EXPLORING NOVEL CANCER-ASSOCIATED FIBROBLAST MARKERS OF COLORECTAL TUMOUR MICROENVIRONMENT IN DIFFERENTIATING THE MECHANISMS OF RIGHT-SIDED AND LEFT-SIDED COLORECTAL CANCER

SAHIRA SYAMIMI BINTI AHMAD ZAWAWI

UNIVERSITI SAINS MALAYSIA

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by

SAHIRA SYAMIMI BINTI AHMAD ZAWAWI

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	degree Celcius
µg	microgram
µl	microlitre
mm	millimitre
ng	nanogram
%	percent
α– SMA	α-smooth muscle actin
ACTA2	actin alpha 2
ACTN1	alpha-actinin-1
ADAR1	adenosine deaminase RNA specific 1
ANTXR1	anthrax toxin receptor 1
AOC3	amine oxidase copper containing 3
AP-1	activator protein 1
APC	adenomatous polyposis coli
AREG	amphiregulin
ASCs	adult stem cells
BCA	bicinchoninic acid colorimetric
BGN	biglycan
BME	basement membrane extract
BMI	body mass index
BMP	bone morphogenetic protein
BRAF	B-Raf proto-oncogene
Buffer RLT	Lysis buffer
Buffer RPE	Washing buffer
Buffer RW1	Washing buffer
CA19-9	carbohydrate Antigen 19–9
CAFs	cancer-associated fibroblasts
CALD1	caldesmon 1
CAV-1	Caveolin-1

CCL	chemokine (C-C motif) ligands
CEA	carcinoembryonic antigen
CIMP	CpG island methylation pathway
CIMP-H	CIMP-high
CIMP-L	CIMP-low
CIN	chromosomal instability
C1S	complement component 1 s
CLEC3B	C-Type Lectin Domain Family 3 Member B
CM	conditioned medium
COL1A1	collagen type 1 alpha chain
COL1A2	collagen type 1 alpha 2 chain
COL11A1	collagen type XI alpha 1
COL12A1	collagen type XII alpha 1 chain
COX2	cyclooxygenase-2
Cre	cyclic recombinase
CRLM	colorectal liver metastasis
CT	computed tomography
CXCL14	chemokine (C-X-C motif) ligand 14
CXCR2	C-X-C motif chemokine receptor 2
DAPI	4′,6-diamidino-2-phenylindole hydrochloride
DCN	decorin
DEGs	differentially expressed genes
DFS	disease-free survival
DMEM	Dulbecco's Modified Eagle Medium
dMMR	dysregulation of DNA mismatch repair
DMSO	dimethyl-sulfoxide
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EHS	Engelbreth-Holm-Swarm
EMT	epithelial-mesenchymal transition
EpCAM	epithelial cellular adhesion molecule

EREG	epiregulin
ERK	extracellular signal-regulated kinase
FAP	familial adenomatous polyposis
FBS	fetal bovine serum
FDR	false discovery rate
FGF	fibroblast growth factor
FIT	fecal immunochemical test
FLRT3	fibronectin leucine-rich transmembrane protein 3
FSP1	fibroblast specific protein-1
FOBT	fecal occult blood test
FOLFIRI	folinic acid, 5-FU, irinotecan
FOLFOX	5-FU, folinic acid, and OXA
5-FU	5-fluorouracil
GREM1	gremlin1
GTPase	guanosine triphosphatase
H&E	haematoxylin and eosin
H3K36	methylation at histone 3, lysine 36
HAT	histone acetyltransferase
HDACs	histone deacetylases
HDMs	histone demethylases
HER2	human epidermal growth factor receptor 2
HIF-1α	hypoxia-inducible factor-1 alpha
HNPP	hereditary non-polyposis colorectal cancer
HPUSM	Hospital Pakar Universiti Sains Malaysia
HPE	histopathological examination
HREC	Human Research Ethics Committee USM
HRP	horseradish peroxidase
IAF	inflammatory fibroblast
IBD	inflammatory bowel disease
iCAFs	inflammatory CAFs
ICI	immune checkpoints inhibitors
IF	immunofluorescence

IGF	insulin growth factor
IGF-1	insulin growth factor-1
IGF-1R-IRS	insulin-growth factor 1 receptor- insulin receptor substrate
ILs	interleukins
IL6	Interleukin 6
JAK	janus kinase
JEPem-USM	Jawatankuasa Etika Penyelidikan Manusia
JNK	c Jun-N terminal kinase
kDa	kilodalton
KRAS	Kirsten Rat Sarcoma
KRT18	keratin 18
LAMP5	lysosomal associated membrane protein family member 5
LC	CAFs from left-sided CRC (cancer)
LCRC	left-sided CRC
LENG8	leukocyte receptor cluster member 8
LN	normal fibroblasts from left-sided colon (normal)
lncRNAs	long ncRNAs
LUM	luminan
LRG1	leucine-rich repeat-containing G protein-coupled receptor 1
LRRC17	leucine-rich repeat-containing 17
MAIT	mucosal-associated invariant T
MAPK	mitogen-activated protein kinase
miR	microRNA
ml	millilitre
MLH1	MutL homolog 1
MHC1	major histocompatibility complex 1
mm	millimeter
MMP2	matrix metalloproteinases 2
MMR	mismatch repair
M-PER	mammalian protein extraction reagent
MSCs	mesenchymal stem cells
MSI	microsatellite instability

MSI-H	MSI-high
MSI-L	MSI-low
MSH2	MutS homolog 2
MTT	3-4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide
MYC	myelocytoma
MyoCAFs	myofibroblast-like CAFs
NADPH	nicotinamide adenine dinucleotide phosphate
NAMPT	nicotinamide phosphoribosyltransferase
ncRNAs	non-coding RNAs
NFs	non-activated or normal fibroblasts
NK	natural killer
NOCs	N-nitroso compounds
nTPM	normalised transcript expression per million
OD	optical density
OS	overall survival
OXA	oxaliplatin
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PDAC	pancreatic ductal adenocarcinoma
PD-1	programmed cell death 1
PDGFR	platelet-derived growth factor
PDGFR-β	platelet-derived growth factor receptor-β
PI3K	phosphatidylinositol 3-kinase
PIK3CA	phosphatidylinositol-4,5-bisphosphate,3-kinase catalytic subunit alpha
PFS	progression-free survival
PLA2G4A/AA	phospholipase a2-IVa/arachidonic acid
PMS6	postmeiotic segregation increased 6
POLR2J3	polymerase (RNA) II polypeptide J3
PRMT	protein methyltransferases
PTEN	phosphatase and tensin homolog deleted on chromosome ten
PVDF	polyvinylidene difluoride

RAS	Rat Sarcoma
RBPs	RNA binding proteins
RBPMS	ribosomal protein with multiple splicing
RC	CAFs from right-sided CRC (cancer)
RCRC	right-sided CRC
RFS	recurrence-free survival
RN	normal fibroblasts from right-sided colon tissue (normal)
RNF43	ring finger protein 43
SD	standard deviation
SDC1	syndecan 1
SEER	Surveillance, Epidemiology, and End Results Program
SEM	standard error mean
SES	socioeconomic status
SF	serum-free
SHH	Sonic Hedgehog
SMAD	Suppressor of Mothers against Decapentaplegic
SPARC	secreted protein acidic and cysteine rich
STAT3	Signal Transducer and Activator of Transcription 3
sVCAM-1	Circulating Vascular Cell Adhesion Molecule-1
TAM	tumour associated macrophages
Tcf	T-cell factor
TILs	tumour infiltrating lymphocytes
TIMP	Tissue inhibition of metalloproteinases
TME	tumour microenvironment
TNFRS11B	tumour necrosis factor receptor superfamily, member 11b
TNM	tumour-node metastasis
TGFβ	transforming growth factor-β
TGN	transgelin
TP53	tumour protein p53
TRAIL	tumour necrosis factor–related apoptosis-inducing ligand
Treg	regulatory T cells
TSGs	tumour suppressor genes

TWIST1	TWIST-related protein 1
UC	ulcerative colitis
VAP-1	vascular adhesion protein-1
VEGF	vascular endothelial growth factor
VIM	vimentin
WHO	World Health Organization
Wnt	Wingless-related integration site
XELOX	5-FU and OXA
ZEB1	zinc finger E-box binding homeobox 1

LIST OF APPENDICES

MENEROKA PENANDA FIBROBLAS BERKAITAN KANSER YANG BAHARU DALAM PERSEKITARAN MIKRO TUMOR KOLOREKTAL BAGI MEMBEZAKAN MEKANISME KANSER KOLOREKTAL SEBELAH KANAN DAN SEBELAH KIRI

ABSTRAK

Kanser kolorektal (KK) adalah penyakit yang heterogen, sering diklasifikasikan kepada KK sebelah kanan (KKKanan) dan KK sebelah kiri (KKKiri), yang berbeza dalam pelbagai aspek, termasuk histologi dan molekul. Persekitaran mikro kanser (PMT) dan fibroblas berkaitan kanser (FBK) yang merupakan komponen selular utama PMT, menyumbang kepada karsinogenesis. Pengumpulan FBK mencerminkan prognosis yang buruk dalam pesakit KK. Oleh kerana peranannya yang penting, FBK sedang dikaji sebagai sasaran utama untuk terapi kanser. Berbeza dengan laporan sebelumnya di mana KKKanan dan KKKiri diklasifikasikan berdasarkan profil mutasi sel kanser epitelial atau histologi, pengaruh FBK dalam membezakan mekanisme kedua-dua entiti KK ini masih belum diterokai. Kajian ini bertujuan meneroka penanda FBK dalam membezakan mekanisme KKKanan dan KKKiri. FBK yang diperoleh daripada FBK dan fibroblas tidak aktif (NFs) yang diperoleh daripada tisu kolon normal bersebelahan telah ditubuhkan. Untuk pengelasan fenotip fibroblas, pewarnaan imunofluoresen (IF) bagi amine oxidase copper containing 3 (AOC3), leucine-rich repeat-containing 17 (LRRC17), dan alpha smooth muscle actin (α-SMA) telah dijalankan dan morfologi fibroblas telah direkod. Fenotip kontraktil dan proliferasi fibroblas dibandingkan melalui pengujian kontraksi kolagen dan 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).

Profil gen sel-sel fibroblas dianalisis melalui analisis mikroarray (Clariom S (Human) assay) untuk menyaring gen yang diekspresikan secara berbeza (DEGs). Biopenanda epitelium, epithelial cellular adhesion molecule (EpCAM) juga disertakan. Sel epitelium KK (SW620) dan kanser serviks epitelium (C33A) dimasukkan sebagai kawalan. Fibroblas yang dirawat dengan TGFβ (10ng/ml) menunjukkan penurunan yang ketara dalam AOC3, peningkatan LRRC17, dan α-SMA, bersama dengan peningkatan kontraktiliti, berbanding dengan medium tanpa serum ($p<0.05$). Medium terkondisi (CM) yang diperolehi daripada fibroblas meningkatkan proliferasi sel SW620. FBK daripada KKKanan (RC) menunjukkan fenotip keradangan yang ketara dengan ekspresi biopenanda kaya kemokin, manakala FBK daripada KKKiri (LC) menunjukkan fenotip FBK miofibroblastik (myoFBK) dengan ekspresi biopenanda myoFBK. Ekspresi RNA binding protein with multiple splicing (*RBPMS*) meningkat dengan ketara dalam fibroblas, dengan corak ekspresi yang berbeza antara fibroblas daripada sisi kolon yang berbeza melalui mikroarray ($p<0.05$), dan disahkan melalui *western blot*. Selain itu, TGFβ1 meningkatkan ekspresi RBPMS dalam LC tetapi menekan ekspresi RBPMS dalam RC. Ini adalah kajian pertama yang meneroka peranan FBK dalam KK berdasarkan sisi kolon. Pengawalan ekspresi RBPMS dalam FBK oleh TGFβ menjelaskan mekanisme yang berbeza antara KKKanan dan KKKiri. Kesimpulannya, RBPMS boleh berfungsi sebagai biopenanda FBK yang novel untuk pendekatan sasaran terhadap KK berdasarkan sisi kolon.

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ABSTRACT

Colorectal cancer (CRC) is a heterogeneous disease, often classified into right-sided CRC (RCRC) and left-sided CRC (LCRC), which differ in many ways, including their histological and molecular phenotypes. Tumour environment (TME) of the colon and cancer-associated fibroblasts (CAFs), the main cellular component of TME have been described to drive CRC progression. Accumulation of CAF reflect poor prognosis in CRC. Due to its vital role, CAFs are being studied as a prime target for cancer therapy. In contrast to previous reports in which RCRC and LCRC were classified based on the epithelial cancer cell mutational profiles or histology, CAFs influence in differentiating mechanism of these two CRC entities has yet to be discovered. This study aimed to explore novel CAF markers of colorectal TME in differentiating the mechanisms of RCRC and LCRC. CAFs derived from CRC and non-activated fibroblasts (NFs) derived from adjacent normal colon tissues were established. For fibroblast phenotypic characterisation, immunofluorescence (IF) staining of amine oxidase copper containing 3 (AOC3), leucine-rich repeat-containing 17 (LRRC17), and alpha smooth muscle actin (α-SMA) were performed and the fibroblasts morphology was recorded. The contractile and proliferation phenotype of the fibroblasts were compared through collagen contraction and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays. Their

gene profiles were analysed via microarray analysis (Clariom S (Human) assay) to screen differentially expressed genes (DEGs). Epithelial marker, epithelial cellular adhesion molecule (EpCAM) was included. Epithelial cell lines of CRC (SW620) and cervical cancer (C33A) were incorporated as controls. Fibroblasts treated with TGFβ (10ng/ml) showed significant AOC3 downregulation, LRRC17, and α-SMA upregulation, along with increased contractility, compared to serum free medium ($p<0.05$). Conditioned medium (CM) derived from fibroblasts promoted SW620 cells proliferation. CAFs from RCRC (RC) denote prominent inflammatory phenotype with chemokines-rich markers expression whereas, CAFs from LCRC (LC) demonstrate myofibroblastic CAFs (myoCAFs) phenotype with myoCAF markers expression. RNA-binding protein with multiple splicing (*RBPMS*) expression was significantly upregulated in fibroblasts, with distinct patterns of expression between fibroblasts from different colon sidedness as indicated by microarray ($p<0.05$), and validated via western blot. Additionally, TGFβ1 promoted RBPMS expression in LC but suppressed RBPMS expression in RC. This is the first study to explore the role of CAFs in CRC based on the colon sidedness. The regulation of RBPMS expression in CAFs by TGFβ elucidate the different mechanisms of RCRC and LCRC. In conclusion, RBPMS could serve as a novel CAFs marker for targeted approach against CRC based on its sidedness.

CHAPTER 1

INTRODUCTION

1.1 Research background

Colorectal cancer (CRC), a multistage of neoplasm characterised by colonic mucosal epithelia (Keum and Giovannucci, 2019), continues to be one of the highest incidences of cases reported and the third leading cause of cancer mortality worldwide (Siegel et al., 2021). In Malaysia, CRC ranks second after breast cancer with predominantly males and elders (Ministry of Health Malaysia, National Cancer Institute, 2023). Despite advances and widespread application of conventional therapy for CRC patients like adjuvant chemotherapy and radical surgery, the 5-year relative survival rate of CRC patients of advanced cases like metastatic CRC remain significantly low (Siegel et al., 2014; Siegel et al., 2021).

The characterisation of CRC differs considerably between right-sided CRC (RCRC) and left-sided CRC (LCRC) attributed to the differences in their embryological origin, histology, and clinicopathological phenotypes. RCRC that is midgut derivative covers the caecum, ascending colon, and proximal two-third transverse colon whereas, LCRC that is hindgut derivative includes the distal one-third of transverse colon, splenic flexure, descending colon, sigmoid colon, and rectum (Baran et al., 2018; Su et al., 2022). RCRC is typically characterised to have advanced tumours and poorly differentiated with sessile serrated adenomas or mucinous adenocarcinomas (Carethers, 2011; Bordaçahar et al., 2015). In contrast, LCRC, tend to have tubular, villous, and typical adenocarcinomas (Marzouk and Schofield, 2011). Further studies corroborated the manifestation of different types of aggressive phenotypes in RCRC in comparison to LCRC (Yahagi et al.,

2016; Petrelli et al., 2017; Hu et al., 2018). These pathological phenotypes or tumour behaviour variations may likely correspond to the molecular variations underlying between RCRC and LCRC. Accumulating evidence has revealed the molecular variations of the tumour-sidedness in CRC including higher rates of Kirsten Rat Sarcoma viral oncogene homolog (*KRAS*) and v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutations in RCRC compared to LCRC (Benedix et al., 2010; Nitsche et al., 2016). As such, RCRC and LCRC are different disease entities attributed to their differences in molecular and pathological features, highlighting the need of upscaling genomic profiling and a better understanding of CRC carcinogenesis.

Owing to its heterogenous molecular features, CRC can be further classified into four consensus molecular subtypes (CMSs), as such CMS4 describes the invasive and metastatic nature of CRC prominently mediated by the stromal components in the tumour microenvironment (TME) including cancer-associated fibroblasts (CAFs) (Guinney et al., 2015). CAFs, a dominant cell type in TME have been demonstrated to crosstalk with cancer cells through the secretion of protumourigenic cytokines and growth factors which have endowed CAFs with abilities in mediating the hallmarks of cancer; sustenance of proliferative signalling, evasion of cell death and growth suppressors, and promotion of metastasis and angiogenesis (Musa and Ali, 2020). Of note, the abundance of CAF is linked to the poor prognosis in CRC patients (Wikberg et al., 2013; Paulsson and Micke, 2014; Isella et al., 2015; Wu et al., 2016; Sahai et al., 2020). Investigation into CAFs may reveal the causal factors that derive the differences between RCRC and LCRC, and provide better insights toward tailoring future prognostic targeted approaches.

1.2 Historical background of CRC research

CRC is one of the solid tumours that still rely on the rigid-based system tumour classification with poorly defined tumour subtypes. The conventional tumour staging based on the tumour-node-metastases (TNM) system has been the basis for CRC classification for years. Almost all versions of the TNM system incorporated similar key principles adopted in Dukes staging system developed in 1932. Besides, CRC has been widely managed with different treatment regimens including surgery, chemoradiotherapy, and immunotherapy (Häfner and Debus, 2016; Werner and Heinemann, 2016).

Over the years, the malignant progression was viewed as a cancer-centric model, focusing on the intrinsic nature of epithelial cells turning malignant, with little to no emphasis on the immediate surroundings of such a complex disease (Jin and Jin, 2020). Henceforth, different angles have been taken by reshaping the notion that cancers are influenced by TME. TME components including CAFs and other components such as endothelial cells, immune cells, and extracellular matrix (ECM) generate the favourable microenvironment for cancer cell growth. Paget (1889) was the first to emphasise the important role of TME in cancer progression with the seed and soil theory. The theory denotes the pivotal interaction between cancer cells (seed) and TME (soil) in cancer progression. Ever since, accumulating evidence further supports and expands the theory with findings highlighting several molecules or mediators derived from TME cellular components that promote carcinogenesis (Lee et al., 2017).

Similar to other solid cancers, colorectal carcinogenesis is highly supported by TME which shapes the dynamic and heterogeneous nature of CRC as further dictated in its sidedness; RCRC and LCRC and hampers the effective management of this cancer (Baran et al., 2018). Throughout the years, the breakthrough of single-cell technologies

has enabled the investigation of single cell subpopulation in cancer including CAFs which ultimately provide a greater understanding of the cancer (Li et al., 2017).

1.3 Problem statement

Patients suffering from RCRC are shown less responsive to conventional therapies such as adjuvant chemotherapy and conferred with worse outcome different to that of LCRC (Nitsche et al., 2016; Baran et al., 2018). Whilst there have been reports on the lower incidences of RCRC, RCRC patients tend to have higher surgical complications, and poor prognosis compared to those with LCRC (Salem et al., 2020; Fernández et al., 2021). Consistently, Benedix et al. (2010) found a higher 5-year survival rate for LCRC (74%) than that of RCRC (73%). This reflects the considerable heterogeneity of CRC subjected to different anatomical regions by which remains a challenge to construe the underlying mechanisms. The complexity and heterogenous nature of CRC imposed a challenge to the current treatment. Henceforth, it leads to such urgency where novel molecular markers are needed to further provide insights on the underlying mechanisms specific to CRC sidedness.

CAFs have been shown to garner the attention of many for their interaction with malignant cells integral to carcinogenesis. Nevertheless, the correlation between CAFs, RCRC, and LCRC has passively been discussed over and by far yet reaching the clinical trial. This may be due to the lack of available specific and reliable markers for CAFs, taking into account the heterogeneous and complex nature of the CAFs population (Musa and Ali, 2020). Moreover, none of the previous studies related have systematically explored the role of CAFs with the highlighting nuances between RCRC and LCRC. Thus,

as the larger scale of evidence is highly in quest, this study has been focusing primely on the role of CAFs in differentiating the mechanisms of RCRC and LCRC.

1.4 Purpose of this study

The purpose of the study is to explore novel CAF markers of colorectal TME in differentiating the mechanisms of RCRC and LCRC. This study mainly involves primary fibroblasts that were first isolated from human samples of RCRC and LCRC, and normal adjacent colon tissues. The established primary fibroblasts were cultured and maintained throughout the study. This study focuses on the in-depth characterisation of primary fibroblasts based on their phenotypical, genomic, and proteomic aspects differentiating between RCRC and LCRC. Thus, this study is expected to bridge the gap of knowledge related to CAFs in RCRC and LCRC.

1.5 Research objectives

1.5.1 General objective

To explore novel CAF markers of colorectal tumour microenvironment in differentiating the mechanisms of RCRC and LCRC.

1.5.2 Specific objectives

1. To establish and characterise primary fibroblast cultures from cancer and normal adjacent colon tissues.
2. To analyse and compare gene expression profiles between CAFs from RCRC and LCRC tumours, and fibroblasts from normal adjacent colon tissues.

3. To validate and compare protein expression profile in CAFs from RCRC and LCRC tumours, and fibroblasts from normal adjacent colon tissues.

1.6 Hypothesis

RCRC and LCRC tumours are represented by differential gene expression signatures and protein markers which indicate differences in CAF phenotype, functional property, and molecular identity that will subsequently influence CRC progression.

CHAPTER 2

LITERATURE REVIEW

2.1 Fundamental of CRC

The term ‘cancer’, ‘malignant tumour’ or ‘neoplasm’ is broadly used to describe the uncontrolled or abnormal growth or proliferation of epithelial cells (Cooper and Hausman, 2007). According to the World Health Organization (WHO), the notion that cancer as a large group of disease originating from any organ is highlighted (World Health Organization, 2024). Given the advent of technology toward a greater understanding of cancer, cancer has been concurrently defined and conformed to the hallmarks of cancer constituted (Hanahan and Weinberg, 2011). The hallmarks include several fundamental principles underlying cancer such as genomic instability, uncontrolled proliferation, activating invasion and metastasis, inducing angiogenesis, and immune evasion (Hanahan and Weinberg, 2011; Hanahan, 2022). This framework also represents the underlying complex malignant neoplasm of the human colon, commonly known as CRC.

By anatomical definition, CRC is the neoplasm characterisation of cells originating from colonic mucosal epithelia and detected in either part of colorectal; proximal colon, distal colon, or rectum (Keum and Giovannucci, 2019). CRC represents a heterogeneous and complex disease due to considerable heterogeneity within tumours (intratumour heterogeneity) and between different individuals (intertumour heterogeneity) (Zheng et al., 2020). The most common types of CRC are adenocarcinoma (>90%) and mucinous adenocarcinoma (20%) (Alzahrani et al., 2021). CRC are mostly sporadic with the remaining CRC cases arise from hereditary contribution (Yamagishi et al., 2016). CRC is a multifactorial disease as explained in the later paragraphs. Colorectal carcinogenesis

preferentially involves the sequential accumulation of various genetic mutations and epigenetic alterations during the progression of a normal colonic crypt to pre-cancerous adenoma, carcinoma, and later invasive cancer (Xi and Xu, 2021).

2.1.1 Epidemiology of CRC

According to GLOBOCAN 2020, CRC is the third leading cancer incidence affecting both males and females worldwide (Sung et al., 2021). By 2040, CRC prevalence is predicted to 3.2 million new CRC cases globally with a marked disparity across ages and a decline in healthy lifestyle and human development (Xi and Xu, 2021). Aside from the fact that CRC is a frequent disease in the elderly, the cases seem to increase in younger population below 50 years old (Siegel et al., 2017). Alarmingly, CRC accounts as the second highest for cancer-related mortality, especially in men of younger populations (Siegel et al., 2023). However, worth to note the positive decline has been documented in age-standardised CRC incidence rate among elders of above 50 years. This indicates improved patients' compliance to early-stage diagnosis and is possibly attributed to their changing lifestyle (Siegel et al., 2023).

In Malaysia, CRC ranks second (14.1% cases) after breast (17.6%) for the most frequently diagnosed cancer and fourth for the most cancer mortality by which in men account higher than women with the respective age-standardised rates; (18.8/100,000) and (13.7/100,000) (Ministry of Health Malaysia, National Cancer Institute, 2023). It has been reported that the leading cases in Malaysia were represented by Chinese (27.35), followed by Malay (18.5), and Indian (17.55) (Abu Hassan et al., 2016). In the period of 2012-2016, Malaysia recorded the rising trend of incidence with an alarming rate of CRC patients

diagnosed late at the advanced stages (stage III and IV) from 58.7 % to 63.7 % (Azizah et al., 2019).

The CRC prevalence remains high in the developing and high-income countries especially within the regions of Asia, Eastern Europe, and South America (Arnold et al., 2017; Center et al., 2009). There is a causal relationship between the CRC incidence and developing countries in which higher CRC prevalence increased morbidity and mortality, linking cancer with increasing development. Whilst, some high developing countries have showed declines in prevalence and mortality rates which are possibly attributed to their prominent socioeconomic transition and continued efforts of early screening program (Arnold et al., 2017).

Despite the advent of current diagnostic and therapy, most CRC cases are still diagnosed at later and advanced stages. This shortcoming is deemed related with limited resources for improved endoscopic technology (Vega et al., 2015). Patients with CRC often suffer from less effective treatments with detrimental side effects such as diarrhoea and significant, unexplained weight loss (Negarandeh et al., 2020). Tumour recurrence is typically noted in 25% of patients with stage II and III CRC and become metastatic within 5 years (Renouf et al., 2013; Sung et al., 2021). These ultimately result in significant increase of morbidity and mortality among CRC cases.

2.1.2 Risk factors of CRC

The multifactorial CRC describes several unmodifiable and modifiable risk factors of the onset of cancer. These risk factors are further described below.

2.1.2 (a) Non-modifiable factors

Age is one of the primary unmodifiable risk factors for CRC, with cases being rare in individuals under 50 (Kuipers et al., 2015). Most CRC diagnoses occur in older adults, with a mean age around 70 years (Bray et al., 2018). Although the exact mechanism remains unclear, ageing and chronic inflammation appear to be interrelated in promoting CRC development. The term 'inflammaging' refers to chronic, age-related inflammation that may foster neoplasia and contribute to tumour progression (Bottazzi et al., 2018). The relative survival rates of CRC patients decrease with increasing age and this may be correlated with the adding of comorbidities and less efficient treatments (Siegel et al., 2021). With regards to tumour biology, senescent cells likely accumulate with age thereby promote the CRC development and metastatic outgrowth (Pretzsch et al., 2022).

By gender, CRC tends to develop more in men compared to women, with men also experiencing higher mortality rates and poorer prognosis. Higher rate of rectal cancer was shown in men than women (Demb et al., 2019; Janati et al., 2022). These differences could be attributed to variation in the lifestyles and diets which co-exist and interact with genetic factors. Also, it was reported that the lesser CRC incidence in women relative to men in elderly may be related to the use of estrogen and progestins in hormone replacement therapy among postmenopausal women (Barzi et al., 2013; Topi et al., 2017). Estrogen is thought to decrease risk of CRC and progestins add on the estrogenic effect. However, the underlying mechanisms of these gender-specific differences remain unclear.

The risk for developing CRC doubles in the individuals who have affected family members with CRC or inflammatory bowel disease (IBD). Majority of CRC cases occur sporadically while hereditary factors are implicated in 10-30% of CRC patients (Grady and Markowitz, 2015; Yamagishi et al., 2016). Patients with a history of CRC in first-

degree family are more likely to develop CRC especially the early-onset CRC, compared to those with no family history of CRC (Janati et al., 2022). In addition, individuals who have been affected with other cancers such as ovarian, breast, and pancreatic cancer also presented with an increased risk of CRC. Other than that, inherited CRC shows site-specific to anatomical colon as in Korean population, where most CRC patients, both men and women with a family history of cancer are distal colon cancer (Shin et al., 2011).

Furthermore, 2-8% of CRC is identified as inherited CRC syndromes which commonly cover autosomal dominant diseases (Hossain et al., 2022). This highlights hereditary non-polyposis colorectal cancer (HNPP), and familial adenomatous polyposis (FAP). HNPP, also known as Lynch syndrome, is associated with one of the classic CRC phenotypes, microsatellite instability (MSI) that leads to CRC. Lynch syndrome that is caused by MSI accounts for up to 60% of lifetime risk of CRC (Rasool et al., 2014; Samadder et al., 2015). Lynch syndrome patients with MutS homolog 2 (*MSH2*) and MutL homolog 1 (*MLH1*) mutations (70%) (common mismatch repair (MMR) genes in MSI) are at a higher risk of developing CRC than those with either *MSH6* or postmeiotic segregation increased 6 (*PMS6*) mutations (Battaglin et al., 2018). The mechanism underlying this phenotype is described in the later subheading. For those with FAP, they are described to be affected with successive multiple colonic polyp development in their mid-teens which then turn into carcinoma. It is estimated that those with untreated FAP have increased risk of CRC (Yang et al., 2020).

Additionally, IBD which consists of ulcerative colitis (UC) and Crohn's disease, is one of the chronic inherited conditions that carry almost triple risk of CRC compared to those with no IBD (Johnson et al., 2013). Chronic mucosal inflammation signified by long-standing and untreated IBD is strongly related to patient predisposition to increasing

risk of CRC. Another factor that can contribute to CRC include individuals with type 2 diabetes mellitus (Schäfer and Werner, 2008). The development of CRC is thought to be of greater risk in these diabetic patients compared to non-diabetic (Peeters et al., 2015). This may be attributed to hyperinsulinemia and the continued secretion of insulin-growth factors (IGFs) in cancer cells which function to increase cell proliferation and decrease cell apoptosis (Yao et al., 2014; Pang et al., 2018).

2.1.2(b) Modifiable factors

Prolonged sedentary behaviour can predispose to CRC and increase risk of CRC-specific mortality (Campbell et al., 2013). The inverse association to CRC risk is documented in people with active lifestyle, increased occupational activity, and decreased sitting hours (Eyl et al., 2018). The sedentary behaviours are demonstrated in cities populated with higher socioeconomic status (SES), hence higher risk of CRC than that of population with poor SES (Eyl et al., 2018). People living in the former condition are more likely to be physically inactive given the continual adoption of high technologies embracing the ideal aspects of low human energy consumption and high comfort (Koc et al., 2016). Though, this view has been mixed as some suggested that people with low SES exhibit higher incidence of CRC, than people with higher SES. The reasons could be multifactorial which include the poor lifestyle with lack of regular exercise and limited access to both health education and healthcare services (Thélin and Sikka, 2015; Carethers and Doubeni, 2020). These could affect metabolic hormones, increase in body fat mass, and as result, leads to obesity which taken together may promote the CRC initiation and progression (Jurdana, 2021).

Additionally, the aftermath of physical inactivity is primarily obesity which can lead to a greater risk of CRC (Rawla et al., 2019). Physical inactivity has been characterised with low energy expenditure and contribute to weight gain. Previous meta-analysis study has shown an overall increased risk of CRC for every 5 units rise in body mass index (BMI) of 18% which particularly affected tumours of colon (Ning et al., 2010). Obesity accounts for 50% and 20% relative risk of CRC in men and women, respectively in comparison to people with normal BMI. This relationship between obesity and CRC appears to be rooted from several factors underlying which include metabolic dysregulation and oxidative stress (Renehan et al., 2008; Lynch and Leitzmann, 2017). Adipocytokines including circulating leptin, adiponectin, and pro-inflammatory cytokines such as interleukins (ILs) (e.g., IL1 and IL6) are higher in obese people than non-obese, generating inflammatory environment conducive for CRC progression (Carr et al., 2018; Sawicki et al., 2021).

Numerous evidence has pointed out that tobacco smoking escalates risk estimates for CRC incidence and mortality (Botteri et al., 2008; Parajuli et al., 2013). In the early-onset CRC, smoking is related to a greater risk for advanced adenoma formation and transition into carcinoma (Zhao et al., 2018; O'Sullivan et al., 2022).

Furthermore, dietary factors have been considerably associated with CRC. It is established that having a healthy dietary pattern consisting of wholegrains, soy, fruits, and vegetables ameliorates risk of CRC (Ramadas and Kandiah, 2009; Godos et al., 2016). In contrast, dietary patterns enriched with processed and red meats, high sugar, and salty foods are increasingly linked to development of colorectal adenomas. Based on previous systematic review and meta-analysis studies, there were positive association between red meat consumption and increased colorectal adenomas incidence (Aune et al., 2013; Zhao

et al., 2018). Heme iron and n-nitroso (NOCs) compounds in red and processed meats which are carcinogenic may cause cytotoxic damage on the colonic epithelial crypts, exposing to oxidative stress and subsequently lead to CRC (Sasso and Latella, 2018). Another study also suggested that diets rich in sugar such as fructose promote angiogenesis as increased level of sVCAM-1, a biomarker for angiogenesis, lead to CRC (Stewart et al., 2022).

It is studied that the combination of these modifiable factors can elevate risk of CRC to a greater extent than one single factor (Yu et al., 2022). Some suggested the pairwise combined effects between smoking and obesity increased the risk of CRC (Gong et al., 2012; Roos et al., 2022; Yu et al., 2022). Contrarywise, several healthy lifestyle factors including regular physical exercise, non-smoking, and healthy diet were observed positively associated with significant low risk of colorectal adenoma (Fedirko et al., 2014). Another risk factor of CRC, alcohol consumption increased risk of CRC among smokers, highlighting the addictive effects from these pairwise combination factors (Viner et al., 2019). Gut and intestinal microbiota were also recognised as emerging factors and susceptible to unhealthy diet, leading to colorectal carcinogenesis (Hossain et al., 2022). For example, *Escherichia coli* and *Bacteroides fragilis* in dysbiosis can result in chronic inflammation and further induce CRC carcinogenesis (Kuipers et al., 2015). Further description of the microbiota distribution in the colon with respect to its anatomical location is included in the later paragraph.

2.1.3 Aetiology and Molecular Pathway of CRC

CRC is a multi-gene, multi-step, multi-mechanistic, and multi-stage process that can be dictated into different CRC subtypes with different clinical and molecular

phenotypes. The gradual accumulation of genetic and epigenetic alterations underlies the colorectal carcinogenesis which describes the transformation of normal colonic crypt to an aberrant crypt and then adenoma-carcinoma (primary cancer) development (Figure 2.1) (Xi and Xu, 2021). Genomic instability stands as one of the major hallmarks in the onset of colorectal carcinogenesis, leading to the activation of oncogenes and inactivation of tumour suppressor genes (TSGs). Common mutated oncogenes in colorectal carcinogenesis include *KRAS*, *BRAF*, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (*PIK3CA*), phosphatase and tensin homolog deleted on chromosome ten (*PTEN*), and small mothers against decapentaplegic (*SMAD*) 2 and 4 which in turn affect common signalling pathway such as mitogen-activated protein kinase (MAPK) and tumour protein p53 (*TP53*) (Morkel et al., 2015; Chandrasinghe et al., 2018; Salvatore et al., 2019). *KRAS* oncogene homolog encodes KRAS protein which is implicated with MAPK signalling pathway via epidermal growth factor receptor (*EGFR*) activation and thus reduce guanosine triphosphatase (GTPase) activity. *KRAS* mutation is often observed to occur at codons 12 and 13 in exon of 2 of the *KRAS* gene and during preceding tumorigenesis which contribute hyperproliferation (Fearon and Vogelstein, 1990; Janssen et al., 2022).

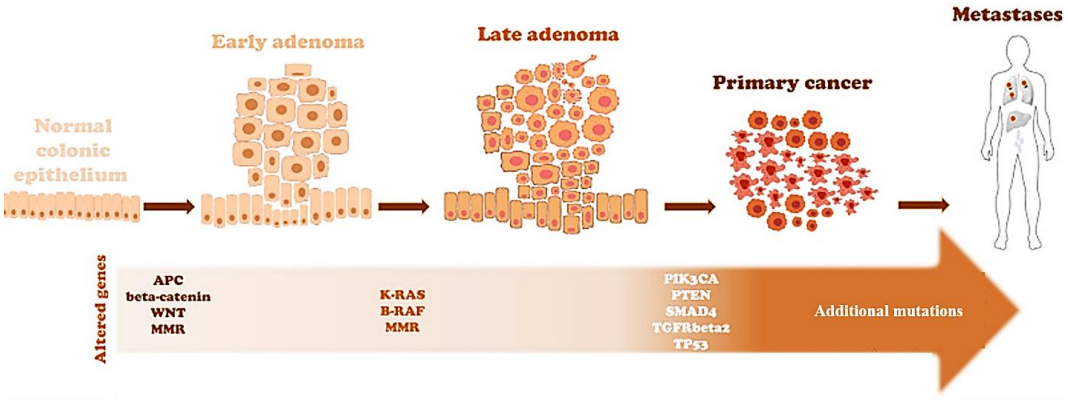

Figure 2.1 Colorectal carcinogenesis from normal colonic epithelium to metastasis with common mutated genes (Ottaiano et al., 2021).

There are three different key pathways leading to the manifestation of CRC namely, chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylation pathway (CIMP). CIN pathway, also known as the classical pathway, describes almost 80% of sporadic CRC, characterised by aneuploidy and frequent loss of heterozygosity at tumour with typical mutations in TSGs such as adenomatous polyposis coli (*APC*) and followed by *KRAS*, *PI3K*, and *TP53* mutations (Fearon and Vogelstein, 1990). CIN phenotype often presents with poor survival and outcome. Early on, Fearon and Vogelstein (1990) proposed the multi-step genetic model of colorectal carcinogenesis which describes multiple genetic events that occur in an ordered or stepwise series. *APC* mutations enable constitutive inactivation of β-catenin/T-cell factor (Tcf)-mediated transcription important in early CRC development and invasion. This is followed by *KRAS* and *PI3K* mutations which results in larger adenomas (polyps) and carcinoma development, respectively. Subsequently, loss-of-function mutations in *TP53* has been regarded for its role in adenoma-carcinoma development, which encodes p53, guardian of the genome, driving the transcription of multiple genes implicated in cell cycle regulation, senescence, differentiation, and migration (Pino and Chung, 2010).

Approximately 15% of all CRCs display MSI instability. MSI refers to the molecular phenotype due to dysregulation of DNA mismatch repair (dMMR) in the short tandem repeat DNA sequences, also known as microsatellite (De'angelis et al., 2018). Majority of MSI tumours are sporadic, caused by epigenetic silencing of *MLH1* via hypermethylation which associated with CIMP. In addition, MSI causes Lynch syndrome via epigenetic silencing of *MSH2* or *MLH1*, one of the major MMR genes responsible for destabilization of microsatellites (Battaglin et al., 2018). These dMMR genes impair DNA repairs, resulting in the accumulation of DNA replication errors and MSI phenotype, characterised by the prominent frameshift mutations in microsatellite DNA (Hampel et al., 2005; De'angelis et al., 2018). Notably, MSI has been recognised for its distinct phenotypes including poor differentiation, right-sided colon location, and synchronous metastases into lymph nodes and peritoneum (Battaglin et al., 2018). CRC patients with MSI are often to exhibit resistance to adjuvant chemotherapy different to that of CRC with CIN, as evidenced by its poor outcome in response to 5-fluorouracil treatment. Investigation into MSI as biomarker for improved prognostication is included which classify three groups including MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stable (MSS). For example, early-stage colorectal adenomas that are often observed with MSI-H/dMMR is associated with tumour immunity and thus better response to immune checkpoints inhibitors (ICI) compared to MSI-L group (Lin et al., 2020). This per group comparison based on MSI status informs the prognostic difference between MSI-H and MSI-L groups in receiving ICI treatment (Ganesh et al., 2019; Lin et al., 2020). Accordingly, patients with MSS had high tumour infiltrating lymphocytes (TILs) compared to MSI-L, hence associated with better prognosis (Dahlin et al., 2011).

Nevertheless, findings were inconsistent and thus remain poorly justified for the prognostic significance of MSI in CRC (Samowitz et al., 2006; Kim et al., 2007).

CIMP tumour is another subgroup or feature of CRC that defines hypermethylation of tumour related genes or TSGs located in the promoter of CpG island sites at the promoter region. The aberrant methylation of cytosine bases in CG-rich sequences (CpG island) is associated with epigenetic transcriptional inactivation, loss of function, and silencing of TSGs resulting in carcinogenesis (Lao and Grady, 2011). CIMP tumours can be classified into CIMP-high (CIMP-H) and CIMP-low (CIMP-L) based on the methylation level. Most CIMP tumours are MSI tumours with either high *KRAS* or *BRAF* mutations (Shen et al., 2015). There are also CIMP in serrated polyps which exhibit $BRAF^{v600E}$ mutations. Thus, it is worthwhile to note that CIMP CRC tends to present similar clinicopathological phenotype to that CRC with MSI which include right-sided colon location, mucinous tumours, and poor differentiation.

These distinct pathways constitute a multi-mechanistic CRC progression. It is clearly understood that there are multiple molecular pathways to develop CRC which prominently involve both genetic and epigenetic alterations. Importantly, these pathways are mutually exclusive hence tumours tend to show overlapping pathway phenotypes as manifested in most CIMP tumours which also harbour MSI phenotypes. Another example is in the case of premalignant polyps such as serrated polyps where they develop via serrated pathway and are associated with MAPK pathway activation from either *KRAS* or *BRAF* mutations and CIMP mutations (Schmitt and Greten, 2021). Tubular adenomas also present overlapping phenotypes with *APC* inactivation and other genetic alterations from CIN.

CRC can be classified using conventional method such as TNM staging as further discussed in the later section. More recent classification using transcriptomic profiling, CRC can be further classified into four CMSs: CMS1 (MSI immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal) (Guinney et al., 2015; Rejali et al., 2023). With respect to genomic changes, CMS1 tumours are defined with hypermethylation status while the rest of CMSs tumours exhibit higher CIN. These different molecular subtypes are said to reflect different signalling pathways. CMS1 is enriched for signatures of immune infiltration (Guinney et al., 2015). Conversely, CMS2 is associated with Wingless-related integration site (Wnt) and myelocytoma (*MYC*) activation, whereas CMS3 is associated with multiple metabolism signatures. CMS4 is characterised predominantly by stromal infiltration and signatures of epithelial-mesenchymal transition (EMT) and ECM remodelling (Guinney et al., 2015). Furthermore, the overrepresentation of certain mutations such as *BRAF* mutations in CMS1 and *KRAS* in CMS3 are also described but limited in its definite clinical value for stratification of a certain subtype. CMS classification has been also shown to have differing implications for prognosis of CRC. CMS3 showed better prognosis compared to other CMSs whereas, CMS4 is often associated with worse prognosis comparative to other CMSs, highlighting the vital implication of high stromal infiltration in ECM remodelling and EMT (Sawayama et al., 2020; Valenzuela et al., 2021). However, findings on prognostic value of CMSs remain inconsistent and heterogeneous which may be due to certain conflicting factors related such as tumour stages, tumour locations, and previous treatment lines.

2.1.4 Diagnosis of CRC

CRC is a complex disease that is asymptomatic at early stages and later develops typical and non-specific clinical manifestations. Abdominal pain, haematochezia, loss of appetite, altered bowel habits, and rectal bleeding are among the common symptoms of CRC that are typical in other diseases (Waters., 2022). In addition, vomiting, weight loss, and abdominal distension have been reported from CRC patients with advanced stages (Chen et al., 2017). It is noteworthy that the clinical manifestation of CRC slightly differs by its anatomical location. Enlarged and palpable abdominal mass are seen mostly in RCRC whereas, in LCRC, the common symptoms include blood stool and obstruction (Sun et al., 2014). Due to late and non-specific symptoms, most CRC patients are often delayed in getting diagnosis, thereby affecting patient treatment outcomes with a low early diagnosis rate.

As part of CRC diagnosis, the clinical examination primarily involves abdominal examination and several screening tests. The digital examination has been also recognised as a vital tool particularly where rectal cancer is suspected. This is followed by non-invasive screening such as faecal immunochemical test (FIT) or faecal occult blood test (FOBT) and screening for tumour markers (e.g., carcinoembryonic antigen (CEA) and carbohydrate Antigen 19–9 (CA19–9)) that provide a clinical value of disease monitoring (Sun et al., 2017). Serum-based CEA has become the standard biomarker for CRC diagnosis essential in predicting recurrence and prognosis (Nicholson et al., 2015). CEA level at <5 ng/ml indicates the normal range whereas >5 ng/ml might predict high recurrence and poor prognosis. CEA at >20 ng/ml might also further indicate the highly metastasize tumours (Khan et al., 2024). Colonoscopy, a minimally invasive procedure of endoscopy examination of the colon and rectum is also incorporated for its feasible

method in pathology tissue biopsy. Further examination using computed tomography (CT) is also done for visualisation of the size of lesions and preoperative staging of CRC (Bnard et al., 2018). To date, a major concern with such screening tools has been addressed related to their low sensitivity in detection of early adenomas or precursor lesion. FOBT and colonoscopy were shown to often miss the early adenomas including proximal and distal adenomas, hence leading to delayed diagnosis of CRC (Dodou and De Winter, 2012; Garborg et al., 2013).

It is paramount to classify the status of tumours correctly for clinically informed decision-making in CRC patient treatment and management. CRC staging is the key determinant for treatment and management approaches, patient prognosis, and disease recurrence (Macrae and Bendell, 2016). TNM system by the American Joint Committee on Cancer (AJCC) is one of the standardised approaches to CRC staging and is widely accepted for clinical practices across countries. The TNM staging system classifies the tumours based on three key characteristics namely tumour (T), nodes (N), and metastasis (M). T describes the extent of tumour invasion, N describes the extent of nodal metastasis, and M describes the metastatic spread. This system applies almost similar anatomical classification principles to that of the Union for International Cancer Control staging system. Stage I is identified as the tumour invades the submucosa (T1) (Figure 2.2) or passing through the thin muscle layer (T2) without metastasis to the nearby lymph nodes (N0) or distant sites (M0). Stage II classification is further divided into stages IIA, IIB, and IIC specifically according to the extent of tumour invasion through the wall of the colon but no metastasis to nearby lymph nodes or distant sites. Stages IIIA, IIIB, and IIIC are tumours that metastasize to the nearby lymph nodes (N1/N1c/N2a) without distant metastasis, and stages IVA and IVB are the tumours that may have or not metastasize to

the lymph nodes but with the presence of metastasis to distant organs such as lung, liver, or lymph nodes. Additionally, stage IVC is introduced in the latest edition (8th edition) of AJCC staging which highlights the peritoneal metastasis (M1c) (Tong et al., 2018). Alongside TNM staging, modified Dukes staging is also incorporated in CRC staging (Akkoca et al., 2014). Despite several modifications, TNM staging has been discussed as a poor tool in prognostic discrimination analyses between multiple factors such as disease subtypes and ages (Zhang et al., 2021).

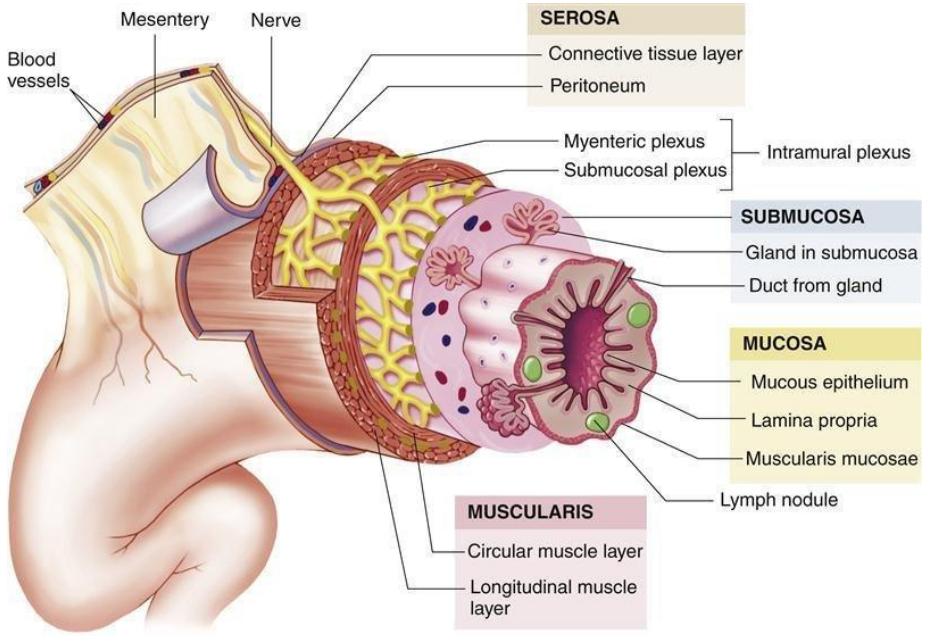

Figure 2.2 Cross-section of the layers of the colon wall (Patton, 2015).

2.1.5 Treatment and Management of CRC

The current therapeutic regime in CRC involves a multimodal approach for different subtypes as such rectal and colon cancers. Surgery is the mainstay curative treatment for a subgroup of patients with resectable and localised tumours (Mármol et al., 2017). In more advanced cases such as T4 colon cancer, preoperative chemotherapy is

usually opted to reduce the tumour load and achieve resectability while, for patients with disseminated unresectable disease as such those with stage III tumours are treated within adjuvant setting utilising the combination of multiple chemotherapeutic drugs that include doublet like XELOX (5-fluorouracil (5-FU) and oxaliplatin (OXA)) and triplet chemotherapy like FOLFOX (5-FU, folinic acid, and OXA) (Benson et al., 2017). To date, FOLFOX tops as the first-line therapy for most CRC patients worldwide especially cases of post-recurrence surgeries (Benson et al., 2017). One of the key drugs in FOLFOX, OXA is a diaminocyclohexane platinum derivative, used to treat metastatic CRC with the improved objective response rates to about 53% following the FOLFOX regimen (Neugut et al., 2019). FOLFOX therapy including FOLFOX4 and modified FOLFOX6 regimens were shown to improve the overall survival rate of patients with metastatic CRC (Schmoll et al., 2014; Souglakos et al., 2019). Plus, immunotherapy and targeted therapy are recognised as favourable treatment options due to their specific targeting of tumour molecular phenotypes, thus leading to better management of the disease. Pembrolizumab and nivolumab, monoclonal antibodies targeting programmed cell death 1 (PD1)-blocking have been reported to benefit patients with metastatic CRC (Li et al., 2022). BRAF inhibitors and anti-EGFR antibodies (i.e., Cetuximab and Panitumumab) have shown efficacy in treating $BRAF^{v600E}$ mutant CRC and metastatic CRC respectively (Muhammad et al., 2013) but recently confounded by acquired resistance (Haibe et al., 2020).

It is an established notion that cancer tends to evolve from the accumulation of genetic and epigenetic mutations, as well as clonal expansion (Beerenwinkel et al., 2015). This emphasises the dynamic and heterogeneous nature of CRC, which has quite deflated the conventional regimens to effectively diagnose and manage the disease. The relative survival rate of CRC patients of advanced cases remains low regardless of different

treatment regimens (Siegel et al., 2014). Personalized profiling and more targeted therapy are needed for better management of such a complex disease. One of the main factors in determining robust therapy and comprehensive profiling for CRC is sidedness.

2.2 Overview of CRC sidedness: RCRC vs LCRC

CRC is a heterogeneous disease. To date, CRC has been increasingly regarded for its two separate entities, entailing RCRC and LCRC. Bufill (1990) addressed CRC development and treatment outcomes that vary depending on its tumour location. RCRC covers the part of caecal colon (caecum), ascending colon, hepatic flexure until proximal two thirds of the transverse colon while, LCRC describes the remaining part of transverse colon, splenic flexure, sigmoid, descending colon, and rectum. It is worth noting that, rectum is sometimes considered separately from LCRC given their differences in surgical intervention and treatment outcomes but the studies attempting to sub-classify them are inconsistent (Salem et al., 2017). Molecular profiling on tumours arising from rectum and other parts of colon were observed in similar pattern (Sanz-Pamplona et al., 2011; Knight et al., 2016). It is far from conclusive to clearly dictate the differences between RCRC and LCRC, albeit accumulating evidence have highlighted some crucial data that could clarify their considerable variations. These include their differences with respect to its anatomical location, epidemiology, histology, and clinicopathological phenotypes, as well as immunological factors that are collectively summarised in Figure 2.3. These differences reflect the heterogenous CRC subjected to its anatomical location which is vital for precise refinement of CRC.