced CRC risk, OR=0.28 for rs4774585 AA genotype in the male gender; OR=0.37 for rs4775936 CT genotype in the female	59
IL7R rs1053496	Case-control	N=112, 108 controls	TaqMan genotyping assay	Reduced CRC risk, OR=0.529, 0.467 and 0.644 for the CT and TT genotypes, and the T allele	53
PARP1 rs8679	Case-control	N=183, 190 controls	TaqMan assay	Reduced CRC risk, OR=0.566 for the TC genotype and 0.695 for the C allele	60
TDG rs1866074 rs3751209	Case-control	N=100, 192 controls	TaqMan genotyping	Reduced CRC risk, OR=0.501 for rs1866074 GG genotype; OR=0.407 for rs3751209 GA genotype in the male gender	50
TLR4 rs10759931 rs2770150	Case-control	N=115, 102 controls	TaqMan allelic discrimination assay	Reduced CRC risk, OR=0.052, 0.018 and 0.085 for rs10759931 GA and AA genotypes, and A allele, respectively; OR=0.074, 0.194, 0.188 for rs2770150 TC and CC genotypes, and C allele only in the female gender	61
TLR9 rs187084 rs352144	Case-control	N=115, 102 controls	TaqMan allelic discrimination assay (PCR)	Reduced CRC risk, OR=0.527 for rs187084 T allele in the female gender; OR=0.067 for rs352144 AC genotype for rectal tumours	51
VDR1 BsmI rs1544410	Case-control	N=100, 100 controls	PCR amplification, followed by Sanger sequencing	OR=0.217 for rs1544410 AA genotype and 0.442 for A allele in the female gender	54

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism, CRC: colorectal cancer, OR: odds ratio

Table 3 - Somatic mutations in colorectal cancer from Saudi patients.

Genes	Mutation rates (mutation type)	Sample sizes	Molecular methodologies	References
KRAS	56.0% (point)	150	PCR followed by amplicon hybridization	68
	28.6% (point)	755	PCR and DNA sequencing	69
	30.1% (point)	498	PCR and DNA sequencing	70
	50.0% (point)	194	Biocartis Idylla™	71
	45.2% (point)	93	NGS	72
	42.2% (point)	83	PCR followed by amplicon hybridization	73
	49.6% (point)	248	Biocartis Idylla™	74
	35.0% (point)	99	Ion PGM sequencing	75
	32.5% (point)	80	Sanger sequencing	76
	42.85% (point)	56	HRM analysis, Sanger sequencing, and shifted termination assays	77
	42.0% (point)	300	LCD-array	78
	43.0% (point)	51	Ion AmpliSeq NGS Panel	79
	25.0% (point)	99	Ion AmpliSeq™	80
NRAS	2.2% (point)	93	NGS	72
	2.0% (point)	248	Biocartis Idylla™	74
BRAF	2.5% (point)	757	PCR and DNA sequencing	69
	2.4% (point)	498	PCR and DNA sequencing	70
	2.2% (point)	93	NGS	72
	0.4% (point)	248	Biocartis Idylla™	74
PIK3CA	19.0% (point)	99	Ion PGM Sequencing	75
	12.2% (point)	418	Sanger sequencing	81
HER2 (ERBB2)	0.0% (gene amplification)	114	FISH	82
	51.0% (point)	99	Ion AmpliSeq™	80
CCND1	1.9% (gene amplification)	114	FISH	82
EGFR	11.0% (point)	99	Ion PGM Sequencing	75
	1.1% (gene amplification)	114	FISH	82
C-MYC	40.0% (point)	99	Ion AmpliSeq™	80
	9.0% (gene amplification)	114	FISH	82
MSI	11.2%	741	Microsatellite analysis by multiplex fluorescent PCR	69
	10.9%	494	Microsatellite analysis by multiplex fluorescent PCR	70
	20.2%	388	Microsatellite analysis by multiplex fluorescent PCR	81
	9.7%	992	Microsatellite analysis by multiplex fluorescent PCR	83
	11.6%	284	Pentaplex MSI analysis system	84
	11.3%	807	Microsatellite analysis by multiplex fluorescent PCR and immunohistochemistry assessment	20
TP53	65.0% (point)	99	Ion PGM sequencing	75
	33.7% (point)	386	Sanger sequencing	81
APC	19.0% (point)	99	AmpliSeq comprehensive	80
	36.0% (point)	99	Ion PGM sequencing	75
	66.0% (point)	99	AmpliSeq comprehensive cancer panel	80
SMAD4	11.0% (point)	99	Ion PGM sequencing	75
	3.0% (point)	99	AmpliSeq comprehensive cancer panel	80
	13.6% (point)	426	High depth capture sequencing	83
	60.8% (SMAD4 gene and 18q deletion)	991	FISH	83
PTEN	13.0% (point)	99	Ion PGM sequencing	75
	3.0% (point)	99	AmpliSeq comprehensive cancer panel	80
	66.1% (point)	386	Sanger sequencing	81
CIMP	CIMP-H=4.8%; CIMP-L=44.6%; CIMP-negative=50.6%	500	Real-time PCR (MethyLight)	70

MSI: microsatellite instability, CIMP: CpG island methylator phenotype, CIMP-H: CpG island methylator phenotype-high, CIMP-L: CpG island methylator phenotype-low, PCR: polymerase chain reaction, PGM: personal genome machine, FISH: fluorescence in situ hybridisation, HRM: high resolution melt, LCD: liquid-crystal display, NGS: next-generation sequencing

CRC develop via the chromosomal instability (CIN) pathway.[10,13] Although a few cases of presumed FAP syndrome in Saudi patients have been reported in the literature, a comprehensive molecular genetic study of FAP syndrome in patients of Saudi origin has yet to be undertaken.[25,26] One case of FAP syndrome caused by homozygous germline mutations in PMS2, the PMS2 c.1376C>G; p.S459X variant was reported in a young Saudi patient.[27] Although this patient had the clinical features of FAP syndrome (thousands of colonic polyps), he lacked germline APC mutations. However, somatic APC mutations were detected in this patient's polyps, which lacked microsatellite instability. Germline compound heterozygous mutations of the MMR gene MSH3 have been reported previously in European patients with clinical features of FAP syndrome, although these individuals did have trinucleotide and dinucleotide repeats-based APC mutations in their tumours.[28] Another patient with FAP syndrome was reported to harbour germline compound heterozygous mutations in the PMS2 gene but without APC mutations, evidence that certain germline MMR gene mutations can be causative of FAP syndrome.[28]

Familial (or non-syndromic CRC). Familial, non-syndromic CRCs, also known as familial CRC type X (FCCTX), form a heterogeneous group of CRCs with unknown molecular pathogenesis.[13,14] It is characterised by familial clustering of CRC in the absence of the known inherited syndromes.[13,14] Current postulation is that FCCTX is either some unknown CRC syndrome or sporadic forms of CRC that cluster in families.[14] Furthermore, FCCTX is hypothesized to result from co-inheritance of numerous low-penetrance genetic alterations.[11,14] Genetic/genomic risk loci described for familial CRC are 2q32.3, 8q24, 8q23.3, 10p14, 11q23, 15q13.3 (CRAC1/HMPS), 16q22.2, 18q21 (SMAD7), and several more.[29-36] The clinicopathological features of FCCTX include a rectal location, male gender and older patient age preponderance, low rate of metachronous carcinoma, low grade histology, and reduced risk of extra-intestinal tumours.[38] Several genetic variants with CRC risks have been described in the Saudi population.[39] Furthermore, some genetic variants have been found to be protective from CRC in the Saudi studies. The genetic variants that have been found to increase CRC risks in the Saudi population include the ABCC1 C218T, ADIPOQ G276T, CYP1A1wt/*2A, GSTM1, KIR 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1, IL17A rs2275913:GA and AA genotypes, NOTCH3 rs1043994: G>A, PARP1 Lys933Asn and Lys945Asn, PRNCR1 rs1456315: CC genotype, RETN rs1862513 and rs375367, TDG rs4135113, TLR9 rs352139, TNFA rs361525 (G238A), TP53 rs1042522, TSLP rs10043985, VDR1 ApaI rs797232 and TaqI rs731236, and XRCC1 A399G.[40-56] Protective variants that have been found in the Saudi population include ABCB1 G2677T, ADIPOQ T45G, CTNNB1 rs44135385, rs2284396, CYP19A1 rs4774585, rs936308, rs4775936, IL7R rs1053496, LRP6 rs2284396, PARP1 rs8679, SFRP3 rs7775, TDG rs1866074, TLR4 rs10759931 and rs2770150, TLR9 rs187084 and rs352144, VDR1BsmI rs1544410 (Table 2).[41,50-61]

Sporadic CRC. It develops through 3 genetic pathways: CIN pathway (70-85% of sporadic CRC), MSI pathway (10-15%), and the CpG island methylation pathway (CIMP, <5%).[9,11] Typically, CIN CRC have numerical and structural chromosomal abnormalities.[9,11] Furthermore, CRCs arising by the CIN pathway are characterised by mutations in KRAS, BRAF, APC, TP53, PIK3CA, and SMAD4.[9,11] The MSI CRC results from MMR downregulation by epigenetic mechanisms. Promoter methylation of MLH1 is the culprit in more than 80% of sporadic MSI cases.[9,11] The karyotype of most MSI CRCs is diploid or near-diploid. However, MSI CRCs typically have widespread frameshift mutations, which are recurrently found in the genetic targets of MSI: MSH3, MSH6, TGFBRII, IGFIIR, MBD4, PTEN, BAX, MLH3, RIZ, CASP5, WISP3, RAD50, GARE, E2F4, etc.[11,62] Moreover, BRAF and APC mutations are common in MSI tumours, but TP53 and KRAS mutations are uncommon.[9,11] The clinicopathological characteristics of MSI CRC include right-sided location, mucinous and medullary histomorphology, presence of tumour-infiltrating lymphocytes, earlier-stage disease, and more favourable outcomes.[11,63] The CpG island methylation pathway CRCs have genome-wide CpG island methylation.[9,11,64] Approximately 30-40% of CIMP tumours arise in the proximal colon, while 3-12% occur in the distal colon.[11,64] Colorectal cancer is classified as CIMP-high, CIMP-low, and CIMP-negative based on MINTS1, MINTS2, MINTS31, CDKN2A, and MLH1 methylation.[11,64] More than 50% of CIMP-positive CRC are microsatellite stable (MSS).[64,65] Some CIMP-positive tumours are, however, MSI as a result of MLH1 methylation. The CpG island methylation pathway-high tumours typically are MSI- and BRAF mutation-positive. On the other hand, CIMP-low tumours are characterised by KRAS mutations, while CIMP-negative CRC are MSS and TP53 mutation-positive.[11,64,65]

However, the 3 genetic/epigenetic pathways of sporadic CRC are not mutually exclusive.[9,11,65] For example, 25% of MSI CRC has CIN, and 12% of CIN

tumours are MSI.[11,66] And whilst CIMP-positive CRC are frequently MSI, 25-33% has CIN.[66,67] However, individual studies have separately evaluated MSI, CIMP and various other genetic aberrations in CRC with their associated clinicopathological and prognostic significances. The following sections describe alterations of oncogenes and tumour suppressor genes that have been investigated in Saudi CRC populations by various research groups.

The rate of KRAS mutation in CRC from Saudi patients is between 28.6-56%, making it one of the most common genes mutated in Saudi CRC cases, with G12D and G12V constituting the major hotspots for KRAS mutations.[68-80,72,73,78,79] KRAS mutations show associations with tumour site, disease stage, metastatic sites, and survival in the Saudi CRC population.[68-71,73,75,78-80] On the other hand, some authors did not find any clinicopathological associations with KRAS mutations.[72,74] Furthermore, novel KRAS mutations not previously described in European CRC populations have been found in the Saudi population. For example, Rasool et al[76] sequenced 51 Saudi CRC cases and identified novel KRAS E31K in 3 cases and S17R mutation in 4 cases. In addition, Naser et al[77] reported 7 novel KRAS gene mutations in a cohort of Saudi CRC patient, including A134V, R135K, Q150X, K147K, E143K, G138G, and R149G. Three of these mutations (A134V, E143K, and R149G) were predicted by in silico tools to be deleterious sequence changes. NRAS mutations were found in 2-2.2% of CRC cases by 2 studies. But no clinical or pathological correlates were described for NRAS mutations by these studies.[72,74] The prevalence of BRAF mutations in the Saudi CRC population has been found to be between 0.4-2.5%, according to 4 studies.[20,69,72,74] One of these studies found that over 90% of the BRAF mutations was the V600E mutation in BRAF exon 15. That study also found a right-sided tumour site association for BRAF mutations.[69] The rate of PIK3CA mutations (exons 9, 20, and 21) in the Saudi CRC cohort is between 12.2-19%.[75,81] PIK3CA mutations were shown to correlate with poor disease-specific survival in one of the 2 studies.[75] A single study investigated the gene amplifications of HER2 (0%), CCND1 (1.9%), EGFR (1.1%), and C-MYC (9%) in Saudi CRC patients.[82] Moreover, the study found no clinicopathological or survival correlates for the gene amplification studied. However, 2 separate studies which investigated EGFR mutations in Saudi CRC patients found a prevalence of 11-40%.[75,80] EGFR mutations in one of the studies were found more significantly in young patients and correlated with poor survival outcomes.[75] Moreover, HER2 (ERBB2) mutations were found in 51% of Saudi CRC cases, but did not show any clinicopathological significance in that study.[80] **Table 3** summarizes the mutation rates, sample sizes, and molecular methodologies from the molecular pathology studies on Saudi CRC cohorts.

A total of 5 studies found a prevalence of 9.7-20.2% for MSI in Saudi CRC cases.[20,69,70,81,83,84] Microsatellite instability was associated directly with right-sided tumours, mucinous histology, BRAF mutations, and survival, but showed an inverse association with TP53.[20,70,81] This clinicopathological association was however not uniform across the studies as some authors did not find any clinicopathological correlates for MSI.[84] TP53 mutations have been described in 19-65% of CRC from Saudi patients by 3 studies.[75,80,81] This rate is comparable to the prevalence of TP53 in other populations.[85] TP53 mutations were found to correlate directly with lymph node metastasis and survival.[75,81] Adenomatous polyposis coli was found mutated in 36-66% of Saudi CRC population, according to 2 studies from Jeddah and Riyadh regions.[75,80] Whilst one study found that APC mutation were left-sided tumours in young females, the other study found no clinicopathological correlates for APC mutations.[75,80] A total of 3 studies which considered SMAD4 mutations in Saudi CRC cohort found SMAD4 prevalence of 3-13.6% of CRC cases.[75,80,83] In one of these studies, SMAD4 deletion (in the setting of chromosome 18q deletion) was also detected in 60.8% of the CRC cases.[83] Both SMAD4 mutations and SMAD4 deletions were associated with loss of smad4 expression. Additionally, SMAD4 (and 18q) deletion was significantly associated with distant metastasis and microsatellite stable (MSS) tumours. Low expression of smad4 (caused by SMAD4 mutations and deletion) was found to correlate with survival. PTEN mutations were found in 3-66.1% of Saudi CRC patients in 3 studies.[75,80,81] In one of the studies, PTEN mutations were associated with microsatellite status.[81] In the other 2 studies PTEN mutations did not show any clinicopathological significance in CRC (**Table 3**).[75,80]

The sequence of morphological evolution of adenocarcinoma from normal colonic epithelium correlates with the genetic/epigenetic pathway of colorectal carcinogenesis, and with tumour location. The traditional adenoma-carcinoma sequence (50-70% of CRC), by which normal colonic epithelium changes to tubular adenoma and then to adenocarcinoma, arise most commonly in the distal colon and correlates with the CIN pathway and with APC, and TP53 mutations.[9,11] The serrated sequence (10-20%) that forms the sessile

serrated lesions and then adenocarcinoma, arises in the proximal colon and corelates with BRAF mutations, MSI, MLH1 methylation, and CIMP.[86,87] The alternative sequence (10-30%), by which normal epithelium transforms to villous, tubulovillous, traditional serrated or serrated tubulovillous adenomas then to adenocarcinoma, correlates with CIMP, APC, BRAF, and KRAS mutations.[11,86,87] In a study of 770 Saudi CRC cohort, the researchers used MSI and CIMP statuses, KRAS and BRAF mutations to classify tumours into traditional, alternate, and serrated pathways of colorectal carcinogenesis.[70] The study found that whilst the traditional pathway (MSS, CIMP-negative, BRAF-negative, and KRAS-negative) was found in 33.4% of cases, the alternate pathway (MSS, CIMP-low, BRAF-negative, and KRAS-positive) was responsible for 11.6% and the serrated pathway (any MSI, CIMP-positive, BRAF-positive, and KRAS-negative) for 0.8% of CRC cases. Approximately 54.2% of cases was unassigned to any group. Stage III CRC, moderately differentiated and left-sided tumours were more significantly found in the traditional and alternate pathways than in the unassigned group. The difference in outcome among the different colorectal carcinogenesis pathways did not reach statistical significance.

The consensus molecular subtypes (CMS) of CRC were described by the CRC subtyping consortium (CRCSC) to include 4 subgroups of CRC, CMS1-CMS4.[88] CMS1 CRCs make up 14% of the CMS of CRC, and are more significantly hypermutated MSI tumours having immune activation signatures, low somatic copy number alterations (SCNA), significantly high rates of BRAF mutations, and receptor tyrosine kinase activation. CMS2 tumours comprise 37% of CRCs and have chromosomal instability (high SCNA with copy number gains and losses) with marked WNT and MYC activation, and consistent enrichment in HNF4A amplification, that are not present in other genetic loci. Approximately 15% of the CMS CRC belong to the CMS3 subtype, which is characterised by tumours with metabolic dysregulation, low SCNA count, higher CIMP-low cluster, high hypermutated tumour cluster, significantly high rates of KRAS mutations, and MAPK pathway activation. CMS4 tumours, which comprise 23% of CRCs, are designated as mesenchymal based on prominent TGFB activation, stromal invasion, and angiogenesis signatures. In addition, the CMS1 tumours are more frequently associated with females, have right-sided location and higher tumour grades, whereas the CMS2 CRCs are left-sided lesions. The CMS4 CRCs are most commonly diagnosed in stages III and IV. Moreover, the patients with the CMS4 tumours have worse overall and relapse-free survivals, whereas the CMS2 patients have better outcomes post-relapse, while the CMS1 patients are characterised by dismal prognosis post-relapse.[88]

Clinical applications of molecular pathology of CRC. Currently, approximately 13 genetic markers, including MLH1, PMS2, MSH2, EPCAM, MSH6, APC, MUTYH, BRAF, KRAS, NRAS, MSI, MLH1 methylation, and HER2, have clinical use in CRC management.[10,18,89-91] The clinical relevancies of these markers are for the identification of inherited CRC syndromes, molecular subtyping of CRC, prognostication of disease, and prediction of response to therapy.[10,18,89-91] Microsatellite instability, somatic BRAF mutation, MLH1 methylation, and germline alterations of MLH1, PMS2, MSH2, MSH6, APC, MUTYH, and EPCAM are clinically useful for the diagnosis of LS and FAP.[10,15,18] Microsatellite instability, somatic BRAF, KRAS, NRAS mutations, and HER2 amplification have therapeutic response prediction values.[18,89-94] The 2021-2022 UK National Genomic Test Directory for Cancer lists approximately 7 clinically actionable gene targets for sporadic CRC.[91] Up to 11 drugs are currently approved by the FDA for targeted CRC therapy and 7 by the National Institute for Health and Care Excellence for the same cancer.[92-96] Companion molecular diagnostic assays are available for mutations in KRAS (cetuximab and panitumumab), NRAS (cetuximab and panitumumab), BRAF (encorafenib), MSI (pembrolizumab, ipilimumab, and nivolumab), NTRK (NTRK inhibitors), HER2 (tucatinib and trastuzumab), and DPYD (fluoropyrimidine).[92-95] However, in the current 2018 Saudi National Cancer Centre Colorectal Cancer Clinical Guidelines only RAS (KRAS and NRAS, presumably) is mentioned as the genetic marker for clinical management overall.[97] RAS mutation testing is recommended to identify the patients that would benefit from panitumumab, an anti-EGFR therapy.[97] All other therapeutically relevant markers have yet to be considered for clinical utility in KSA. Furthermore, diagnostic markers of hereditary cancer syndromes, such as APC, MUTYH, BRAF, HER2, KRAS, NRAS, MSI, MLH1 methylation, MLH1, PMS2, MSH2, and MSH6 mutation are not routinely used in the management of presumed hereditary CRC, and are not yet captured in the 2018 guidelines for CRC management.[25,26,97]

In conclusion, the depth and width of molecular pathology research on CRC that has emanated from Saudi Arabia has not been in tandem with the urgency of calls made by researchers for the health authorities to expedite actions to stem the rising incidence of CRC

in the Saudi population.[3-6] The prevalence rates found for genetic mutations in the Saudi CRC population, as well as the clinical correlates for these mutations have varied widely. The reasons for this variability are immediately evident from the small numbers of per-gene(s) studies, which are predominantly hospital-based and small-sized, to the varied methodologies that were applied in the studies. The paucity of CRC molecular pathology data from Saudi patients is revealed in the inability of the Saudi National Cancer Centre to develop a comprehensive biomarker-based CRC management guidelines that reflects personalised or precision medicine.[7,8] Large multicentre and multiregional translational studies which utilise multiple methodologies are therefore warranted to define the prevalence rates and the clinicopathological significances of genetic mutations in the Saudi CRC population. The findings from these translational studies should clarify CRC biology in Saudis, form the bases for diagnostic and risk-stratification guidelines, and inform the strategies to pursue subsequent large-scale clinical trials for the evaluation of the efficacy of targeted therapeutics in Saudi CRC patients.

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