ELUCIDATION OF THE ROLE OF LONG NONCODING RNA KCNMA1-AS2 IN COLORECTAL CANCER

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ELUCIDATION OF THE ROLE OF LONG NONCODING RNA KCNMA1-AS2 IN COLORECTAL CANCER

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LIST OF SYMBOLS

%	Percentage
bp	Base pair
℃	Degree Celsius
cm	Centimetre
g	Gram
h	Hour
kb	kilobase
L	Liter
M	Molar
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
ng	Nanogram
nm	Nanometre
nM	Nanomolar
rpm	Revolutions per minute
s	Second
U	Unit
V	Volt
x g	G force
µg	Microgram
µL	Microlitre
µM	Micromolar

LIST OF ABBREVIATIONS

5-Fu	5-fluorouracil
ABCB1	ATP binding cassette subfamily B member 1
AJCC	American Joint Committee on Cancer
Alb	Albumin
AMPK	Adenosine monophosphate-activated protein kinase
ANCR	Angelman syndrome chromosome region
ANRIL	Antisense non-coding RNA in the INK4 locus
ARAP1	ArfGAP with RhoGAP domain, Ankyrin repeat and pH domain 1
AS	Antisense
ATCC	American type culture collection
BAZ2B	Bromodomain adjacent to zinc finger domain 2b
BCAT1	Branched chain amino acid transaminase 1
Bcl-2	B-cell lymphoma 2
BCYRN1	Brain cytoplasmic RNA 1
BioCarta	Biocarta pathway database
BioCyc	Biocyc genome database collection
BLACAT1	Bladder cancer associated transcript 1
BLCAP	Bladder cancer-associated protein
BSA	Bovine serum albumin
CA199	Carbohydrate antigen 199
CACS15	Cancer susceptibility candidate 15
CAFs	Carcinoma-associated fibroblasts
Car10	Carbonic anhydrase 10
CCAL	Colorectal cancer-associated lncrna
CCAT1	Colon cancer associated transcript 1
CDK	Cyclin-dependent kinase
CDKN2B	Cyclin dependent kinase inhibitor 2B
cDNA	Complementary DNA
CEA	Carcinoembryonic antigen
ceRNA	Competing endogenous RNA
ChIRP	Chromatin isolation by RNA purification

CPS1-IT1	Carbamoyl-phosphate synthase 1 Intronic Transcript 1
CRC	Colorectal cancer
CREB1	Cyclic AMP-responsive element-binding protein 1
CRISPR	Clustered regularly interspaced short palindromic repeats
CRNDE	Colorectal neoplasia differentially expressed
CT	Computerized tomography
CTR1	Copper uptake protein 1
CUL4A	Cullin 4A
CYTOR	Cytoskeleton regulator RNA
DANCR	Differentiation antagonizing non-protein coding RNA
DDN	Dendrin
DDP	Cisplatin
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DNase	Deoxyribonuclease
dsDNA	Double stranded DNA
DTT	Dithiothreitol
DUXAP8	Double homeobox A pseudogene 8
E2F	E2 transcription factor
EDTA	Ethylenediaminetetraacetic acid
EGCG	Epigallocatechin gallate
EMT	Epithelial-mesenchymal transition
ERG	ETS-related gene
ERK	Extracellular signal-regulated kinase
EtBr	Ethidium bromide
EZH2	Enhancer of zeste homolog 2
Fas	Fas cell surface death receptor
FBS	Fetal bovine serum
FC	Foldchange
FGD5	Fyve, rhogef and pH domain containing 5
FGF9	Fibroblast growth factor 9
FISH	Fluorescence *in situ* hybridisation
FL	Firefly luciferase
FLVCR	Feline leukemia virus subgroup C receptor

FOXC2	Forkhead box protein C2
FOXD1	Forkhead box d1
FOXO3a	Forkhead box class O 3a
FunDO	Functional disease ontology annotation database
G0 phase	Gap phase
G1 phase	Gap 1 phase
G2 phase	Gap 2 phase
G4	G-quadruplex
GAD	Genetic association database
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GAS	Growth arrestspecific
GC	Gastric cancer
H3K27ac	H3 acetylation on lysine 27
H3K27me3	Trimethylation of lysine 27 on histone H3
HAND2	Heart and neural crest derivatives expressed 2
Hb	Haemoglobin
HER2	Human epidermal growth factor receptor 2
HHIP	Hedgehog-interacting protein
HIF1A	Hypoxia inducible factor 1 subunit alpha
HMGA	High mobility group A
HMGA1	High mobility group at-hook 1
HNF4α	Hepatocyte nuclear factor 4α
HOTAIR	HOX transcript antisense RNA
HOTTIP	HOXA transcript at the distal tip
Hu/ELAV	Human/embryonic lethal abnormal vision
HuR	Human antigen R
IF	Immunofluorescence
IL	Interleukin
IRS1	Insulin receptor substrate-1
KCNMA1	Potassium calcium-activated channel subfamily M alpha 1
KEGG	Kyoto encyclopedia of genes and genomes
Klf	Krüppel-like factor
KO	Kegg ortholog
LB	Lysogeny broth

LIMK2	LIM domain kinase 2
LINP1	LncRNA in non-homologous end joining pathway 1
LncRNA	Long noncoding RNA
LUCAT1	Lung cancer associated transcript 1
M phase	Mitotic phase
MAGI2	Membrane-associated guanylate kinase inverted 2
MALAT 1	Metastasis associated lung adenocarcinoma transcript 1
MAPK	Mitogen-activated protein kinases
MBNL1	Muscleblind like splicing regulator 1
MEF2C	Myocyte enhancer factor 2c
MEG3	Maternally expressed 3
miR	Mirna, microrna
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MTHFR	Methylenetetrahydrofolate reductase
mTOR	Mammalian target of rapamycin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUT	Mutant
MYC	Myc proto-oncogene
NaAc	Sodium acetate
NC	Negative control
NCL	Nucleolin
ncRNA	Noncoding RNA
NEAT1	Nuclear paraspeckle assembly transcript 1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHGRI GWAS Catalog	National human genome research institute home genome-wide association studies database
NR2F	Nuclear receptor subfamily 2 group f member
NTP	Nucleoside triphosphate
OMIM	Online mendelian inheritance in mandatabase
OR3A4	Olfactory receptor, family 3, subfamily A, member 4
ORF	Open reading frame
OXA	Oxaliplatin
PANDAR	Promoter of cdkn1a antisense dna damage activated rna

PANTHER	Protein analysis through evolutionary relationships database
PBS	Phosphate-buffered saline
PC	Pancreatic cancer
PCR	Polymerase chain reaction
Pen-Strep	Penicillin-streptomycin
PI	Propidium iodide
PI3Ks	Phosphoinositide 3-kinases
PID	Pathway interaction database
piRNA	Piwi-interacting RNA
PKB/Akt	Protein kinase B
PRNCR1	Prostate cancer-associated non-coding RNA1
PSA	Polysialic acid
PTEN	Phosphatase and tensin homolog
PVT1	Plasmacytoma variant translocation 1
qPCR	Real-time polymerase chain reaction
RAB14	Ras-related protein Rab-14
RBPs	RNA-binding proteins
Reactome	Reactome pathway database
RIP	RNA immunoprecipitation
RL	Renilla luciferase
RNase	Ribonuclease
ROR	Retinoic acid-related orphan receptor
RPLP0P2	Ribosomal protein lateral stalk subunit p0 pseudogene 2
rRNA	Ribosomal RNA
S phase	Synthesis phase
SCARNA2	Small Cajal body-associated RNA 2
siRNA	Small interfering RNA
SLC7A11	Solute carrier family 7, member 11
SNAI	Snail family transcriptional repressor
SND1	Staphylococcal nuclease and Tudor domain containing 1
SNHG	Small nucleolar RNA host gene
snoRNAs	Small nucleolar RNA
Sp1	Specificity protein 1
SphK1	Sphingosine kinase 1

Spt16	Suppressor of Ty 16
SRSF6	Serine and arginine rich splicing factor 6
ST8SIA2	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2
STAT3	Signal transducer and activator of transcription 3
TINCR	Tissue differentiation-inducing non-protein coding RNA
TMPO	Thymopoietin
TNFRSF10A	TNF Receptor Superfamily Member 10a
TNM	Tumour, node, metastasis
tRNA	Transfer RNA
TSLNC8	Tumor suppressor long noncoding RNA on chromosome 8p12
TUG1	Taurine upregulated 1
UCA1	Urothelial cancer associated 1
UICC	Union for international cancer control
UICLM	Up-regulated in colorectal cancer liver metastasis
URGCP	Upregulator of cell proliferation
USM	Universiti Sains Malaysia
UTR	Un-translated terminal region
VELUCT	Viability enhancing in lung cancer transcript
Wnt	Wingless-related integration site
WCRF	World Cancer Research Fund
WRAP53	WD Repeat containing antisense to TP53
WT	Wild-type
WWC2	WW-and-C2-domain-containing family of proteins
Xist	X-inactive specific transcript
XXMU	Xinxiang Medical University
YAP1	Yes-associated protein 1
ZFAS1	Zinc finger antisense 1
ZNF561	Zinc finger protein 561

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ELUSIDASI PERANAN RNA PANJANG BUKAN PENGEKODAN KCNMA1-AS2 DALAM KANSER KOLOREKTAL

ABSTRAK

Ekspresi luar biasa RNA panjang bukan pengekodan (lncRNA) seringkali didokumentasikan dalam kajian berkaitan kanser kolorektal. Pemerhatian ini dikaitkan dengan tumorigenesis yang menggalakkan perkembangan sel tumor melalui proses percambahan, apoptosis, pergerakan kitaran selular dan migrasi. Terdapat pertambahan jumlah pembuktian yang mencadangkan peranan penting lncRNA dalam mengawalatur tumor dari aspek molekular dan selular. Sebahagian daripada lncRNA ini berfungsi sebagai onkogen dengan menunjukkan peningkatan ekspresi, manakala sebahagian yang lain pula bertindak sebagai perencat tumor melalui jumlah ekspresi yang rendah dalam tisu kanser. LncRNA berupaya mengawalatur pelbagai proses dengan menyasarkan molekul-molekul miRNA, sekaligus bertindak sebagai molekul RNA endogen saingan. Kajian tesis ini bertujuan untuk mengenalpasti molekul-molekul lncRNA baharu, merungkai serta mengesahkan peranan mereka dalam mekanisma molekular kanser kolorektal. Sampel-sampel tisu tumor dan bukan tumor yang diperoleh daripada tiga pesakit kanser kolorektal telah diproses untuk tujuan penjujukan RNA. Keputusan analisis ini menunjukkan peningkatan ekspresi sebanyak 148 lncRNA dan penurunan ekspresi 146 lncRNA yang lain. Pengesahan ekspresi yang dibuat, dengan menggunakan 10 sampel pesakit dan 4 jenis turunan sel berkaitan kolorektal kanser, telah mengenalpasti LINC00668 sebagai lncRNA kawal atur menaik. Berikutnya, kaedah penindasan siRNA telah dijalankan untuk menganalisis kesan perubahan ekspresi LINC00668 terhadap fungsi sel kolorektal kanser. Ujian MTT digunakan untuk mengukur percambahan sel. Ujian apoptosis Annexin-V/PI

dilakukan untuk menentukan apoptosis sel. Untuk menganalisis kitaran sel, ujian kitaran sel dijalankan, dan ujian penyembuhan luka digunakan untuk menilai migrasi sel. Hasil analisis menunjukkan tiada kesan signifikan LINC00668 terhadap percambahan sel atau apoptosis. Maka, LINC00668 dikeluarkan daripada senarai untuk kajian seterusnya dalam tesis ini. Dalam kesemua 10 sampel pesakit yang dikaji, lncRNA KCNMA1-AS2 telah menunjukkan penurunan ekspresi yang signifikan dalam tisu tumor berbanding tisu bukan tumor. Pola penurunan ekspresi ini juga diperhatikan dalam kesemua turunan sel kolorektal yang dikaji. Oleh itu, KCNMA1-AS2 dianggap sebagai lncRNA kawal atur menurun. Berikutan itu, ekspresi KCNMA1-AS2 telah diubah dan dinaikkan melalui proses transfeksi. Hasil daripada perubahan ini, terdapat penindasan yang ketara dalam percambahan dan migrasi sel, peningkatan sel apoptosis dan lebih banyak sel yang terhenti di fasa S. Seterusnya, kaedah 'dual luciferase assay' dan analisis qPCR telah dijalankan untuk mengesahkan perlekatan/pengikatan KCNMA1-AS2 dengan miR-1227-5p. Analisis bioinformatik melalui 3 pangkalan data yang digunakan telah mengenalpasti 58 sasaran hiliran yang berpotensi bagi miR-1227-5p. Daripada jumlah ini, 10 sasaran telah dikaitkan dengan kanser. Daripada subset ini pula, MTHFR dan ST8SIA2 diramalkan berperanan secara spesifik dalam kolorektal kanser. Secara keseluruhannya, kajian ini telah berjaya mengenalpasti lncRNA baharu iaitu KCNMA1-AS2, yang berpotensi untuk dibangunkan sebagai biopenanda diagnostik dan terapeutik untuk kolorektal kanser. Kajian lanjut pada masa hadapan perlu dijalankan dengan menyeluruh untuk memahami mekanisme jaringan perhubungan antara molekul-molekul lncRNA, miRNA dan mRNA secara mendalam dalam kolorektal kanser.

ELUCIDATION OF THE ROLE OF LONG NONCODING RNA KCNMA1-AS2 IN COLORECTAL CANCER

ABSTRACT

The aberrant expression of long noncoding RNAs (lncRNAs) is frequently documented in colorectal cancer (CRC). This anomalous expression is associated with tumourigenesis by promoting detrimental biological behaviours in tumour cells, including proliferation, apoptosis, cell cycle progression, and migration. A growing body of evidence suggests that lncRNAs play pivotal roles in tumour regulation at both molecular and cellular dimensions. Some lncRNAs function as oncogenes and exhibit increased expression, while others act as tumour suppressors and show reduced levels in cancerous tissues. Furthermore, lncRNAs can regulate processes by targeting miRNAs, serving as competing endogenous RNAs. This study seeks to discover novel lncRNA entities and comprehensively examine and validate their roles and underlying molecular mechanisms in CRC. Tissue samples, both tumourous and non-tumourous, from three CRC patients were submitted for sequencing. The sequencing results indicated 148 up-regulated and 146 down-regulated lncRNAs. Following expression validation in samples from ten patients and four CRC cell lines, LINC00668 was identified as an up-regulated lncRNA. An siRNA knockdown was performed on LINC00668 to assess if altering its impact on CRC cell functions. The MTT assay was used to measure cell proliferation. The Annexin-V/PI apoptosis assay was performed to determine cell apoptosis. To analyse the cell cycle, a cell cycle assay was conducted, and a wound healing assay was utilised to evaluate cell migration. However, no significant effects on cell proliferation or apoptosis were observed post-knockdown of LINC00668, leading to its exclusion from subsequent research. In all 10 patients,

lncRNA KCNMA1-AS2 demonstrated a significant decrease in expression within tumour tissues compared to non-tumour counterparts, which was also consistently observed across all CRC cell lines when compared to normal colon cells. As a result, KCNMA1-AS2 was identified as a down-regulated lncRNA. Following the overexpression of lncRNA KCNMA1-AS2, there was a marked suppression in cell proliferation and migration, an enhancement in cell apoptosis, and a greater number of cells were arrested in the S phase. Furthermore, the binding of KCNMA1-AS2 to miR-1227-5p was confirmed using dual luciferase reporter assays and qPCR analyses. Subsequent bioinformatics analyses identified 58 potential downstream targets of miR-1227-5p across three databases. Out of these, 10 were pinpointed as being specifically cancer-related. From this subset, MTHFR and ST8SIA2 were putatively predicted linked to CRC. This study identifies a novel lncRNA, KCNMA1-AS2, that shows potential as a diagnostic and therapeutic biomarker for CRC. Future research must be more exhaustive to thoroughly understand the intricate lncRNA-miRNA-mRNA network mechanisms.

CHAPTER 1

INTRODUCTION

1.1 Background

Carcinoma of the colon and rectum is a common malignancy of the gastrointestinal tract. The early symptoms are not noticeable, and as the tumour grows, patients may experience changes in bowel habits, rectal bleeding, diarrhoea, alternating constipation and diarrhoea, and local abdominal pain. In later stages, patients may display systemic symptoms such as anaemia and weight loss. Regrettably, the initial indications of colorectal cancer (CRC) are often indistinct, which causes numerous patients to be diagnosed at advanced stages during their initial medical consultation (Walsh and Terdiman, 2003).

Previously considered "transcriptional noise", it is now recognised that noncoding RNAs (ncRNAs) play significant roles in colorectal development and progression due to their involvement in gene splicing and modification. Long noncoding RNAs (lncRNAs) are a type of ncRNA known for regulating gene expression and having unique functions. LncRNAs, are over 200 nucleotides long and can be classified into subclasses based on their position and transcription direction relative to other genes: sense, antisense, bidirectional, intronic, and intergenic (Herrera-Solorio *et al.*, 2017). LncRNA has been verified to participate in a multitude of biological processes, including proliferation, metastasis, differentiation, inflammation, angiogenesis, metabolism and chemoresistance (Miao *et al.*, 2021).

1.2 Problem statement

Owing to limited awareness of CRC, patients are often diagnosed at advanced stages of the disease. It is essential to recognise new biomarkers or therapeutic targets.

LncRNAs have been one of the hotspots in molecular mechanism research of tumours over the past decade. It has been confirmed that lncRNAs play a regulatory role in gene expression in living organisms and are closely related to the occurrence and development of tumours. Although thousands of lncRNA have been discovered, the mechanisms of many remain unclear.

Studies have discovered that numerous lncRNAs exhibit altered expression in CRC (Weng *et al.*, 2017; Yang *et al.*, 2018b). These aberrantly expressed lncRNAs, acting as vital modulators, are involved in several biological activities, influencing tumour cell proliferation and cell apoptosis, as well as cell migration, invasion, metastasis and drug resistance modulation (Morin, 2019; Deng *et al.*, 2020). Delving into the roles of lncRNAs in CRC might offer fresh perspectives for its clinical application. Furthermore, lncRNAs hold promise as potential indicators for early detection and assessment of prognosis of CRC.

1.3 Aim and Objectives

The objective of this project was to identify novel lncRNA and explore the function and significance of lncRNAs in CRC. The hypothesis of this project posited that certain up-regulated and down-regulated lncRNAs in CRC could significantly impact the function of CRC cells.

The specific objectives of this project were:

(1) To establish the expression profile of lncRNAs and identify some specific potential lncRNAs in tissue samples from patients with CRC.

(2) To verify the distinct expression of lncRNAs in both normal colorectal tissues and cancerous tissues and cell lines.

(3) To establish cell line model systems for silencing and overexpression of target lncRNAs.

(4) To investigate the functions of target lncRNAs in CRC cell lines such as proliferation, apoptosis, cell cycle and migration.

(5) To determine and confirm the target miRNAs of lncRNAs in CRC cell lines, and subsequently investigate the corresponding downstream messenger RNA (mRNA) pathways and functions by using bioinformatics analysis.

The aim of this research project is to identify novel lncRNA molecules, investigate and validate their role in the molecular mechanisms of CRC. The bioinformatics analysis is employed to discover their downstream targets and pathways.

CHAPTER 2

LITERATURE REVIEW

The gene is the fundamental unit of protein coding. Upon completion of human genome sequencing, only 3-5% of the entire genome is comprised of DNA that can encode proteins. Moreover, most genes lack coding protein functions. Previously, it was believed that 90% of the RNA transcribed from the non-coding region of the human genome's gene was considered "transcriptional noise." However, it is now widely recognized that ncRNAs are intricately associated with the onset and advancement of numerous diseases, as gene splicing and modification play vital roles (Weng *et al.*, 2017).

ncRNA is a collective term for a group of RNA molecules that, while transcribed from DNA, do not undergo translation into proteins. These noncoding RNAs are classified into microRNA (miRNA), lncRNA, small interfering RNA (siRNA), and piwi-interacting RNA (piRNA), small nucleolar RNA (snoRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) (Beňačka *et al.*, 2023). In recent years, the study of ncRNA has emerged as a pioneering area within molecular biology, yielding numerous significant findings. However, the majority of researchers predominantly focus on miRNA, often overlooking the more abundant and equally important lncRNA.

2.1 Introduction of lncRNA

LncRNA is a category of RNA molecules identified in recent years as regulators of gene expression and other unique functions. As research in this area continually progresses and expands, thousands of lncRNAs have been discovered, but the functional roles of only a limited number of them have been elucidated. LncRNA, which is over 200 nucleotides long, is primarily transcribed by RNA polymerase II. Due to the

absence of a clear open reading frame (ORF), lncRNA molecules are structurally incapable of encoding proteins. They can be found throughout the nucleus and cytoplasm (Kurokawa., 2015). The discovery of lncRNA H19 and Xist in mouse occurred for the first time during the late 1980s and early 1990s (Brannan *et al.*, 1990; Brockdorff *et al.*, 1991). Subsequently, lncRNA has garnered substantial interest and emerged as a popular subject of research, the number of articles related to lncRNA published over the past 20 years was shown in Figure 2.1.

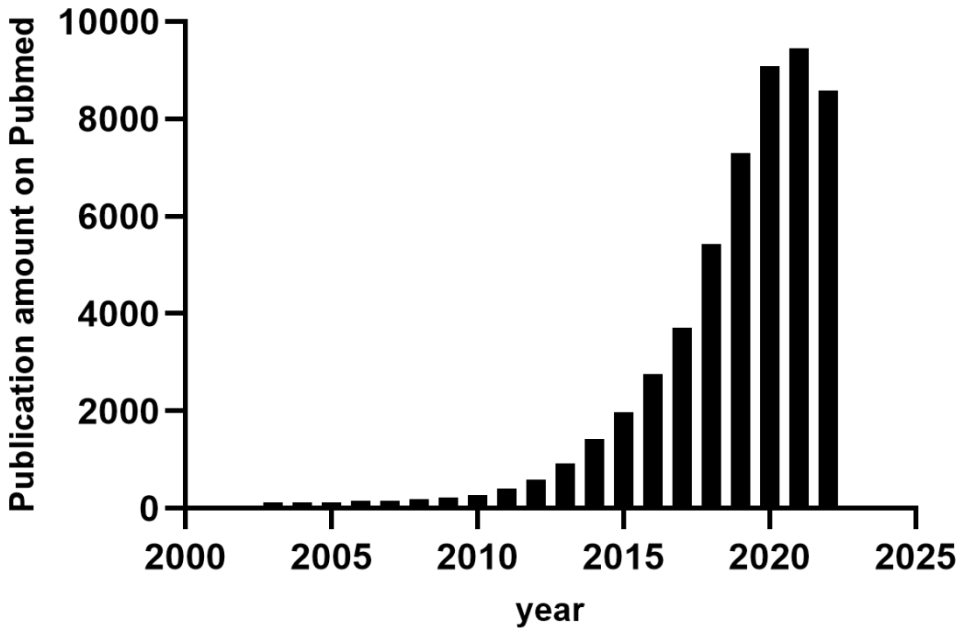

Figure 2.1 Number of articles related to lncRNA published over the past 20 years. The term "lncRNA" was used as a search query on PubMed, and publications from 2003-2022 were tallied and graphed. Research pertaining to lncRNA has seen a significant uptick in recent years.

LncRNA can be classified into five categories depending on its position relative to coding genes. These categories include antisense, sense, intergenic, intronic, and bidirectional (divergent) (Herrera-Solorio *et al.*, 2017). The categories of sense, antisense, bidirectional or divergent lncRNA reflect the transcription orientation of the lncRNA in relation to nearby protein-coding genes, which may be in the same, opposite, or alternate directions. On the other hand, intronic and intergenic lncRNA

classifications determine whether the lncRNA originates from the intron region of a gene or from the intergenic region between two genes (Figure 2.2A) (Miao *et al.*, 2021).

Based on current research and the aforementioned structural features of lncRNA, it is believed that lncRNAs may have multiple distinct origins. However, the widely accepted international consensus includes the following five types: (1) lncRNAs originating from disrupted protein-coding gene structures, with lncRNA formation occurring at the opposite end; (2) lncRNAs arising from chromatin recombination in the original gene sequence due to exon deletion; (3) lncRNAs produced by the sequential connection of replicons from two neighbouring genes; (4) pseudogenes created during the replication process of ncRNA, where some RNAs are reverse-transcribed; (5) some transposons are inserted into protein-coding gene sequences, transforming them into partially functional lncRNAs (Rinn and Chang, 2012).

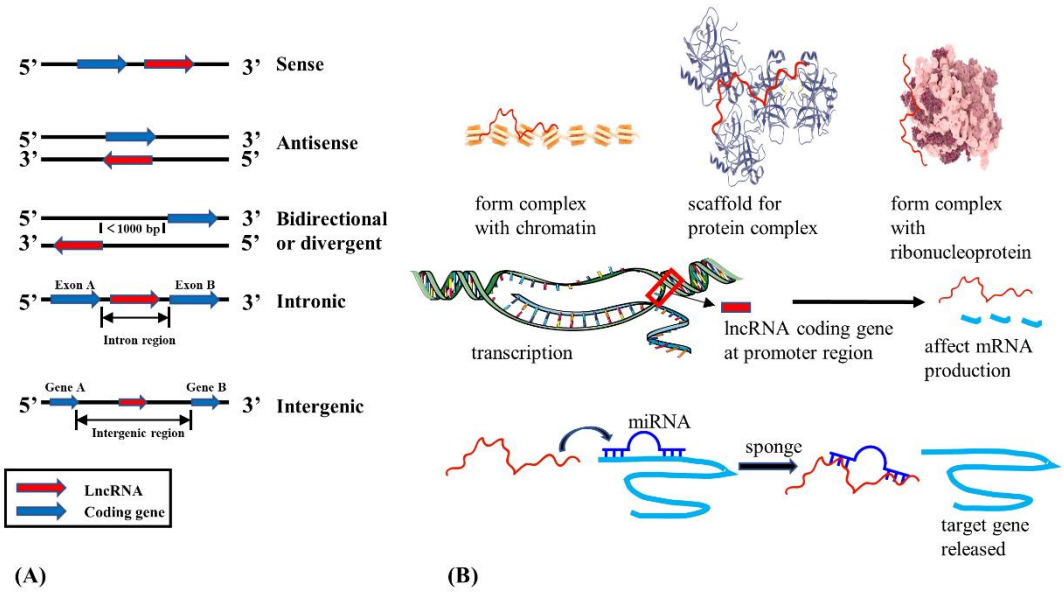

Figure 2.2 Classification of lncRNA according to their position within the genome and the general function.

(A) LncRNAs can be categorised based on their transcription direction relative to neighbouring protein-coding genes and their origin within the gene structure. Sense, antisense, and bidirectional (or divergent) lncRNAs indicate whether the lncRNA is transcribed in the same direction, opposite direction, or in different directions relative to nearby protein-coding genes. Intronic and intergenic lncRNAs denote whether the lncRNA is transcribed from the gene's intron region or from the intergenic region between two genes. (B) The roles and functions of lncRNA. LncRNA can influence gene expression through various methods: by forming complexes with chromatin, distinct proteins, or ribonucleoproteins; by acting as a cis-acting element that interferes with the transcription of downstream mRNA production; and by serving as a sponge for miRNA, which enables the release of target genes from miRNA-mediated regulation. Adapted from Miao *et al*., 2021.

There is a growing body of evidence indicating that lncRNAs play diverse roles (Figure 2.2B) in biological processes, including facilitating changes in chromatin structure, serving as a scaffold to facilitate interactions between various proteins, and modulating transcriptional regulation (Chen and Carmichael, 2010). LncRNAs can have an impact on mRNA production at the transcriptional level. When certain lncRNA genes are situated in the promoter region upstream of the coding gene, they can be transcribed into corresponding lncRNAs that act as cis-acting elements, interfering with transcription of genes downstream. This interference can ultimately lead to alterations in the production of mRNA (Martens *et al.*, 2004). Earlier investigations have

demonstrated that lncRNA is capable of creating a complex with ribonucleoprotein in order to regulate gene expression (Ponting *et al.*, 2009). The formation of a complex between lncRNA and pre-mRNA enables the regulation of gene expression at the post-transcriptional level (Beltran *et al.*, 2008). In addition, lncRNA can indirectly affect downstream processes by binding to miRNA. miRNAs can target and bind to lncRNAs, which can then act as a reservoir to sequester miRNAs, preventing them from binding to their endogenous targets. The sequestration of miRNAs by lncRNAs can disrupt the typical regulatory functions of miRNAs, ultimately impacting gene expression. Thus, lncRNAs can function as molecular "sponges" by absorbing miRNAs, which in turn prevents them from targeting other RNAs and affecting their expression (Zhang *et al.*, 2018a).

2.2 LncRNAs in cancers

Figure 2.3 illustrates the diverse functions and roles of lncRNAs in different cancers, including their aberrant expression, chemoresistance, and various other functions, as reported in numerous studies (Miao *et al.*, 2021). LncRNAs participate in several biological processes, such as cell proliferation, migration, and invasion (Gao *et al.*, 2017a), differentiation (Chen *et al.*, 2017c), metastasis (Chen *et al.*, 2017d), inflammation (Du *et al.*, 2017; Carpenter., 2022), angiogenesis (Li *et al.*, 2017d), and metabolism (Fan *et al.*, 2017), and as a result, they can modulate the pathophysiological mechanisms in cancer and other human ailments. For instance, previous studies have indicated that lncRNAs are involved in the AMPK signalling pathway, which plays a role in the regulatory mechanisms of human aging and age-related diseases (Li *et al.*, 2024). Additionally, research on diabetic cardiomyopathy has shown that high glucose-induced upregulation of the lncRNA MIAT is responsible for IL-17 production in

cardiomyocytes. Knocking down MIAT significantly attenuated IL-17 expression, ameliorated cardiac fibrosis, and improved cardiac contractility (Qi *et al.*, 2020). The atypical expression of lncRNAs is commonly observed in cancer, and it is linked to the development of tumours by stimulating malignant biological activities in tumour cells, such as proliferation, invasion, and metastasis (Weidle *et al.*, 2017; Xiao *et al.*, 2017a). The dysregulated expression and corresponding function of cancer-related lncRNAs can determine whether they act as oncogenes or tumour suppressor genes. Certain lncRNAs act as oncogenes and are up-regulated, whereas others function as tumour suppressors and are down-regulated in cancer tissues.

miRNA is a class of ncRNA molecules consisting of 19-25 nucleotides. These molecules are involved in a wide range of biological processes, including regulation of the cell cycle, cellular differentiation, development, metabolism, and aging of the body (Ho *et al.*, 2022; Stafa *et al.*, 2024). miRNAs also have the potential to serve as molecular markers for tumours. miRNAs have been extensively implicated in various aspects of tumorigenesis and development, including angiogenesis, metastasis, invasion, and apoptosis (Tüfekci *et al.*, 2014). They exert their effects by either inhibiting or promoting the expression of oncogenes. Changes in miRNA expression levels have been found to be closely associated with tumour progression (Lee and Dutta, 2009). Additionally, lncRNAs have the ability to act as regulators by targeting miRNAs through a mechanism known as "sponging" or competing endogenous RNA (ceRNA). In doing so, they can reduce the regulatory effect of miRNAs on target mRNA (Su *et al.*, 2021).

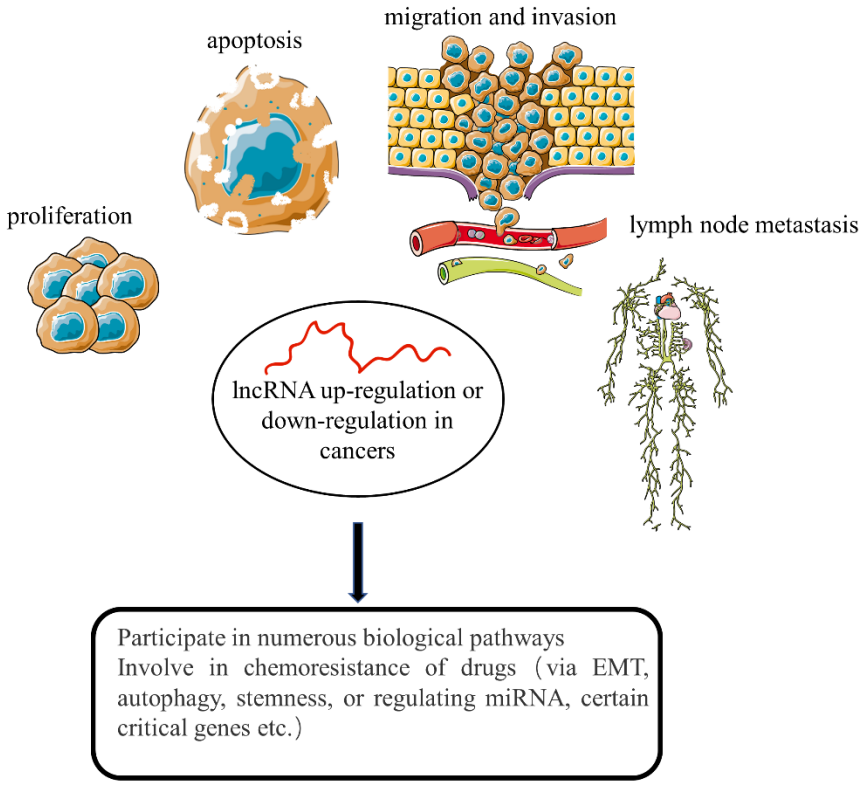

Figure 2.3 The function of lncRNA in cancers.
LncRNAs are involved in a wide array of biological processes relevant to cancer research, such as cell proliferation, apoptosis, migration, invasion, and lymph node metastasis. Dysregulation of lncRNAs is commonly observed in cancers, and they have been demonstrated to play crucial roles in multiple pathways as well as in chemoresistance investigations. Adapted from Miao *et al.*, 2021.

Although lncRNAs have not yet been utilised in clinical trials, they have been extensively examined at the laboratory level to address chemoresistance. Previous studies have reported that certain lncRNAs, such as HCG11 and LEIGC, are associated with regulating cancer cell resistance to 5-FU. These lncRNAs can act as either suppressors or inducers of cell sensitivity to the drug by altering various mechanisms, including metabolism, proliferation, and EMT (Eptaminitaki *et al.*, 2022). These investigations suggest that lncRNAs possess significant potential for future clinical research. In the realm of diagnosis, lncRNA expression boasts the benefits of specificity and sensitivity, making it a crucial tumour marker, particularly when traditional imaging methods struggle to detect tumours. These lncRNAs have the potential to serve as

therapeutic targets due to their critical roles in modulating the malignancy and tumorigenesis of various cancers through interactions with miRNAs. In terms of treatment, despite the effectiveness of chemotherapy as a cancer treatment, the development of chemoresistance remains a significant obstacle, often resulting in cancer recurrence and ultimately, treatment ineffectiveness (Zheng, 2017). Whether it is innate, congenital drug resistance or acquired, secondary drug resistance, both are associated with the abnormal activation of intracellular signalling pathways. The modulation of lncRNA can alter the drug efflux system, enhance drug metabolism, affect DNA repair, disrupt cell cycles, induce abnormal cell apoptosis, promote epithelial-mesenchymal transition (EMT) (Mitra *et al.*, 2015), and interact with miRNA as a sponge, all of which contribute to the development of drug resistance (Li *et al.*, 2017e; Xiao *et al.*, 2018). One of the fundamental mechanisms of chemotherapy involves inducing apoptosis. Cancer cells may enhance their survival through autophagy, resulting in reduced sensitivity to chemotherapy (Yang and Klionsky, 2010). Additionally, the notable activation of specific signalling pathways such as Erk/MAPK and p38/MAPK pathways has been associated with chemoresistance (Chung *et al.*, 2012).

Undoubtedly, lncRNAs play a crucial role in various types of cancer, extending beyond mentioned. It is not feasible to provide a comprehensive description of all instances within this limited context. However, a few examples of some common cancers will be presented to highlight the significance of lncRNAs. A concise review of research conducted on lncRNAs in breast cancer, lung cancer, and digestive system cancer are provided here. The focus is on elucidating the diverse functional roles of lncRNAs in genetic alterations, molecular mechanisms, and signalling pathways implicated in tumour progression, metastasis, and therapy resistance.

2.2.1 LncRNAs in breast cancer

Breast cancer stands as one of the most malignant diseases and remains the most prevalent cancer affecting women globally. The World Cancer Research Fund International (WCRF International) reported that breast cancer was the second most common cancer worldwide in 2022 and it is the most prevalent cancer among women. Previous research has identified numerous lncRNAs exhibiting abnormal expression patterns and exhibiting diverse functions in the context of breast cancer (Liu *et al.*, 2015). The group of patients displaying elevated expression levels of the lncRNA LINP1 exhibited a higher incidence of lymph node metastasis, more severe pathological differentiation, shorter overall survival, and a poorer prognosis in comparison to the group with low LINP1 expression (Liu *et al.*, 2018e). The dysregulated expression of PRNCR1-2 (Pang *et al.*, 2018), HIF1A-AS2 (Wang *et al.*, 2019f) promoted breast cancer cell proliferation, invasion, and migration. Furthermore, certain lncRNAs demonstrate functionality across multiple pathways. Multiple previous publications have consistently highlighted the significant down-regulation of the lncRNA MEG3 in breast cancer (Hussain *et al.*, 2023). As a tumour suppressor, MEG3 is involved in various pathways, further emphasising its crucial role in the disease. A prior publication indicated that the upregulation of MEG3 hindered breast cancer growth and triggered cell apoptosis by intensifying endoplasmic reticulum stress. This process was facilitated by the activation of the NF-κB and p53 pathways (Zhang *et al.*, 2018c). Previous findings have illustrated that the overexpression of MEG3 resulted in the downregulation of miR-21 through the PI3K/Akt pathway. Consequently, this downregulation suppressed cell proliferation and effectively inhibited tumour growth (Zhu *et al.*, 2019). In addition, the overexpression of MEG3 was found to inhibit EMT in cells by acting as a sponge and negatively regulating endogenous miR-421. This

regulatory mechanism effectively constrained cell proliferation and invasion (Zhang *et al.*, 2017a). It was demonstrated that lncRNA MALAT1 regulated BLCAP mRNA expression through its binding to miR-339-5p (Zheng *et al.*, 2019). In another study , it was revealed that LINC00461 acted as a sponge for miR-20a-5p, consequently positively regulating the expression of integrin β3 (Dong *et al.*, 2019b). Additionally, it was discovered that Linc00339 functioned as a miR-337-3p sponge, thus influencing its expression (Wang *et al.*, 2019c).

Several lncRNAs have been found to contribute to drug resistance in breast cancer metastasis, making them potential therapeutic targets for overcoming drug resistance. Tamoxifen, the gold standard for endocrine therapy in breast cancer, is not exempt from this challenge, as drug resistance poses a significant obstacle and limitation to its effectiveness. In breast cancer, there is a notable upregulation of the lncRNA UCA1 (Hosseini *et al.*, 2021). The investigation demonstrated that the suppression of UCA1 led to a notable increase in tamoxifen effectiveness, achieved by halting the cell cycle progression at the G2/M phase and prompting apoptosis in the cells. These results highlight UCA1's promise as a valuable focal point for therapeutic interventions in the context of breast cancer therapy (Li *et al.*, 2019c). Previous investigations have suggested that the activation of the lncRNA TINCR contributes to trastuzumab resistance in patients with advanced HER2+ breast cancer. Therefore, a feasible approach to overcome trastuzumab resistance during treatment is to knock down or silence TINCR (Dong *et al.*, 2019a).

2.2.2 LncRNAs in lung cancer

WCRF International also reported that lung cancer was the most common cancer worldwide in 2022. It was the most prevalent cancer among men and the second most common cancer among women. Lung cancer, which encompasses both small-cell

and non-small cell types, ranks as the second most widespread form of cancer worldwide, afflicting both males and females. Its occurrence and fatality rates are escalating, cementing its status as one of the most substantial and prevalent cancer categories on a global scale. Research into lung cancer has delved deeply into the pivotal involvement of lncRNAs, providing valuable insights into their underlying mechanisms. A noteworthy discovery pertains to the inhibition of lung cancer cell growth, mobility, and infiltration by the lncRNA BX357664. Intriguingly, its levels were notably reduced in tumour tissue compared to healthy tissues, suggesting its potential as a lncRNA with tumour-suppressive properties in the context of lung cancer. Patients with higher expression levels of BX357664 exhibited earlier tumour stages, lower incidence of lymph node metastasis or distant metastasis, better prognosis, and higher overall survival rates, as reported (Yang *et al.*, 2019a). Notably, certain lncRNAs exhibit remarkably low expression levels but exert a substantial loss-of-function phenotype. One such example is VELUCT, which, upon silencing, leads to a significant reduction in cell viability (Seiler *et al.*, 2017).

Previous research studies have provided insights into the intricate functions of lncRNAs and have also identified their downstream target genes. The upregulation of the lncRNA DANCR in lung cancer tissues has been extensively documented, and its ability to inhibit p21 expression has been identified. This inhibition accelerates the progression of non-small-cell lung cancer (Guo *et al.*, 2019). Another publication has proposed a similar functional role for DANCR, albeit through a distinct mechanism. In this case, DANCR was found to promote tumour invasiveness by regulating the expression of HMGA-2 (Zhang and Jiang, 2019). The previous discovery revealed that the lncRNA FAM83A-AS1 not only elevates the protein level of FAM38A but also activates ERK1/2 phosphorylation, which occurs downstream of FAM83A.

Consequently, this cascade enhances the proliferation and invasion capabilities of lung adenocarcinoma cells (Shi *et al.*, 2019).

Numerous lncRNAs have been pinpointed as contributors to the advancement, development of tumours, and metastasis in lung cancer by selectively influencing specific miRNAs. Particularly, the lncRNA FOXC2-AC1 has demonstrated its importance in the malignant progression of lung cancer by modulating miR-107 (Wu *et al.*, 2019). Another lncRNA, CAR10, was observed to bolster the metastasis of lung adenocarcinoma by interacting with miR-30 and miR-203. This interaction subsequently resulted in the modulation of SNI1 and SNI2 expression (Ge *et al.*, 2019). Multiple studies have provided evidence that DANCR interacts with several miRNAs, including miR-216a, miR-758-3p, and miR-496, exacerbating the progression of lung cancer (Jiang *et al.*, 2017b; Lu *et al.*, 2018; Wang and Jiang, 2018; Zhen *et al.*, 2018).

The existing body of literature extensively explores how lncRNAs regulate various pathways. When lncRNA FLVCR1-AS1 is silenced, it impedes the Wnt/β-catenin signalling pathway, leading to reduced proliferation, migration, and invasion of lung cancer cells (Lin *et al.*, 2019). Conversely, in lung cancer cells, heightened expression of lncRNA SNHG1 activates the Wnt/β-catenin signalling pathway, fostering the advancement of lung cancer (Cui *et al.*, 2017). A prior study revealed that LINC00963 stimulates the activation of the AKT/mTOR signalling pathway, thus facilitating lung cancer metastasis (Yu *et al.*, 2017b).

Chemoresistance represents a significant contributor to treatment failure in lung cancer. Fortunately, several lncRNAs have been identified as being associated with cisplatin resistance, which is considered the gold standard for lung cancer treatment. Previous publications have implicated several lncRNAs, namely AK126688, NEAT1, and HOTAIR, in cisplatin resistance. Specifically, knockdown of AK126688 in lung

cancer cells resulted in a substantial reduction in the apoptosis rate induced by cisplatin (Yang *et al.*, 2013). In its capacity as a ceRNA, NEAT1 was found to upregulate EGCG-induced CTR1, leading to enhanced cisplatin sensitivity in both *in vitro* and *in vivo* settings of lung cancer cells. (Jiang *et al.*, 2016). Repression of HOTAIR has been identified as a means to reverse cisplatin resistance by modulating the miR-326/SP1 pathway (Li *et al.*, 2016a). Additionally, higher expression of HOTAIR has been observed in cisplatin-resistant non-small cell lung cancer patients compared to non-resistant patients. Decreasing HOTAIR expression enhanced cisplatin sensitivity, and experimental evidence suggests that this effect may be mediated through the upregulation of Klf4 (Liu *et al.*, 2016).

2.2.3 LncRNAs in digestive system cancers

Numerous studies have demonstrated the involvement of lncRNA in the regulation of tumours at these levels, indicating its potential as a significant indicator for the diagnosis, treatment, and prognosis of digestive system cancer (Lv and Huang, 2019; Deng *et al.*, 2020; Gao *et al.*, 2020). CRC is a prevalent form of cancer worldwide, with an annual diagnosis of approximately one to two million new cases. This places CRC as the third most common cancer and the fourth leading cause of cancer-related deaths, accounting for approximately 700,000 deaths each year (Mármol *et al.*, 2017). The survival rate for individuals diagnosed with advanced-stage CRC, characterised by metastasis to distant organs, stands at 13.1% (Simon, 2016). During this stage, treatment typically shifts towards palliative care, and the associated financial burdens become substantial. Global statistics indicate that approximately 70% of patients with gastric cancer (GC) succumb to the disease within a 5-year follow-up period (Verdecchia *et al.*, 2007). A multitude of factors, such as smoking, alcohol intake, dietary patterns, and *Helicobacter pylori* infection, have been strongly linked to the susceptibility of

developing GC. The elevated mortality rate associated with GC can be ascribed to the complex interaction between factors related to the individual and their environment. Unfortunately, patients with GC are often diagnosed at advanced stages, characterised by the occurrence of metastasis and a generally unfavourable prognosis. In such cases, palliative chemotherapy serves as the primary treatment approach (Digklia and Wagner, 2016). Pancreatic cancer (PC), although relatively uncommon, exhibits aggressive behaviour with a rapid growth rate, often leading to late-stage diagnosis. Due to the challenges associated with early detection, the majority of patients miss the opportunity for surgical intervention at the time of diagnosis. Notably, patients diagnosed with grade III differentiation experience particularly low survival rates, with only 7.6% surviving (Li *et al.*, 2022c). Epidemiological investigations have unveiled a connection between the incidence and advancement of pancreatic cancer and an array of risk factors. Factors unrelated to genetics, such as prolonged tobacco use, a diet rich in fats, and the presence of chronic pancreatitis or coexisting diabetes, have been linked to the onset of pancreatic cancer (Rebelo *et al.*, 2017).

The identification of an effective and highly sensitive biomarker is crucial and pressing in order to enhance the diagnosis and treatment of digestive system cancers. In recent years, advancements in molecular biology technology have led to an upsurge in research conducted at the molecular and cellular levels. The regulatory mechanism of miRNA sponges has emerged as an important pattern in gene regulation. Through their ceRNA function, many studies have focused on the modulation of lncRNA expression. By targeting specific lncRNAs, researchers aim to inhibit the proliferation and invasion of cancer cells and prevent the progression of tumour processes. Significantly, certain lncRNAs are common to various types of digestive system cancers, yet they exert their functions through distinct mechanisms. One illustrative example is CRNDE, which has

been observed to enhance the migratory and invasive abilities of CRC cells when overexpressed. CRNDE functions as a regulator by targeting miR-136, and further in-depth mechanistic studies have revealed that this interaction leads to the regulation of its endogenous target, E2F transcription factor 1 (Gao *et al.*, 2017a). Furthermore, studies have reported that CRNDE can also modulate miR-217 (Yu *et al.*, 2017a) and miR-181a-5p (Han *et al.*, 2017) in CRC. Interestingly, a previous study suggests that CRNDE knockdown resulted in a significant inhibition of PC cell proliferation, migration, and invasion, observed in both *in vitro* and *in vivo* experiments. Furthermore, it has been discovered that CRNDE exerts a positive regulation on IRS1 expression by functioning as a miR-384 sponge, as reported (Wang *et al.*, 2017a). In addition, in the context of GC, miR-145 has been identified as a target of CRNDE acting as a sponge. One of the mechanisms by which CRNDE enhances the growth of tumour cells is its competitive interaction with miR-145. By sequestering miR-145 within the cell, CRNDE effectively neutralizes the inhibitory effects of miR-145 on E2F3. This, in turn, allows CRNDE to exert its oncogenic functions by regulating the miR-145/E2F3 axis (Hu *et al.*, 2017).

Another lncRNA, GAS5, operates as a tumour suppressor and has been observed to be diminished in both CRC tissues and CRC cell lines. Elevated levels of GAS5 have demonstrated a substantial reduction in CRC cell proliferation and an enhancement in apoptosis. Mechanistically, GAS5 exerts its inhibitory effect on CRC by modulating the activity of miR-182-5p through the regulation of FOXO3a (Cheng *et al.*, 2018b). It has been proposed that in GC, the GAS5/miR-222 axis plays a regulatory role in the proliferation of GC cells through the PTEN/Akt/mTOR pathway (Li *et al.*, 2017g). In PC, GAS5 positively regulates the PTEN-induced tumour-suppressor pathway by modulating miR-32-5p, thereby suppressing PC metastasis (Gao *et al.*,

2017b). Furthermore, GAS5 has been identified as a ceRNA for miR-221 in both CRC and PC (Liu *et al.*, 2018b; Liu *et al.*, 2018c).

lncRNA plays a crucial role in combating chemoresistance in digestive system cancer as well. In digestive cancers, particularly CRC and GC, 5-fluorouracil (5-Fu) is widely utilised as the most frequently employed chemotherapeutic drug. In a prior investigation, it was illustrated that decreasing the expression of lncRNA CRNDE while elevating the levels of miR-181a-5p led to heightened responsiveness of CRC cells to 5-Fu treatment. Conversely, when CRNDE was overexpressed and miR-181a-5p was inhibited, the susceptibility of CRC cells to 5-Fu therapy was diminished (Han *et al.*, 2017). By inhibiting EMT, the overexpression of LEIGC enhanced the sensitivity of GC cells to 5-Fu treatment (Han *et al.*, 2014). Autophagy has been recognised as a significant contributor to chemotherapy outcomes. In CRC, the up-regulation of SIRT1-mediated autophagy can be facilitated by lncRNA H19 through the modulation of miR-194-5p, thereby resulting in the development of resistance to 5-Fu (Wang *et al.*, 2018c). Attenuation of autophagy and increased sensitivity to 5-Fu treatment can be achieved by knocking down lncRNA NEAT1. This is accomplished through the targeting of miR-34a in CRC (Liu *et al.*, 2020b). Down-regulation of lncRNA FGD5-AS1 exhibited a notable anti-tumour effect on GC proliferation, both *in vitro* and *in vivo*. Additionally, it suppressed the chemoresistance response to 5-Fu in GC (Gao *et al.*, 2020).

Oxaliplatin (OXA) is a platinum-based compound frequently employed in the treatment of CRC and GC (Dy *et al.*, 2009). Similar to 5-Fu, a significant number of patients undergoing treatment with OXA experience the development of chemoresistance and metastasis (Goldberg *et al.*, 2004). The lncRNA CCAL has been linked to apoptosis and reduced chemoresistance to OXA in CRC cells, indicating its potential as a therapeutic target for reversing chemoresistance (Deng *et al.*, 2020).

LncRNA BLACAT1 exhibits up-regulation in both OXA-resistant GC tissue and cells. However, the knockdown of BLACAT1 demonstrates the ability to inhibit ABCB1 expression, invasion, and confer OXA sensitivity, resulting in increased apoptosis in both *in vitro* and *in vivo* (Wu *et al.*, 2018). The maintenance of cancer stemness and the development of chemoresistance are strongly influenced by the factor of stemness. An illustrative example is lncRNA H19, which not only overcomes resistance to 5-Fu but also confers resistance to OXA in carcinoma-associated fibroblasts (CAFs). The overexpression of H19 significantly promoted OXA resistance in CRC, whereas the knockdown of H19 suppressed tumour growth in a xenograft model. The study revealed that exosomes derived from CAFs upregulate the expression of H19, stemness, and OXA resistance in CRC cells, both *in vitro* and *in vivo* (Ren *et al.*, 2018).

Cisplatin (DDP)-based chemotherapy serves as the cornerstone of GC treatment. However, the emergence of cisplatin resistance can contribute to tumour recurrence (Amable, 2016). A substantial body of evidence suggests that exosomes derived from cancer cells can play a role in promoting tumour progression and metastasis (Kahlert and Kalluri, 2013). Additionally, exosomes derived from chemosensitive or chemoresistant cells have been found to influence therapeutic response by transferring specific lncRNAs (Qu *et al.*, 2016; Xu *et al.*, 2016). Observations in DDP-resistant GC cells revealed the presence of EMT and elevated levels of lncRNA HOTTIP. The down-regulation of HOTTIP demonstrated a reduction in cisplatin sensitivity. Exosomal HOTTIP was found to activate HMGA1, thereby inducing resistance to DDP in (GC cells (Wang *et al.*, 2019a). FOXD1-AS1 was found to promote the resistance of GC cells to DDP. The depletion of FOXD1-AS1 reversed DDP resistance both *in vitro* and *in vivo* by targeting the PI3K/AKT/mTOR pathway (Wu *et al.*, 2020a). DDP is occasionally employed in CRC treatment, although not as commonly as 5-Fu and OXA.

Another noteworthy instance of autophagy involvement in chemoresistance is observed in CRC, where lncRNA SNHG14 stimulates autophagy in CRC cells through the miR-186/ATG14 axis (Han *et al.*, 2020).

Gemcitabine-based chemotherapy is considered the primary treatment for PC. However, similar to CRC and GC, the development of gemcitabine resistance presents a significant obstacle in the treatment of PC (Ju *et al.*, 2015). In PC tissues and gemcitabine-resistant cell lines, there was observed an overexpression of lncRNA SLC7A11-AS1. The knockdown of SLC7A11-AS1 has been shown to enhance the sensitivity of PC cells to gemcitabine, indicating that SLC7A11-AS1 holds promise as a target for overcoming gemcitabine resistance in PC (Yang *et al.*, 2020c). The study revealed that PVT1-induced gemcitabine resistance in PC was linked to heightened activation of the Wnt/β-catenin signalling pathway and increased autophagic activity. PVT1 directly targeted miR-619-5p, and the introduction of miR-619-5p resulted in the reversal of gemcitabine resistance in PC (Zhou *et al.*, 2020). Furthermore, numerous other lncRNAs including TUG1, HOTAIR, GAS5, and HOTTIP, have also been implicated in the regulation of gemcitabine resistance in PC (Li *et al.*, 2015; Wang *et al.*, 2017b; Gao *et al.*, 2018; Yang *et al.*, 2018a). A similar example can also be observed in CRC, specifically with lncRNA AGAP2-AS1. AGAP2-AS1 functions as a ceRNA for miR-497, which in turn targets fibroblast growth factor receptor 1. Silencing AGAP2-AS1 has been shown to reduce gemcitabine resistance and induce G1/M phase cell cycle arrest in CRC cells (Hong *et al.*, 2020).

2.3 Colorectal cancer

CRC is a malignant digestive system disease that arises from the genetic and epigenetic alterations of the colon epithelial mucosa due to various factors such as

environmental and hereditary causes. The causes of CRC are complex. Factors such as age over 40, geographical location, and gender can all influence the incidence of this disease (Hall *et al.*, 2015). In addition, factors such as unhealthy lifestyle habits (such as long-term smoking, high sugar and fat intake, and sustained emotional stress), parasites, chemical pollution, and genetics can also lead to the onset of the disease. The development of CRC can be influenced by genetic factors in both sporadic and familial cases (Lichtenstein *et al.*, 2000). Sporadic cases account for approximately 70%-80% of all CRC instances, predominantly affecting individuals over the age of 50. Dietary and environmental factors have been implicated in the aetiology of these cases (Müller *et al.*, 2016). In contrast, about 20%-30% of CRC cases have a hereditary component. Studies have shown that germline mutations or epimutations in DNA mismatch repair (*MMR*) genes, such as *MLH1*, *MSH2*, *MSH6*, or *PMS2*, are associated with hereditary nonpolyposis CRC (Valle, 2014). Several factors contribute to this variation, including differences in risk factor exposure and demographic profiles, as well as genetic susceptibility and mutations, which affect prognosis and treatment response (Baidoun *et al.*, 2021). In 2020, global cancer statistics showed that out of 36 prevalent cancer types, CRC was responsible for more than 1.9 million new cases and 935,000 deaths, making up approximately 1/10 of all cancer cases and deaths. In terms of incidence and mortality, CRC ranks 3rd and 4th, respectively (Sung *et al.*, 2021). CRC ranks as the most prevalent cancer among Malaysian men with an age-standardised incidence rate of 14.8 per 100,000. For Malaysian women, it's the second most prevalent with a rate of 11.1 per 100,000. Additionally, it stands as the third leading cause of cancer-related deaths in Malaysia (Schliemann *et al.*, 2020). In China, CRC is the third most frequently diagnosed cancer. In 2020, it was estimated that there were 555,477 new CRC cases and 286,162 deaths attributed to CRC (Chen *et al.*, 2022).

Unfortunately, the early symptoms of CRC are usually subtle, leading to many patients already being in the advanced stages of the disease at the time of their initial medical consultation, resulting in an alarmingly low rate of early detection. The progression of colorectal cancer tends to be slow, and patients often show no specific symptoms in the early stages. As the tumour grows, symptoms such as abdominal discomfort, unexplained diarrhoea, and signs of peritoneal irritation gradually appear. Around 20% of patients are diagnosed with distant metastasis when initially diagnosed with colorectal cancer (CRC), indicating its significant metastatic nature and aggressive behaviour. In the absence of treatment, metastatic CRC exhibits a median survival duration of just 9 months and a 5-year survival rate as low as 3% (Chakedis and Schmidt, 2018).

2.3.1 Screening and diagnosis of CRC

2.3.1(a) Routine tests

Digital rectal examination can determine the size of the tumour, its encroachment on the circumference of the intestine, distance from the anal edge, and surface characteristics, among other things. However, it is relatively subjective and can be difficult to accurately determine the depth of invasion. Its accuracy depends on the experience of the physician. In patients with suspected cancer, the diagnostic accuracy of digital rectal examinations was 63.45% (Ying *et al.*, 2023). This method is unable to evaluate lymph node metastasis.

Faecal occult blood testing is regarded as the most straightforward, cost-effective, and efficient non-invasive initial screening technique for colorectal cancer (Benson *et al.*, 2008). This test can be categorised into two methods: chemical and immunological. The chemical faecal occult blood test, symbolised by the guaiac resin method and predominantly performed using the phenolphthalein method, is applicable

for clinical faecal occult blood screening in instances of upper gastrointestinal bleeding and routine health inspections in the general population. Yet, this method is influenced by certain dietary elements and medications, leading to a substantial false-positive rate (Haug and Coupé, 2020). The immunological faecal occult blood test, chiefly utilising the colloidal gold technique, uses specific antibodies to identify human haemoglobin or transferrin, and this method is less susceptible to food and medication interference. However, the haemoglobin immunoassay is not applicable for upper gastrointestinal bleeding cases. This is because the enzymes excreted by intestinal bacteria can degrade haemoglobin, thus eradicating its immunogenicity, which in turn results in the inability of the haemoglobin to bind with antibodies, leading to false-negative outcomes (Yang *et al.*, 2018b). For faecal occult blood screening for colorectal cancer, the presence of bleeding is a prerequisite. Non-bleeding tumours may be overlooked, and a variety of conditions like inflammation, haemorrhoids, gastrointestinal bleeding, and differing extents of bleeding in some healthy people could also result in false positives (Taksler *et al.*, 2018). As a result, there is a need for enhancement in the sensitivity and specificity of faecal occult blood testing.

2.3.1(b) Tumour marker tests

Confronted with the growing incidence of CRC, the development of novel diagnostic concepts and indicators for early detection and treatment has become a priority for many scientific and clinical researchers. Before surgery, CRC patients typically undergo routine blood tests, liver and kidney function tests, and tumour marker tests. Clinical research has found that CRC patients, especially those in the late stages, often show a decrease in haemoglobin (Hb) (McSorley *et al.*, 2019) and albumin (Alb) and an increase in tumour markers (Lech Pedersen *et al.*, 2020). Tumour markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199) are widely