Diagnostics and Therapeutic Advances in GI Malignancies
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Ganji Purnachandra Nagaraju
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Novel Therapeutic Approaches for Gastrointestinal Malignancies

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Diagnostics and Therapeutic Advances in GI Malignancies

This series will highlight the recent innovations in the diagnostics and therapeutic strategies for different Gastrointestinal (GI) cancers.

Gastrointestinal cancers are a group of cancers that affect the digestive system and include gastric cancer, colorectal cancer, liver cancer, esophageal cancer, and pancreatic cancer. GI cancers are the leading health problem in the world and their burden is increasing in many countries. This heavy burden is due to the lack of effective early detection methods and to the emergence of chemoradioresistance. Attempts at improving the outcome of GI cancers by incorporating cytotoxic agents such as chemo drugs have been so far disappointing. These results indicate that the main challenge remains in the primary resistance of GI cancer cells to chemotherapy in the majority of patients. Therefore, improvement in the outcomes of these malignancies is dependent on the introduction of new agents that can modulate the intrinsic and acquired mechanisms of resistance.

The increased understanding of the biology, metabolism, genetic, epigenetic, and molecular pathways dysregulated in GI cancers has revealed the complexity of the mechanisms implicated in tumor development. These include alterations in the expression of key oncogenic or tumor suppressive miRNAs, modifications in methylation patterns, the upregulation of key oncogenic kinases, etc.

The individual books in this series will focus on the genetic basis of each gastrointestinal cancers, molecular pathophysiology, and different biomarkers to estimate cancer risk, detection of cancer at microscopic dimensions, and suitable and effectiveness of the therapies. In addition, the volumes will discuss the role of various signaling molecules/pathways and transcriptional factors in the regulation of the tumor microenvironment and effect on the tumor growth.

Lastly, it will elaborate the use of molecularly targeted drugs that have been proven to be effective for the treatment of GI cancers, with a focus on the emerging strategies.

This edition will provide researchers and physicians with novel ideas and perspectives for future research that translates the bench to the bedside.

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This book is dedicated to our families,
our teachers, and friends.

Preface

Gastrointestinal malignancies (GI) refer to a group of highly aggressive neoplasm of the gastrointestinal tract. It is a major public health issue worldwide and a leading cause of mortality and morbidity. The current therapeutic strategies manifest uncertain results and poor overall survival rate. There are several factors, including environmental and genetic factors, that promote GI cancers. Regardless of the traditional therapies such as surgery and radio- and chemotherapy, the five-year survival remains low in many patients with GI cancer. Previous studies have suggested that GI malignancies are heterogeneous and are found to develop recurrence and metastasis. This is due to the resistance developed by tumor cells. In this book, we will try to gather and put forward the novel therapeutic strategies against GI cancer.

GI malignancies include esophageal, pancreatic, gastric, liver, and colorectal cancers. These cancers are found to be extremely lethal and malignant. Fatality caused by GI cancer is due to the aberrantly acting transcription factors and tumor suppressor genes. The present book focuses on the role of a few selected transcription factors like STAT3 and HIF-1α. These transcription factors play a crucial role in developing resistance against chemo drugs and promote metastasis. The tumor microenvironment in the altered epithelium stroma constitutes cytokines, growth factors, matrix metalloproteinases, and angiogenic factors that promote angiogenesis and metastasis and inhibit apoptosis. Therefore, a better understanding of the tumor microenvironment and its functions is very much essential to design improved therapeutic strategies to treat GI cancers. Furthermore, the book focuses on a few chapters that include modulators for tumor microenvironment that are essential for designing therapeutic agents. Adiponectin, secreted by intra-abdominal adipose tissues, undergoes an inflammatory transformation as well as acts in an anti-inflammatory way. Further, authors have described the role of adiponectin in GI malignancies in the book. Additionally, exploring molecular mechanisms and signaling pathways that induce tumorigenesis is helpful in drug delivery and targeted therapies.

The GI malignancies are highly fatal due to their delayed diagnosis in advanced stages, which is mainly due to the nonavailability of biomarkers. Biomarkers indicate the condition of the disease along with the response shown during the treatment. Authors have focused on the epigenetic biomarkers and their development in diagnosing GI-related cancers. Moreover, recent advanced technologies that are developed through research, including meta-analysis and bioinformatics, are also included. The chapter on meta-analysis gives a clear picture of determining the increased risk for cancer in the patient. Similarly, bioinformatics is essential for identifying genes involved in progression and that interacts with drugs. Thus, it plays a crucial role in drug designing and targeted therapy. At present, researchers are widely concentrating on phytochemicals to avoid toxic side effects and protect the healthy cells from chemo drugs. However, the bioavailability of phytochemicals is always a limitation and is not encouraged clinically. The development of nanotechnology comes in rescue for the delivery of phytochemicals, which increases the half-life and bioavailability of the phytomedicine used at the tumor site. This book provides the importance of phytochemicals and applications in using nanotechnology for their delivery.

Altogether, this book provides an in-depth understanding of the therapeutic options currently available. We have explored current advancements in a precise way and included applications for GI malignancy therapy. It is our great pleasure to present this comprehensive summary of novel therapeutic strategies to the science community for the benefit of patients and their families.

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Contents

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Chapter 1
Targeting Pathways in GI Malignancies

Neha Merchant and Ganji Purnachandra Nagaraju

Abstract Gastrointestinal (GI) malignancy is one of the most fatal diseases around the world. Increasing awareness about the tumor pathogenesis has led to the identification of various targeting pathways, which could serve as potential therapeutic strategy in combating this disease. Choosing the correct targeted therapy depending on the biomarkers can instigate an era of customized medicine and change the way oncology is practiced. Various targeting agents have been approved for treating gastrointestinal malignancies that particularly target tumor angiogenesis. Numerous other agents are still in their developmental phases. In this chapter, we summarize important targeting pathways in GI malignancies and how targeting these pathways could improve the overall gastrointestinal cancer treatment outcome.

Keywords GI malignancies · Esophageal cancer · Gastric cancer · Colorectal cancer · Signaling pathways · MAPK · PI3K/Akt · EGFR and HER2 · VEGF · HGF/MET · RhoA · JAK/STAT · VEGF · Notch · TGF-β · Wnt

Abbreviations

CDH1	Cadherin-1
CIN	Chromosomal instability
CRC	Colorectal cancer
EBV	Epstein-Barr virus
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor

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ERK	Extracellular signal-regulated kinase
FGFR2	Fibroblast growth factor receptor 2
GC	Gastric cancer
GI	Gastrointestinal
GIN	Gastric intraepithelial neoplasia
Grb2	Growth factor receptor bound protein 2
GTPase	Guanosine triphosphate
HER2	Human epidermal growth factor receptor 2
HGF	Hepatocyte growth factor
HIF-1α	Hypoxia inducible factor 1 alpha
JAK2	Janus kinase 2
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MSI	Microsatellite instable
mTOR	Mammalian target of the rapamycin
NICD	Notch intracellular domain
PI3K	Phosphoinositide 3-kinase
PI3KCA	Phosphatidylinositol 3-kinase catalytic alpha polypeptide
RhoA	Ras homologue A
RTK	Receptor tyrosine kinase
SOS	Son of seven-less
STAT	Signal transducer and activator of transcription
TGFB1	Transforming growth factor beta 1
TGF-β	Transforming growth factor beta
VEGF	Vascular endothelial growth factor

1.1 Introduction

Gastrointestinal (GI) malignancy is a broad term that includes a group of malignancies that affect the intestinal tract. GI malignancies include cancers of the esophagus, gastric tract, colon and rectum, liver, and the pancreas [1]. In the USA and the world, GI cancers pose an extreme public health risk, leading to an estimated 333,680 fatalities in 2020 in the USA [2]. GI malignancies have the largest incidence rate in the USA, according to the American Cancer Society, and is ranked second among the leading causes of malignancy-associated fatalities. Risk factors associated with GI malignancies vary among the type of cancer such as smoking, age, diet, alcohol consumption, obesity, and chronic pancreatitis [3]. Diagnosis of GI malignancies also depends on the type of cancer, which, upon determination, confirms the cancer stage and a subsequent treatment plan. Traditional treatment strategies include surgery, chemotherapy, and radiotherapy [4]. These options are not very effective due to the activation of resistant signaling pathways [5]. Therefore, modern therapies are emerging to combat the shortcoming of traditional strategies, which include combination therapy, immune therapy, targeted therapy, and nano therapy. In this

chapter, we will be discussing some important pathways for specific GI cancers as well as overlapping pathways in GI malignancies.

1.2 Important Targeting Pathways in Esophageal Cancer

1.2.1 MAPK Signaling Pathway

MAP-Kinase of MAPK pathways comprise three different pathways, namely ERK, SAP/JNK, and p38 [6]. These pathways are activated via growth factors, cytokines, altered temperature, and hypoxia by several cell surface receptors [7]. In esophageal cancer, the MAPK pathway is activated by the gastric and bile acid [8, 9], as well as by the cytotoxic agent etoposide [10]. Receptor tyrosine kinase (RTK), integrins, and G-protein-related receptors are cell surface receptors that are known to stimulate the MAPK pathway [7]. A cascade of phospho-proteins is triggered by GTPase signal transducer proteins such as RAS and RAF following the initiation of cell surface receptors. GTPase signal transducer proteins act as a hub that receives signals from various cell surface receptors [11]. They amplify signals forward via different signaling pathways. The ERK MAPK pathway is active in around 60% of esophageal cancers [10]. Active ERK MAPK signaling pathway tumors exhibit metastases and poor prognosis, suggesting that blocking the ERK MAPRK pathway in esophageal cancer can have crucial therapeutic benefits.

1.2.2 PI3K/Akt Pathway

The PI3-Kinase pathway is stimulated via RTKs and RAS. RTK and RAS activation is followed by AKT phosphorylation through PI3K. PI3K pathway activation triggers glycolysis, cell growth, and proliferation, particularly via cMyc and HIF-1α (hypoxia inducible factor 1 alpha) activation [12]. PI3K pathway components are often up regulated in esophageal cancer. Phosphorylated AKT expression is also elevated in esophageal cancer tissues as compared to normal Barrett's and epithelial tissue [8]. The PI3K/Akt pathway mutations are frequently seen in esophageal cancers [13], which makes it an interesting pathway to investigate further and recognize how these signaling pathways interact with one another [13]. More vitally, targeting PI3K/Akt signaling pathways can have synergistic effects on esophageal cancer.

1.3 Important Targeting Pathways in Gastric Cancer

1.3.1 EGFR and HER2 Signaling Pathway

Gastric cancers (GC) often overexpress the EGFR. It is involved in pathological processes such as metastasis, tumor cell motility, and invasion [14, 15]. GC is characterized either by EGFR amplification or mutation [16–18]. EGFR binds to various ligands such as TGF-α and epidermal growth factor (EGF), and triggers signal transduction cascades, which can stimulate PI3K and MAPK signaling pathways. Subsequently, EGFR is a vital factor in the migration, proliferation, differentiation, and survival of malignant cells [18, 19]. A dominant oncogene pathway in most GC cases is the RTK-RAS pathway and the genes associated with these pathways are mutual to each other in gastric malignancies [16]. These genes include MET, EGFR, FGFR2, HER2, and KRAS, which are generally elevated in CIN molecular categories of GC [20]. HER2 overexpression and amplification are exhibited in GC cases, which is linked to poor prognosis and disease aggressiveness [21].

1.3.2 The VEGF Pathway

The VEGF growth factor family consists of VEGFA, which encodes a protein identified as a disulfide-linked homodimer. It acts explicitly on endothelial cells and regulates multiple effects such as elevated vascular absorptivity, endothelial cell development, angiogenesis, and vasculogenesis. Thus, VEGFA promotes cell migration and inhibits apoptosis. VEGFA overexpression is often reported in the majority of GC cases. It is also known as an initial marker during the advancement of GC [22–24]. Moreover, VEGFA expression is linked with lymph node metastasis and poor prognosis [25]. Other growth factors are also overexpressed in GC cases like VEGFC and VEGFD [26]. Elevated expression levels of these growth factors have been associated with lymphatic invasion [27]. GIN-GC (Gastric intraepithelial neoplasia) exhibits recurrent amplification of VEGFA, and these patients are excellent candidates for VEGF-targeted therapies [28].

1.3.3 The PI3K/AKT/MTOR Pathway

The PI3K intracellular kinase family facilitates the modulation of cell migration, metabolism, survival, proliferation, and differentiation [29]. The downstream of stimulated RTKs is subunit p110α of PI3K like EGFR and HER2 [30]. Subunit p110α is a stimulator of AKT and a downstream effector of the mTOR pathway [31]. The PI3K/AKT/MTOR pathway is stimulated by RTK activation, loss of

PTEN functionality because of mutations, PI3KCA-activating applications and mutations, and AKT1 activating mutations [32]. The PI3K/AKT/MTOR signaling pathway is generally activated in gastric malignancies along with overexpressed PI3KCA [33–35] and AKT phosphorylation [36, 37]. Modifications in PI3KCA are detected in molecular subtypes of GC such as EBV and MSI [20].

1.3.4 The HGF/MET Pathway

The HGF/MET pathway is exemplified via a combined action of two proteins such as hepatocyte growth factor (HGF) and MET, which is its single known receptor [38]. Both HGF and MET are known to modulate cellular processes including migration, angiogenesis, metastasis, proliferation, and invasion [39]. These proteins thereby lead to the stimulation of multiple pathways such as PI3K-AKT, STAT, MAPK, and v-src [40]. Some recent investigations have highlighted the role of MET through crosstalk, along with other cell membrane proteins and receptors like EGFR and TGFB1 [41], which contribute toward drug resistance and carcinogenesis [42]. HGF and MET are often overexpressed in advanced stages of GC [43, 44]. MET overexpression is also linked with poor prognosis in advanced gastric malignancy cases [45, 46]. Various mechanisms trigger the inappropriate MET signaling. The genomic rearrangement of *MET* results in its stimulation through the kinase domain dimerization and allows MET to elude the general down regulation mechanism [47]. Precursor GC lesions and some adjoining normal mucosa exhibit TRO-MET chromosomal translocation [48]. GC lesion-related genetic mechanisms include gene mutation, MET and MHGF gene transcriptional upregulation, and gene amplification [49]. Consistently, some advanced GC cases have exhibited MET gene amplification along with subsequent protein overexpression and kinase activation [50]. Some CIN gastric malignancy lesions exhibit MET amplifications and some MSI GC subtypes exhibit MET mutations [20].

1.3.5 The RhoA Signaling Pathway

The Ras homologue A or the RhoA pathway plays a key role in GC growth, migration, apoptosis, and adhesion [51]. Rho GTPases are key intracellular signaling molecules, which can modulate cell motility, cytoskeleton organization, and cell cycle. In GC, the Rho activity disrupts the epithelial layer, fosters motility, and induces degradation of the ECM (extracellular matrix) in order to promote metastasis [52]. Recent investigations have revealed that CDH1 mutations and RHOA mutations are strongly correlated to the histologic diffuse type GC [53, 54], which enriches the genomically stable (GS) subcategory of GC [20]. Overall, the RhoA signaling pathway is crucial in regulating cell death of GC. Therefore, inhibiting the RhoA signaling pathway could become a novel therapeutic treatment for GC cases.

1.3.6 The JAK/STAT Pathway

JAK is overexpressed in the GC subcategory Epstein-Barr virus (EBV) [55]. The JAK/STAT pathway is noticed in various tumors such as GC, which makes JAK2 inhibitors a possible therapeutic target for GC patients [56, 57]. JAK2 is a very influential kinase depending on the interactions between growth hormone receptors and cytokine receptors [58]. Upon JAK2 activation via phosphorylation, the following actions takes place: stimulation of the STAT phosphorylation, gene expression that is participated in cell proliferation and apoptosis arrest [59]. The JAK/STAT signaling pathway plays a significant role in cytokine as well as growth factor cascade by regulating various cellular processes such as cell survival, proliferation, differentiation, and migration [60]. Various animal and in vitro investigations have exhibited that uncontrolled JAK/STAT pathway is a major driving strength for several malignancies including GI cancers [61, 62]. In GC, the aberrant STAT3 expression contributes to cell survival and proliferation and promotes inflammation, metastasis and EMT transition [23, 63, 64]. Multiple investigations have confirmed that STAT3 plays a crucial role in precancerous pathology of the stomach, which indicates that it can serve as a powerful predictive marker for initial detection of GC [65]. It has also been confirmed that limiting STAT3 activities can even aid in preventing the malignancy [66]. Therefore, many preclinical and clinical investigations targeting the JAK/STAT pathway are underway. Hence, it has been suggested that aberrant JAK/STAT targeting in GC can exhibit great potential in treating patients with advanced stages of gastric malignancies and the inhibitors of the JAK/STAT pathway that are in their clinical trial stages for solid tumors must be tested for their efficiency and efficacy.

1.4 Important Targeting Pathways in CRC

1.4.1 EGFR/MAPK Signaling Pathway

The EGFR pathway in involved in multiple cellular processes including survival and metastasis. Abnormality in EGFR pathway controls neoplastic cell growth, and proliferation [67]. Multiple receptors like EGFR are situated upstream of MAPK signaling pathways [68]. EGFR pathway's adaptor protein complex consists of Grb2 and SOS [69]. The complex stimulates Ras-GTP through binding to the phosphorylated tyrosine molecules [70]. Upon activation of RAS, a cascade of stimulating ERK, RAF, and MEK begins via phosphorylation [71]. According to research, it has been revealed that the Raf-ERK-Ras pathway leads toward controlling cellular differentiation, growth, and survival [72]. When the Raf-ERK-Ras cascade is dysregulated, it can cause malignant transformation and tumor progression via elevated cell proliferation, angiogenesis, metastasis, anti-apoptosis, invasion, and prolonged survival [73]. The EGFR/MAPK pathway is associated with oncogenic

processes, and thereby plays a key role in CRC progression [74, 75]. Aberrant expression of EGFR/MAPK pathway can be used as a therapeutic target for CRC cases [76, 77].

1.4.2 Notch Pathway

Notch signaling is one of the extremely conserved pathways that is accountable for precise cellular interaction [78]. Adequate functionality of the Notch signaling pathway is required for normal cellular advancement, proliferation, variation, and apoptosis [79]. When the Notch ligands bind to the Notch receptors, such as Notch-1, -2, -3, and -4, of the target cell, the Notch signaling stimulation begins via γ-secretase protein complex activation and Notch receptor cleavage [80]. Subsequently, this stage is necessary to produce Notch's active (NICD) form [81]. Following NICD production, it translocates itself inside the nucleus and interacts with the CSL (inactive form), forming a complex [82]. The Notch pathway mediates the conservation of intestinal advancement and homeostasis via the modulation of the variation of goblet cells and stem cells [83]. Previous research has suggested that Notch ligands, receptors and certain downstream targets such as Hes-1, NICD, and Deltex are highly in CRC cells [84]. The Notch pathway either has an oncogenic or a tumor suppressor role [82]. Notch-1 has been reported as an oncogene in CRC [85]. A recent investigation has reported that the Notch expression is elevated in the initial CRC stages as compared to advanced stages [82]. The Notch signaling pathway promotes CRC by regulating cell apoptosis and cell cycle through P21 and PUMA gene regulation [85]. Hence, inhibiting the Notch signaling pathway could have some crucial therapeutic benefits in CRC [82, 86].

1.4.3 PI3K Signaling Pathway

PI3K signaling is activated via the EGFR pathway. As a heterodimeric molecule, PI3K consists of the following classes, I–III [87]. These classes can be characterized by the variations in their structure and functions [88]. Class Ia is an extremely implicated subtype in human malignancies including CRC [89]. Class Ia consists of two other sub-categories for PI3K: p85, which is regulatory, and p110, which is a catalytic subunit [90]. P85 isoforms are encoded via different genes such as *PIK3R1*, *PIK3R2,* and *PIK3R3,* and various types of p110 such as α, β, γ, and δ, producing PIK3-CA, -CB, and -CD, respectively [91]. Akt regulates the PI3K properties on tumor development and progression [92]. Akt phosphorylation is correlated with cell proliferation and inhibition of cell death in CRC [92]. PI3K activation can be stimulated via Extracellular factors through RTK or Ras. Upon the binding of p85 to RTK at the intracellular level, the inhibitory effect of p85 on p110 is removed, causing PI3K activation. Stimulated PI3K phosphorylates PIP2 and produces PIP3

[93]. AKT is subsequently activated via PIP3, resulting in survival. AKT modulates downstream targets like mTOR that promotes metabolism, growth, angiogenesis, and protein translation [94]. The PI3K pathway is downregulated via PTEN, which is a tumor suppressor, through dephosphorylating of PIP3 [95]. Studies have described that PI3K exhibits in CRC advancement and progression [96]. Many investigations have reported that inhibiting the PI3K pathway leads to reduced CRC cell augmentation and elevated rate of apoptosis [97].

1.4.4 TGF-β Pathway

TGF-β contributes to controlling many biological processes including migration, adhesion, apoptosis, and differentiation [98]. It has been previously studied that TGF-β pathway decreases CRC epithelial cell proliferation and induces apoptosis and differentiation [99]. TGF-β induces variation and apoptosis, and inhibits normal intestinal epithelium cell proliferation. Thus, TGF-β is known to act as a tumor suppressor in such environments [99, 100]. Therefore, CRC is one of those malignancies that form resistance against TGF-β-induced growth inhibition [101]. Moreover, studies have revealed that advanced stages of CRC highly express TGF-β, thereby producing various mitogenic growth factors such as TGF-α, EGF, and FGF. As such, in the advanced stages of CRC, TGF-β acts as a tumor promoter [102].

1.4.5 Wnt Pathway

The Wnt/β-catenin plays a critical role in maintaining tissue and hair, intestine, skin, and so on regeneration [75]. The Wnt/β-catenin pathway is divided into two categories: the canonical β-catenin-dependent and the non-canonical β-catenin-independent pathways [103]. The crypt stem cell compartment in the normal cell is maintained by the canonical Wnt pathway. This pathway also plays an opposing role in the pathology and the physiology of the cells. Abnormalities in this pathway can lead to CRC [104]. Moreover, aberrant Wnt activation is observed in human malignancies, particularly CRC. Many published studies have revealed that hyperactive Wnt plays a critical oncogenic part in CRC [105]. Consequently, Wnt signaling activation is necessary for tumor development in advanced stages of CRC, thereby contributing as an effective therapeutic target for CRC cases [104].

1.5 Conclusion

Valuable understanding into the intracellular pathways and the molecular classification of gastrointestinal malignancies is emerging through advanced and novel technological development. These advancements aid in developing new therapeutic strategies targeted toward treating gastrointestinal cancers. However, resistance toward traditional treatment options such as chemo and radio therapy remains a substantial challenge in the treatment of gastrointestinal malignancies as a result of the heterogeneity of such tumors. Therefore, there is an urgent need for new and advanced treatment options that rely on genetic as well as epigenetic aberrations, which regulate various pathways, and/or combinations of multiple pathways in GI malignancies.

Conflict of Interest None.

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Chapter 2
Immunotherapy in Gastrointestinal Malignancies

Ritu Sarin and Sujatha Peela

Abstract Gastrointestinal (GI) tumors present a high rate of morbidity and mortality worldwide. Currently used treatment modalities include surgical resection, chemotherapy, and radiation therapy, and offer modest or poor overall outcomes. The success of immunotherapy in the treatment of solid tumors such as melanoma and lung cancer in the last decade has galvanized the investigative immunotherapeutic approaches in patients with gastrointestinal malignancies. The GI tumors with high microsatellite instability (MSI) have particularly been responsive to the immunotherapeutic approaches prompting the use of precision medicine in reducing the tumor burden globally. Various combination strategies in clinical trials currently are aiming to study the effect of various targeted monoclonal antibody-based or immune checkpoint inhibitor-based approaches to improve the overall outcome in GI malignancies.

Keywords Gastrointestinal tumors · Immunotherapy · Monoclonal antibody · Checkpoint inhibitor

Abbreviations

CAR-T cells	Chimeric antigen receptor carrying-T cells
CR	Complete response
CRC	Colorectal cancer
DCR	Disease control rate

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EAC	Esophageal adenocarcinoma
EC	Esophageal cancer
ESCC	Esophageal squamous cell carcinoma
GC	Gastric cancer
hEGFR2	Human epidermal growth factor receptor 2
HNPCC	Hereditary non-polyposis colorectal cancer
mAb	Monoclonal antibody
MSI	Microsatellite instability
MSS	Microsatellite stable disease
NKs	Natural killer cells
ORR	Objective response rate
OS	Overall survival
PC	Pancreatic cancer
PFS	Progression-free survival
TILs	Tumor infiltrating lymphocytes
VEGF	Vascular endothelial growth factor
VV	Vaccinia virus

2.1 Introduction

Gastrointestinal malignancies include the cancers of organs that aid in digestion and absorption of nutrition. These include esophagus, gastric, intestine (colon), rectum, anus, pancreas, and liver. Gastric, colorectal, and liver malignancies are among the five most common cancers worldwide. Among these, colorectal cancer presents the highest incidence in developed countries, and stomach and liver cancers are predominant in developing nations [1, 2]. According to a recent report published by *American Cancer Society*, 2020, gastrointestinal malignancies are the third most leading cause of death in males and females in the United States [3]. The risk factors for these malignancies include, but are not limited to, poor diet, chronic inflammation, genetics, and infection with *Helicobacter pylori*. Current GI malignancies' treatment modalities include surgery, radiation, chemotherapy, molecular targeted therapy, and combination approach [4]. Despite several treatment approaches, the overall survival of GI cancer patients has improved modestly [5]. The imminent and ever-increasing global tumor burden due to GI malignancies has pressured the scientific and clinical community to look for alternative strategies to reduce the burden and improve overall treatment outcome.

The seeds of immunotherapy were laid by the pivotal work of William Bradley Coley in 1891 when he demonstrated the ability of the immune system to treat bone cancer [6]. More recently, significant work by James Allison and Tasuku Honjo in identifying immune checkpoint molecules as potential cancer treatment modality won them the 2018 Nobel Prize. Current cancer immunotherapeutic approaches aim toward overcoming the inhibitory blockade on the immune system in malignant condition either by resetting the immune response to tumor antigens or by mitigating

the immunosuppressive effects of the tumor microenvironment. The success of immunotherapy in improving the prognosis of patients in a broad range of solid and hematologic tumors in the last decade has brought immunotherapy to the forefront in treating GI malignancies. The results have been promising, with many studies researching the role of immunotherapy alone or as a combination therapy.

Cancer immunotherapy involves molecular targeted antibodies, cancer vaccines, adoptive cell transfer (ACT), tumor cytolytic viruses, immune checkpoint inhibitors (ICN), cytokines, and adjuvants [7]. These are currently being used as either monotherapies or as a combination treatment.

2.2 Current Immunotherapeutic Strategies

2.2.1 Immune Modulators

(a) *Immune check point inhibitors*: CTLA-4, PD-1, and its ligand PD-L1 are immune checkpoint molecules that play a role in preventing autoimmunity and promoting self-tolerance. Tumor cells evade the immune cells by overexpression of these immune checkpoint molecules. CTLA-4 functions by inhibiting naïve T cell activation and promoting suppression through T reg cells whereas PD-1 and PD-L1 function by inhibiting the activation of effector T cells [8, 9].
Anti-immune checkpoint immune modulators are targeted against these checkpoint proteins and act by lifting the brakes on the immune T cells. Anti-CTLA-4 (Ipilimumab) has been shown to provide clinical benefits in most and durable response in a portion of patients with metastatic melanoma; however, it has not had much success in gastric malignancies [10, 11]. Anti-PD1 inhibitors (Pembrolizumab and Nivolumab), on the other hand, work by inhibiting the immune checkpoint targeting the PD-1/PD-L1 pathway that promotes self-tolerance, and hence lifting the brakes on the effector T cells. Anti-PD1/PD-L1 inhibitors have had greater overall success in the treatment of malignancies and have proven to be effective in treating gastric malignancies, resulting in longer progression-free survival [10, 12]. Anti-CTLA-4 and anti-PD-1/PD-L1 combination therapies have also proven to be more effective than monotherapies [10].

(b) *Immune costimulators*: OX-40 is a costimulatory molecule on T-cells that binds to OX-40L on antigen-presenting cells to provide activating signals to the T cells. OX-40/OX-40L-based costimulation exerts its activating effects in a bidirectional approach specifically enhancing the Th_1 and Th_{17} cell-mediated responses and antagonizing T-reg-mediated suppression. Agonistic anti-OX-40 monoclonal antibody ligation to OX-40 molecule on T cells provides activating signals to T cells, enabling their potential anti-tumorigenic activity in the tumors. PF-04518600 (PF-8600) is an investigational, fully human, monoclonal antibody (mAb) immunotherapeutic OX-40 (CD134) agonist developed by Pfizer and is presently under many clinical studies for its efficacy against solid tumors.

2.2.2 Targeted Antibodies

(a) *Antiangiogenic monoclonal antibody*: Vascular endothelial growth factor (VEGF) is critical to tumor angiogenesis. Monoclonal antibodies that target the VEGF/VEGF-R pathway have demonstrated success with inhibiting tumor growth. Monoclonals under this category include bevacizumab, a recombinant humanized anti-VEGF-A antibody, and ramcirumab, which targets the VEGF/VEGF-R2 pathway. A meta-analysis of bevacizumab from four clinical studies that enrolled 2101 unresectable lung cancer patients predicted its efficacy in improving progression-free survival when administered at low doses, whereas administration at high doses was predicted to increase two-year overall survival rate thus stimulating efforts to study its safety and value in the treatment of various other malignancies including gastric malignancies [13].

(b) *Anti-Her2 mAb*: Human epidermal growth factor receptor 2 (hEGFR2) uses the tyrosine kinase-based signaling pathway. hEGFR2 is overexpressed on many cancer cell types and the dimerization of the receptor causes autophosphorylation of tyrosine residues within the cytoplasmic area of the receptor prompting cellular proliferation and enhanced tumorigenesis [14]. Anti-Her2 (Herceptin or Trastuzumab) is used for inhibiting the growth of Her2+/neu+ tumors. Other monoclonal antibodies in this category include cetuximab, a human/mouse panitumumab, and chimeric, a fully human mAb that blocks EGFR. Both the monoclonal antibodies have demonstrated modest improvements in survival.

(c) *TROP2 Abs*: TROP2 is encoded by the TACSTD2 gene. It is a transmembrane protein that is also a transducer of intracellular calcium-signaling pathway, and it is overexpressed on a variety of tumors and is understood to play a role in tumor progression, renewal, and survival. IMMU-132 (Sacituzumab govitecan) that targets TROP2 is an investigational anti-Trop-2-SN-38 Ab-drug conjugate currently under many clinical trials to study its efficacy in improving overall response and survival outcomes [15].

(d) *Bispecific Abs (BiTE/bsAb)*: Two monoclonal antibodies targeted against two unique tumor antigens are fused together to make BiTE or bispecific antibodies [16].

2.2.3 Cancer Vaccines

Tumor cells express unique tumor-connected antigens and that differentiate them from normal cells. This has potential for prophylactic as well as therapeutic vaccination. The aim of malignance vaccination is to boost the preexisting immunity or induce a strong anti-tumor response against the neo-antigens or targeted differentiation antigens [17]. Current vaccination strategies include injecting peptides resultant from the patient's tumor connected antigens or tumor connected antigen encoding gene with in vitro generated DCs. Currently, OncoVax and dendritic cell

vaccines such as autologous TriMix DCs in combination therapy are being explored in clinical trials [18].

OncoVax requires patients' own tumor cells with BCG as an adjuvant. Sipuleucel-T was the first dendritic cell-based vaccine filled with a protein combination of prostatic acid phosphatase and a macrophage-colony stimulating factor. Sipuleucel-T was approved by FDA for use in asymptomatic or minimally symptomatic castration-resistant prostate malignance. A lack of clinical benefits, especially during late-stage cancer with Sipuleucel-T led to the discontinuation of its use in clinical setting [19] and led to more recent approaches directed toward creating optimally neo or tumor-antigen-loaded more mature DCs.

2.2.4 Oncolytic Viruses

Oncolytic viruses are used to supplement the effect of immunotherapeutic agents. These viruses specifically attack tumor cells and reveal hidden tumor antigens during the process of their lytic cycle, thus acting as potential in situ therapeutic agents [20].

2.2.5 Adoptive T cell therapy

Another approach uses introducing the patient's whole immune cells expanded in vitro to destroy the tumors. In more recent approaches, a chimeric antigen receptor carrying T cells (CAR-T cells) was reprogrammed to identify the target tumor cells and destroy them. The main adverse events associated with this are cytokine release syndrome and neurological toxicity. Other immune cells that are currently being investigated for their potential in killing the tumor are natural killer cells (NKs) and tumor infiltrating lymphocytes (TILs).

2.3 Current Immunotherapeutic Approaches in GI Malignancies

2.3.1 Esophageal Cancer (EC)

Esophageal cancer (EC) is the seventh most common malignancy ranking as the eighth leading cause of death worldwide [21, 22]. Esophageal cancer may present in either of two types:

Esophageal squamous cell carcinoma (ESCC)—cancer in the squamous cell lining, or
Esophageal adenocarcinoma (EAC)—cancer in the mucus producing cells.

Current therapeutic options comprise surgical resection, radiation, chemotherapy, or combination for localized cancer treatment. In early stages with localized cancer, surgery remains the most common treatment choice; however, in advanced stages of EC, combination chemo and radiotherapy has an improved overall survival. However, the prognosis is not favorable with either ESCC or EAC to either form of systemic therapy due to the resistance of cancer caused by the high rate of mutation [23]. The high rate of mutation though makes it a favorable target for immunotherapeutic approach [24]. Further evidence regarding the abscopal effect of radiation in other cancer types suggests that immune cells may be effective in overcoming the tumor burden (TMB), thus forming a rationale for immunotherapy in ESCC or EAC [25]. The treatment of esophageal cancer that has progressed to advanced stages and is resistant to surgery is done using commonly used three immunotherapeutic approaches.

Pembrolizumab and Nivolumab are two FDA-approved anti-PD1 inhibitors for the advanced stages of treatment. The success of pembrolizumab in Keynote 180 (overall response rate ORR: 9.9%), a Phase II multicentric clinical study on patients with advanced and metastatic EAC and ESCC [26], and Keynote 181, a Phase III randomized multicentric clinical study [27], led to the its approval by FDA as a second line of treatment for recurrent esophageal cancer that progressed following systemic chemotherapy administration. Similarly, a phase II study with Nivolumab that enrolled esophageal carcinoma patients that had been pretreated also showed anti-carcinoma effects. Anti-CTLA-4 (tremelimumab) is another checkpoint inhibitor that is currently being used in combination therapy in various clinical trials. Immune-linked adverse events of immune checkpoint inhibitors generally may cause colitis, pneumonitis, hepatitis, nephritis, renal dysfunction, endocrinopathies, and severe dermatologic reactions.

Ramucirumab has also been approved as an orphan drug by FDA for the treatment of patients with advanced gastric cancer (GC) or gastroesophageal junction adenocarcinoma as either a monotherapy or in combination with nivolumab. In advanced gastroesophageal cancer due to low toxicity and increased tumor cell toxicity, it is considered as a second line of treatment [28].

Overexpression of Her2 is particularly observed in gastric and gastroesophageal cancers. In advanced gastroesophageal cancer patients that are molecularly selected for the expression of Her2 on the surface of cancer cells, anti-Her2 mAb is being used as first line of treatment. Trastuzumab (Herceptin) was adopted as choice treatment in Her2 positive patients based on improved overall success in terms of response and progression-free survival in the ToGA study, a phase III investigation that combined trastuzumab with chemotherapy in patients with Her2 positive and as monotherapy in metastatic gastroesophageal cancer patients [29].

2.3.2 Colorectal Cancer (CRC)

Colorectal cancer (CRC), the malignancy of colon and rectum occurs, mostly in the mucus-producing glands (>95%). It is the third most common malignancy worldwide [3, 30]. CRC patients are commonly associated with the occurrence of Lynch syndrome, hereditary non-polyposis colorectal cancer (HNPCC), demonstrate high microsatellite instability due to germ line mutations in one of the following mismatch repair genes—MSH2, MLH1, PMS2, and HSH6 [31]—and are associated with an improved diagnosis compared to the microsatellite stable disease (MSS) [32]. Several FDA-approved options exist for the treatment of MSI CRC cases. These range from immunomodulators to targeted mono or combination therapies. Due to an increased level of expression of PD-L1, PD-1, Lymphocyte activating gene-3, CTLA-4, and IDO, immunotherapeutic modulators including the checkpoint inhibitors can be used to activate the immune system [33]. Phase I clinical investigation of 39 patients with an anti-PD1 inhibitor produced durable complete response against CRC [34]. Pembrolizumab and Nivolumab are approved for MSI-H advanced colorectal cancer patients. Cetuximab has been approved by the FDA for the treatment of metastatic CRC with wild type KRAS. Bevacizumab is being used as a first line of therapy for patients with advanced colorectal cancer. Panitumumab is approved for patients with advanced EGFR positive colorectal cancer. Combination therapy that includes several viral platforms is currently being tried in clinical settings to study their oncolytic activity on colorectal tumors.

These include:

1. Adenovirus (common cold virus): The Ad11p/Ad3 chimeric adenovirus, in combination with nivolumab, is being verified as phase I dose-escalation trial (NCT02636036) and the LOAd703 oncolytic adenovirus monotherapy is being tested in a phase I/II trial of CRC patients.
2. Herpes simplex viruses have shown oncolytic effect on CRC stem cells, New-Castle virus (conjunctivitis and flu-like symptoms causing virus), and Reovirus (gastrointestinal and respiratory tract symptoms causing viruses). Injection of Pexa-Vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus (VV), in CRC has been shown to be safe with fewer immune-adverse events. The Pexa-Vec-durvalumab combination is in phase I and with tremelimumab is in phase II in patients with refractory metastatic CRC (NCT03206073).
3. Reovirus, double-stranded RNA oncolytic virus—It preferentially replicates and causes apoptosis in colorectal cancer KRAS mutant cells forming crystalline arrays of virions within viral inclusions and causing lysis of the host cell [35]. In a phase I dose escalation study, Reovirus serotype 3—Dearing Strain (Reolysin)—has been studied in combination with FOLFIRI (Folinic acid, Leucovorin, and Irinotecan) and bevacizumab, an anti-VEGF-A agent, in FOLFIRI-naive patients with KRAS mutant metastatic CRC (NCT01274624). This was particularly effective where cetuximab and bevacizumab have failed due to KRAS mutations in the tumor.

2.3.3 *Hepatocellular Cancer and Biliary Tract Cancer (Cholangiocarcinoma)*

Hepatocellular carcinomas are one of the leading causes of cancer-related mortality globally with an estimated 0.8 million deaths annually (https://www.cancerresearch.org/immunotherapy/cancer-types/liver-cancer). In 2019, hepatocellular and biliary tract cancers accounted for a total of 2.4% of newly reported cancer cases and caused 31,780 cancer-related deaths (5.2%) in the U.S. (https://seer.cancer.gov/statfacts/html/livibd.html). The common risk factors for HCC include viral infection with Hepatitis, B or C virus, obesity, autoimmune hepatitis, and alcoholic cirrhosis [36]. Less than half of the liver cancer cases are diagnosed early; the surgical treatment of these cases therefore presents challenges, with over 70% cases being unresectable or unsuitable as transplantation candidates due to increased tumor burden or impaired liver function. The treatment regimen for unresectable HCCs has included the cytotoxic chemotherapeutic agents: single agent (doxorubicin and 5-fluorouricil) and more recently tyrosine kinase inhibitors such as sorafenib as a first line of treatment. Failure of sorafenib as a second line of treatment, and increasing data on the success of immunomodulators, prompted the approval of ramucirumab, a direct VEGFR2 antagonist, for treating advanced, unresectable HCC in patients with at least 400 ng/mL of detectable alpha fetoprotein levels [37]. Current data on FDA-approved immune checkpoint inhibitors in the treatment of HCCs or BTCs comes from the results of three published studies. In 2017, a phase 1/2 dose-escalation and dose-expansion study, CheckMate-040 (NCT01658878), led to the approval of nivolumab for use in advanced HCC with or without chronic hepatitis as a second line of treatment. The study reported an objective response rate (ORR) of 20%; complete response (CR) 1%; disease control rate (DCR) 64%; median progression free survival, 4 months; grade 3–5 adverse events, 19% [38].

In 2017, another key study led to the approval of the anti-PD1 inhibitor pembrolizumab in non-CRC patients with advanced MMR-deficient cancer. The phase II study that also included solid unresectable mismatch repair-deficient tumors from cholangiocarcinoma patients showed promising results. Two-year overall survival (OS), Progression-free survival (PFS) and estimates measured using the Response Evaluation Criteria In Solid Tumors (RECIST v1.1) guidelines were 53% and 64%, respectively. The complete response and disease control rates measured in the study following the anti-PD1 treatment were 21% and 77%, respectively underscoring the efficacy of pembrolizumab based treatment [39].

In Keynote-224, a phase II clinical study that enrolled 104 patients with advanced hepatocellular carcinoma, the efficacy of pembrolizumab was tested as a second line of treatment. The study demonstrated an ORR of 17%; CR, 1%, DCR, 69%; median progression free survival, 7 months, and grade 3–5 adverse events, 26%.

The role of non-FDA-approved Tremelimumab, an anti-CTLA-4 monoclonal antibody in the treatment of HCC and Cholangiocarcinoma, is currently under investigation in many studies. Tremelimumab resulted in a partial response of 17.6% and DCR of 76.4% in a phase II trial pilot study that recruited patients with

advanced HCC and HCV infection [40]. Combination studies of tremelimumab with durvalumab in patients with advanced HCC or BTC as a second line of treatment or after previous therapy are currently underway. Oncolytic viral platforms currently under clinical trials for the treatment of liver cancer include adenoviruses, herpes simplex viruses, and vaccinia viruses.

2.3.4 Pancreatic Cancer (PC)

Pancreatic cancer (PC) has the greatest fatality rate worldwide and is the third leading cause of malignance-related deaths in the USA [41]. Globally, PC is the seventh foremost cause of malignance-related deaths. The risk issues include diabetes, chronic pancreatitis, tobacco use, and inherited genetic syndromes [42, 43]. Traditionally, patients with unresectable pancreas have been treated with chemotherapy including gemcitabine and FOLFIRINOX [44]. Immunotherapeutic advances and success met with clinical trials in other cancers have galvanized the investigative approaches in the treatment of PC. However, due to the poor antigenicity and a strong immune-suppressive tumor microenvironment of pancreatic tumors, immunotherapy has not currently met with success as in other GI malignancies [45].

Immune checkpoint inhibitor blockade has met with limited success in the treatment of PC. The Ipilimumab (anti-CTLA-4 blockade) monotherapy proved ineffective in the treatment of advanced PC. Similarly, the phase I trial with anti-PDL1 in a dose escalation study showed no clinical benefit in patients with advanced PC [46].

The safety and efficacy of a whole cell-based cancer vaccine approach that employs GM-CSF-expressing engineered pancreatic cancer cells to further induce APC antigen uptake and T-cell priming (GVAX) was assessed in a phase I study. The phase I study confirmed that GVAX was safe and effective in promoting anti-tumor immunity. A phase II trial using GVAX showed limited effectiveness in a subgroup of patients with extended disease-free survival had improved tumor antigen-specific CD8+ T cells [47]. Currently, many clinical trials that employ GVAX and combination therapy are underway to study their efficacy in the treatment of locally advanced or metastatic pancreatic cancers.

Adjuvant multipeptide-based vaccines as an alternative approach to whole cell vaccines is also being investigated in the treatment of PC. An adjuvant multipeptide KRAS vaccine has also shown some success with anti-RAS response in 58% of the patients in a phase I/II trial [48]. A phase II study of 30 Japanese patients who were administered the peptide cocktail vaccine OCV-C01 containing epitope peptides derived from KIF20A, VEGFR1, and VEGFR2, together with gemcitabine in the adjuvant treatment for resected PC patients showed 58.6% of patients developed cytotoxic CD8+ T lymphocytes.

Combination therapies using immune checkpoint inhibitor blockade and vaccines have also met with some success. A phase I study studied the efficacy and safety of ipilimumab in combination with GVAX in PC comparison to ipilimumab alone. The

study, conducted on 30 patients, displayed that the combination treatment was safe with improved efficacy [49].

Studies in mice models of PC injected with GVAX in combination with anti-PD-1 showed an increased preponderance of IFN-γ-producing CD8+ in the tumor-infiltrating lymphocytes at the tumor sites, underscoring the status of combination treatment regimens for Pancreatic Adenocarcinoma (PAC) [50]. Vaccination with GVAX two weeks prior to surgical resection also resulted in increased PD-1-expressing tumor frequency. Based on these, GVAX is currently being investigated for its potential in improving patient survival outcomes in immunotherapeutic trials with or without immune checkpoint blockade, nivolumab for patients with resectable PC (NCT02451982; clinicaltrials.gov).

Studies of the pancreatic tumor microenvironment have shown increased colony-stimulating factor-1 expression by pancreatic tumor cells and its receptor CSFR1 expression on tumor-linked macrophages and myeloid-derived suppressor cells implicating its role in immune suppression. Blockade of the CSFR1-CSF pathway was revealed to progress chemotherapy-stimulated antitumor immunity in animal models [51]. Preclinical PC models further showed that prior treatment with tyrosine kinase inhibitors to block CSF-CSFR1 interaction increased PD-1 and CTLA-4 expression, making them better candidates for immune checkpoint blockade. Consistently, combination treatment with gemcitabine, CSF1R blockade, and either anti-PD1 or anti-CTLA4 treatment caused a synergistic effect. Currently, clinical trials with IMC-CS4, anti-CSF1R in conjunction with anti-PD1 and GVAX treatment for borderline resectable PC; PLX-3397 (Pexidartinib), another anti-CSF1R agent in combination with anti-PD-L1 for patients with advanced PC and CRC are underway. In PC, CXCR4 is expressed on endothelial and cancer cells and causes carcinoma-associated fibroblast immunosuppression. A dose escalation trial for the CXCR4 antagonist (Plerixafor) is also currently in phase I test for patients with PC (NCT03277209) to target CXCL12/CXCR4 interaction in order to reverse malignance-linked fibroblast immunosuppression. In yet another approach, triggering CD40, a molecule expressed on the surface of CD4+ T cells, has been revealed to improve the efficacy of vaccines in aiding anti-tumor immunity [52], leading to the phase I trial of a CD40-agonist (R07009789) for patients with resectable pancreatic cancer.

Other tumor-associated antigens that are presently being examined in clinical trials include ERBB/HER receptors, PDGFRα, VEGF/VEGF-R, and mesothelin for the treatment of PC. Oncolytic viruses under clinical studies for the treatment of PC include Adenovirus, simplex virus, Herpes, Reovirus, Parvovirus, and Vaccinia virus.

2.4 Combining Immunotherapy with Precision Medicine

Although the first immunotherapeutic treatment for cancer was approved in 2011, four immune checkpoint inhibitors received FDA approval for treatment not very long ago [53, 54]. While there are over 70 immunotherapy drugs are in clinical

investigations, it remains to be seen why some individuals respond better to these compared to others. The increasing number of studies showed that tumors with mutations in DNA mismatch repair or dMMR (microsatellite instability MSI) respond better to immune checkpoint inhibitors. The clinical data from a study to determine the efficacy of anti-PD1 blockade conducted on 12 different tumor types based on their dMMR status indicated that the tumors were susceptible to the blockade consistent with the deficiency in the DNA mismatch repair system [39]. Similarly, recently, identified MR1-restricted pan tumor targeting T cells could be studied in more detail regarding their numbers and origin in different types of tumors [55]. Combination therapy using the pan-T cells and immune check-point inhibitors may even unleash their potential in the treatment of metastasized tumors.

It is thus apparent that individualized or personalized medicine could play a significant role at the forefront of immunotherapy enabling identification of tumor mutation burden, genetic or epigenetic profile of tumors that renders them susceptible to the immunotherapeutics. Tissue agnostic drug approvals could be more relevant given the heterogenous nature of the tumors and the efficacy of immune checkpoint blockade therapy. Future approaches may rely on the identification of immunogenic neoantigens or tumor mutational burden to have deeper insights into understanding the tumor microenvironment and its role in causing immune suppression. Such information on tumor neoantigens and mutations could be vital to breaking the immunosuppression using combination therapy with immune therapeutics paving the path toward optimal patient outcomes.

2.5 Conclusions

Immunotherapy is emerging as a cornerstone of ongoing treatment strategies in GI malignancies. Combination approaches that combine the traditionally favored surgical resection to non-metastasized tumors with radiation or chemotherapy hold promise. Identification of biomarkers, protein expression profiles, and genetic and epigenetic profiles with advances in next-generation sequencing technology may be useful in providing agnostic therapies that treat cancer based on their genetic and molecular profiles rather than their type, stage, or origin. Precision medicine along with immunotherapy may thus hold the key to unlock the treatment strategy for the prolonged battle against cancer.

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Chapter 3
Adiponectin in Gastrointestinal Malignancies

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Abstract Obesity and its related metabolic deregulation have poor prognosis for several cancers, including gastric cancer (GC) and colorectal cancer (CRC). Adiponectin (also known as adipoQ, Acrp30, GBP-28, and apM1) secreted into the bloodstream from the adipose gland, is a protein hormone that regulates glucose levels and fatty acid degradation. Adiponectin, encoded by the *ADIPOQ* gene in human, is also involved in anti-inflammatory, anti-metabolic diseases, anti-atherogenic, anti-angiogenic, tumor growth restriction, pro-apoptotic, and insulin-sensitizing functions. Adiponectin levels in human serum depend on nutrition, exercise, abdominal fat, and heredity. Current epidemiologic and preclinical interpretations indicate the potential link between obesity and GC/CRC. In addition, low adiponectin levels may contribute to high GC and CRC rates in obese people who have a decreased response to insulin, resulting in type 2 diabetes. Adiponectin and its interactions may have anti-cancer effects through a large amount of cellular signaling pathways. This chapter summarizes the association of adiponectin with GC and CRC. Further, we will also suggest that adiponectin is a biomarker or therapeutic molecule in GC and CRC.

Keywords Adiponectin · Obesity · Gastric cancer · Colorectal cancer

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Abbreviations

Acrp30	Adipocyte complement-related protein of 30 kDa
AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
AMPK	AMP-activated protein kinase
apM1	Adipose most abundant single gene transcript 1 located on chromosome 3q27
BMI	Body mass index
CRC	Colorectal cancer
GBP-28	Gelatin binding protein of 28 kDa
GC	Gastric cancer
HMW	High molecular weight
JNK	c-Jun N-terminal kinase
LMW	Low molecular weight
MDM2	Murine double minute 2
mTOR	Mammalian target of rapamycin
PI3K	Phosphoinositide 3-kinase
PPAR	Peroxisome-proliferator-activated receptor

3.1 Introduction

Obesity, defined as a body mass index (BMI) of 30 or above, is one of the main risk factors for the development of several types of cancer, including gastric cancer (GC) and colorectal cancer (CRC) [1–3]. Obesity is a common chronic inflammation condition induced by overloading fatty tissue accumulation when calorie demand exceeds energy consumption. The prevalence of obesity is not only a problem in developed countries but also a major health problem around the world. Obesity is also expected to increase considerably over the next few decades [4–7]. According to tumor cross-section studies in patients with overweight or obesity, adipose-related factors can cause tumors to develop and grow. Interactions between the developing tumor and the microenvironment involve a multifaceted interplay among different cells, mediators, and other components [8, 9]. In particular, new evidence suggests that adipocytes and macrophages in the tumor microenvironment enhance inflammation and rebuild the metabolism of cancer cells to support tumor progression [3]. An inverse relationship between adiponectin levels in serum and GC/CRC has been observed, suggesting that adiponectin may be a link between obesity and cancer [1, 10–14].

Adipose tissue, originally considered a passive reservoir for fat metabolism, is an essential active endocrine organ participating in the production of many metabolic and inflammatory mediators such as adipocytokines, free fatty acids, and chemokines [15, 16]. Adipocytokines including adiponectin act as major mediators

in many obesity-related diseases such as type 2 diabetes and cancer [6, 17, 18]. Adipose tissue containing multiple cell types such as adipocytes, preadipocytes, endothelial cells, and immune cells can be classified into three different types: white adipose tissue, brown adipose tissue, and beige adipose tissue [19]. Brown and beige adipose tissues are involved in temperature control but white adipose tissue is considered the main energy storage site in the form of triacylglycerides (also called neutral fats) [19]. Accumulating evidence has thus uncovered the complexity of adipose tissue and its contribution to various metabolic disorders.

3.2 Adiponectin and Adiponectin Receptors

Adiponectin, a 244 amino acid protein, was discovered by four research groups in the mid-1990s and has four different names; adipoQ, Acrp30 (adipocyte complement-related protein of 30 kDa), GBP-28 (gelatin binding protein of 28 kDa), and apM1 (adipose most abundant single gene transcript 1 located on chromosome 3q27) [20–24]. In 1999, Arita et al. named it adiponectin, the most commonly used name these days [25]. Adiponectin consists of four distinct domains: an N-terminal signal peptide (17aa), a species-specific variable domain (28aa), a collagen-like domain of 22 Gly-X-Y repeats (65aa), and a C-terminal globular domain (137aa) that interacts with adiponectin receptors [23] (Fig. 3.1). The adiponectin collagen-like region of adiponectin allows for oligomerization of protein through disulfide bonds, and hydroxylation and glycosylation of four conserved lysine residues. This is critical for the formation of their high molecular weight

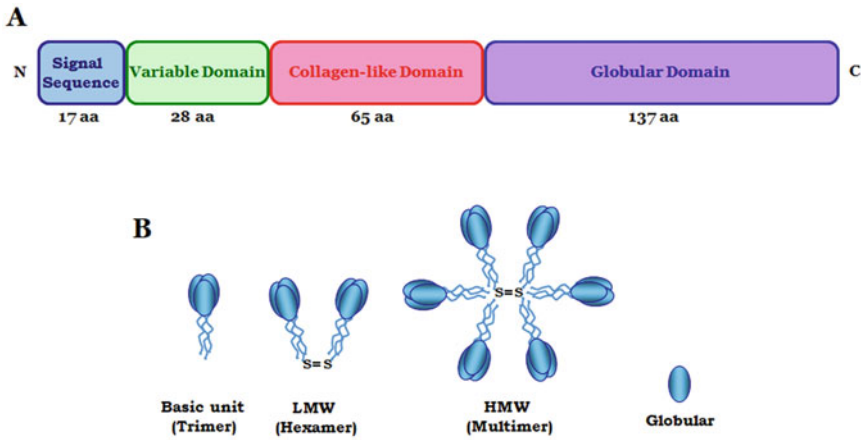

Fig. 3.1 (**a**) Molecular structures of adiponectin including signal sequence, variable domain, collagen-like domain, and globular domain. (**b**) Adiponectin can exist as a trimers, hexamers, or multimers. *LMW* low molecular weight, *MMW* middle molecular weight, *HMW* high molecular weight, *aa* amino acid, *S=S* disulfide bond, *N* N-terminus, *C* C-terminus

complexes [26, 27]. Glycosylation and sialyation are essential to confirm biological activity and binding to receptors [27, 28] and thus play a critical role in ensuring the integrity of the adiponectin-signaling cascade.

Adiponectin is primarily produced in white adipose tissue and lower amounts are produced in brown adipose tissue [24]. Adiponectin is also expressed in much smaller amount in liver, colon, cerebrospinal fluid, skeletal muscle, cardiac tissue, bone marrow, salivary glands, fetal tissue, placenta, and breast milk [29–37]. Adiponectin is synthesized as a single subunit that converts to oligomers before secretion and circulates in serum in four isoforms: simple trimer complex (90 kDa), low molecular weight (LMW; 180 kDa; complex of two trimers), high molecular weight (HMW; 360–400 kDa; complex of up to six trimers), and globular form [38, 39] (Fig. 3.1). These forms of adiponectin might play various biological roles during the initiation of downstream signaling pathways, which might determine their final roles in tumor development [40]. The hexamer HMW has less secretion and greater pre-inflammatory functions in males than females, while the LMW has higher anti-inflammatory properties [41, 42]. Adiponectin is expressed at high levels (up to 30 μg/mL) in healthy people (usually about 0.01% of the total human plasma protein content), whereas adiponectin expression levels are low in patients with CRC (15.9 μg/mL) [43, 44], suggesting that adiponectin levels could be employed as a potential diagnostic tool for CRC.

Three adiponectin receptors have been found: the two classical receptors (i.e., AdipoR1 and AdipoR2) and one receptor similar to the cadherin family (i.e., T-Cadherin) [35, 45–47]. Two main adiponectin receptors, AdipoR1 (375aa; 42.4 kDa) and AdipoR2 (311aa; 35.4 kDa), are seven transmembrane receptors with an inner N-terminal region and an outer C-terminal region. They are expressed ubiquitously but differ in distribution among cell types and in affinity for various forms of adiponectin [48]. The receptor is a type IV-A protein and contains seven transmembrane domains with an inner N-terminal outer C-terminal region [49]. AdipoR1/R2 have a distant relationship with G protein-coupled receptors and have no homology with other mammalian proteins. Studies using knockout mouse models clearly show that activation of AdipoR1/R2 plays various metabolic roles in vivo [50]. AdioR1, expressed mostly in skeletal muscle, is also noticed in endothelial cells and other tissues. AdipoR1 shows high affinity for globular adiponectin and low affinity for full-length adiponectin [51]. AdipoR1 and adiponectin complexes stimulate lipid oxidation through AMP-activated protein kinase (AMPK) activation [52]. AdipoR2 expressed in the liver exhibits moderate affinity for both globular and full-length adiponectin. AdipoR2 increases peroxisome-proliferator-activated receptor (PPAR) ligand activity by enhancing insulin sensitivity through AMPK activation [52]. AdipoR1/R2 form homodimers and heterodimers. T-cadherin has high affinity for HMW and is primarily expressed in endothelium and smooth muscle [53]. Consequently, the biological impacts of adiponectin depend on the tissue-specific expression of adiponectin receptors, and the relative circulation amounts and the properties of adiponectin [49]. Study of the function of AdipoR1/R2 and signaling pathways may provide additional information on the function of adiponectin signaling during tumor development and metastasis

[40]. Increased understanding of these mechanisms could inform future research avenues for clinical and therapeutic development based on adiponectin pathways.

3.3 Connection Between Obesity and Gastrointestinal Malignancies

Evidence from experimental models indicates that inflammation provides an important link between obesity and gastrointestinal cancer. Inflammation is a well-studied route to protect against invading pathogens such as bacteria and viruses. Inflammation from chronic infections can cause carcinogenesis. The link between *Helicobacter pylori* infection and GC is a good example [54]. Comparably, autoimmune diseases can cause chronic inflammation, leading to the risk of CRC [54]. Some relations between carcinogenesis and chronic inflammation are mediated by a multifaceted network of soluble adipocytokines, including adiponectin synthesized and secreted by adipocytes [55], highlighting its role in tumorigenesis.

The balance between increase (i.e., proliferation) and decrease in cell numbers (i.e., apoptosis) is essential for normal cell development. Thus, augmented proliferation or diminished apoptosis is a cause of carcinogenesis [56]. Several identified factors of obesity can induce phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, which can lead to carcinogenesis through numerous downstream signaling pathways. Study of PI3K/AKT downstream target molecules in obesity and their function in carcinogenesis will help develop new approaches to prevent obesity-associated CRC. Several studies have described their roles in cell cycle, cell growth, and cell survival. p53 blocked by PI3K/AKT through stimulation of the oncogenic protein murine double minute 2 (MDM2) induces apoptosis in response to DNA damage [57]. Low expression or mutation of p53 in obese people plays a critical role in obesity-related cancer [58], indicating its involvement in cancer development.

3.4 Adiponectin and Obesity-Related Gastrointestinal Malignancies

3.4.1 Adiponectin and Gastric Cancer

Obesity has long been considered a risk element for GC [59, 60]. The risk of GC occurring in the gastric cardia seems to be associated with obesity due to increased gastroesophageal reflux [1]. Studies on the incidence and spread of GC have shown that adiponectin levels decrease in patients with GC [61]. The defensive mechanism by adiponectin is believed to inhibit endothelial cell proliferation and migration, which induces catalytic caspase activation, resulting in cell death [62]. Adiponectin has anti-angiogenic properties and a decrease in adiponectin levels can inevitably

promote the development of GC [62]. TNF-α inhibits adiponectin production by adipocytes [63]. This inhibition is further exaggerated when cancer cells produce a variety of inflammatory cytokines in cachexia [64]. These reports propose that TNF-α may be responsible for the association between adiponectin and GC.

Adiponectin also has the effect of inhibiting proliferation in GC. Its expression is inversely associated with recognizable clinical signs in undifferentiated GC. Adiponectin is thought to inhibit cell growth through AdipoR1 [65]. As a result, AdipoR1 is being studied as a new anti-cancer therapeutic target in GC. Patients with an immediate decrease in pre-operative adiponectin levels were susceptible to infection after GC surgery [66]. The ratio of post-operative to pre-operative levels of adiponectin after surgery appeared to be the most reliable predictor of post-operative infection. This reduced level suggests higher energy and inflammatory reactions after disordered surgery, which could increase the propensity of infections. Studies on adiponectin receptors have revealed that expression of AdioR1/R2 in GC is higher than that in normal gastric mucosa [67]. Adiponectin receptors are repeatedly expressed in GC with tumor invasion and lymph node metastasis. Otani et al. [68] proved that AdipoR1/R2 were reduced transcriptionally in GC compared to normal healthy counterparts through mouse models. Immunohistochemical investigation also confirmed these molecular results [68]. AdipoR1/R2 expression was reduced when GC cell lines such as MKN-74 and NUGC-3 were treated with a transforming growth factor (TFF-β). Reducing AdipoR1/R2 expression by GC cells may be a strategy that deviates from the anti-proliferative effects of adiponectin at an early stage of tumor development. Another study used ELISA to assess plasma adiponectin levels in GC patients and normal healthy people and found that adiponectin levels were significantly lower in GC patients than normal healthy people [1]. In addition to these examinations, they also found that adiponectin levels tended to decrease with tumor stage progression. These studies concluded that low serum adiponectin levels correlate with increased susceptibility to GC and highlight its role in the development of GC, especially in the undifferentiated type malignancy in the upper gastric region. In addition, rigorous research is essential to determining the precise role of adiponectin and its receptors in GC and metastasis.

3.4.2 Adiponectin and Colorectal Cancer

CRC is the third most common cancer worldwide and its incidence is consistently associated with the incidence of obesity [69, 70]. Recently, a multicenter case-control study investigating the association between the adiponectin pathway and the risk of CRC found an increased risk of CRC by more than 50% in patients with low concentrations of adiponectin [2]. Adiponectin is expressed by both CRC and the colorectal mucosa [71]. Post-translationally modified globular domains from full-length adiponectin were significantly higher in colorectal tumors than colorectal mucosa from the same CRC patients, suggesting that colorectal tumors locally

transform adiponectin into globular adiponectin at a higher level than in healthy colorectal mucosa. Nearly all CRC transcriptionally express both AdipoR1 and AdipoR2 and the adiponectin levels were lower in non-metastatic CRC patients than in healthy people [72]. Another case-control study found that men with low levels of adiponectins had a higher risk of CRC than men with high levels of adiponectin, suggesting that low adiponectin levels may be an auxiliary marker for CRC reappearance [14, 73]. Therefore, it is important to further clarify the signal transduction pathways of adiponectin in obesity-related CRCs. Leptin and adiponectin may be implicated in the connection between obesity and CRC because the carcinogenic effect of leptin is seen only at low levels of circulating adiponectin [74]. The adipose tissue content determines insulin sensitivity and the circulating HMW adiponectin levels [38, 75]. Risk of CRC was reduced by improved insulin sensitivity through the HMW adiponectin, which may mediate the association between CRC and adiposity [76]. Low levels of blood HMW adiponectin have been recognized as a possible risk factor for early cancer in CRC patients, demonstrating that it is associated with early and advanced cancer progression [77]. More clinical research is necessary to elucidate the link between low adiponectin and CRC tumors.

Adiponectin directly inhibits many intracellular signaling transduction pathways that promote CRC [78], primarily through AdipoR1/R2 expressed in colon cancer tissue as well as in normal colon epithelium [79]. Adiponectin-related genetic defects are associated with CRC [80]. Mice without the adiponectin gene and its receptors, AdipoR1/R2, had increased colorectal polyps stimulated by fat compared to normal mice [81]. Low adiponectin levels in obesity are not enough to regulate ROS production, stimulating cancer cell proliferation [82].

In many studies, researchers have shown that low adiponectin levels are related with an increased risk of CRC [83–85]. For instance, supplementation of adiponectin in adiponectin-deficient mice repressed the development of colorectal polyps [86, 87]. Adiponectin KO mice showed more tumors with infiltrating cells than wild type mice. The potential intestinal oncogene, c-Jun N-terminal kinase (JNK), activated in obesity plays an important role in insulin resistance and obesity. Lipotoxic stress resulting from high-fat diet causes JNK to rise abnormally at low adiponectin levels in muscle, liver, and adipose tissue [86]. Activation of JNK in CRC plays a potent role in progression of CRC [88]. Although activation of JNK may not inevitably reproduce tissue inflammation, it might be theoretically assumed that low adiponectin levels observed in obesity may fail to regulate JNK activity in some tumor tissues [89]. Protein expression profiling including pAMPK, pSTAT3, and Cox2 additionally supported these data [90]. Thus, adiponectin may play an important role in preventing CRC by regulating genes associated with obesity-related carcinogenesis, suggesting that lack of adiponectin contributes to CRC.

This effect of adiponectin can be mediated by inhibiting the mammalian target of rapamycin (mTOR) complex, a downstream target of AMPK required for adiponectin action. Adiponectin induces G1/S cell cycle arrest by inhibiting CRC cell growth and activating AMPK to inhibit the mTOR pathway [78, 91]. AMPK/mTOR is the downstream target of the PI3K/Akt signaling pathway. In addition,

colon epithelial cell proliferation was also inhibited by adiponectin [83]. Adiponectin treatment inhibited tumor growth, resulting in the formation of larger central necrotic areas by controlling metabolic, inflammatory, and cell cycle signaling transduction pathways [92]. Adiponectin administration also reduced angiogenesis assessed by CD31 staining and VEGF transcript levels in tumors from mice, suggesting that adiponectin and its analogs may be therapeutic agents preventing the onset of CRC.

3.5 Conclusions and Adiponectin as a Therapeutic Agent

This chapter clearly highlights the possibility that restoring or increasing adiponectin may exhibit therapeutic advantages in obesity-related malignancies with reduced levels of adiponectin secreted from the adipose gland. Unfortunately, it is difficult to synthesize adiponectin into a drug that can be used by humans. Due to this limitation, studies are currently underway to identify pathways that increase endogenous circulating adiponectin levels to remedy obesity-related cancers [93]. For example, the adiponectin mimic ADP335, a novel short peptide based on adiponectin, restricted the proliferation of several cancer cell lines positive for adiponectin receptors [94]. ADP335 also inhibited the growth of cancer tumor xenografts by 31% [95]. Moreover, the SPPARMINT131 (also known as T131 and AMG131), a new class of non-thiazolidinedione PPAR ligands, increased adiponectin concentrations [96]. Additionally, strategies to improve the expression or sensitivity of AdioR1/R2 for adiponectin and agonists of AdioR1/R2 may provide new therapeutic approaches to obesity-related malignancies. For instance, SPPARM, selective PPAR agonists, and L-4F, an apolipoprotein peptide mimetic, are pharmacological agents that can enhance circulating adiponectin levels or regulate adiponectin signaling pathways through its receptors, which are specific treatment metabolites for obesity-related diseases and malignancies [97]. Furthermore, treatment of obesity-related GI cancers with drugs that inhibit the PI3K/Akt complex can be useful as modulators or sensitizers to chemotherapy and/or radiotherapy in patients with unresectable GI cancer. As research indicates, the PI3K/Akt signaling pathway plays a key role in the development of GI cancers and constitutes an important therapeutic target. Hence, pharmacological and clinical advances in this area can be beneficial for obese patients who are at higher risk for several cancers. Although the development of adiponectin analogs could help in preventing GI cancer, the synergistic nature of various risk factors in obesity can contribute to cancer progression. Therefore, international standardization of adiponectin levels and analytical procedures will be required to commercialize adiponectin as a prospective diagnostic tool for obesity-associated cancers such as GC and CRC.

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Chapter 4
Small Molecule-Targeted Therapies for GI Cancers: Success and Failures

Binayak Kumar, Deepu Sharma, Jyotsna Gorantala, and Sri Krishna Jayadev Magani

Abstract Cancer is the second major cause of deaths next to noncommunicable diseases worldwide. The major treatment regimens followed to counter this disease include surgical resection, radiation therapy, and chemotherapy. These treatment regimens can be employed individually or in combinations. The heterogeneity in gastrointestinal (GI) cancers and development of resistance to chemotherapeutics agents and the secondary complication due to their toxic activity in normal cells lead to the research for discovery of novel therapeutics. With increasing knowledge of the aberrant signaling pathways in cancers, the novel approach to avoid the toxic effect of the chemotherapeutic drugs in normal cells was to look for targeted therapeutics. Targeted therapies include the use of either monoclonal antibodies against receptors or extracellular molecules present on cancer cells or using inhibitor molecules that target aberrant pathways in cancers. This chapter mostly focusses on discussing the role of protein kinase inhibitors a class of small molecule inhibitors in cancers with their functional significance and limitations.

Keywords Targeted therapy · Kinase inhibitors · Gastrointestinal cancers · Vascular endothelial growth factor receptor · Epidermal growth factor receptor · Platelet derived growth factor receptor

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Abbreviations

DTC	Differentiated thyroid carcinoma
EGFR	Epidermal growth factor receptor
FGFR	Fibroblast growth factor receptor
GI	Gastrointestinal
GISTs	Gastrointestinal stromal tumor
HCC	Hepatocellular carcinoma
mAbs	Monoclonal antibody based
MAP	Mitogen-activated protein
Mcl-1	Myeloid cell leukemia 1
mCRC	Metastatic colorectal cancer
MMPs	Matrix metalloproteinases
NICE	National Institute for Health and Care Excellence
PDGFR-Rs	Platelet-derived growth factor receptors
RTK	Receptor tyrosine kinases
VEGFRs	Vascular endothelial growth factor receptors

4.1 Introduction

Gastrointestinal (GI) cancers rank third among all the cancers with respect to their incidence and ranked second with highest mortality rate next to lung cancer [1]. GI cancer refers to cancer of the gastrointestinal tract and its associated organs, which includes esophagus, stomach, small intestine, large intestine, rectum, anus, liver, gall bladder, and pancreas. Researcher have been continuously searching for a better therapy of cancer since centuries and the first success was seen in the 1900s in the form of radiation therapy. In 1940s, chemotherapy was introduced as an alternate option for the cancer therapy. The major treatment regimens followed to counter this disease include surgical resection, radiation therapy, and chemotherapy. The completion of human genome project in 2003 mapping the entire human genome and the increasing knowledge of signaling pathways in cancers has opened up scope for exploring novel approaches for the development of new drugs for various cancers.

Radiation therapy uses high energy particles or waves to kill the tumor cells. It targets rapidly dividing cells in the specific phase of cell cycle by damaging their genome. Tumor cells in a localized area are subjected to radiation so that healthy tissues are least harmed. Hence, radiotherapy is a localized therapy; it is not useful for scattered cancerous tissues or cells. However, this therapy does not differentiate between cancerous and healthy cells. Many of the healthy tissues like gastrointestinal tract and hair follicles have rapidly growing cells that are susceptible for the radiotherapy. Recurrence and resistance to radiotherapy are the reported drawbacks of this therapy. The spatial arrangement of GI tract in the body along with other vital organ systems surrounding it makes it difficult for radiotherapy in GI tract cancers.

Another important therapy in various cancers is the usage of chemotherapeutic drugs. These drugs mainly target different phases of cell cycle. One of the main

features of the cancer cells is their uncontrolled cell division. Chemotherapeutic drugs targets the cells that undergo rapid cell division. However, like in case of radiation therapy these drugs too do not differentiate between cancerous and healthy cells. It could lead adverse effects on healthy cells similar to that reported in radiation therapy. These two methods of the cancer therapies are categorized as conventional and traditional therapies.

Chemotherapy involves the use of single chemotherapeutic agent (single-agent chemotherapy) or a combination of several chemotherapeutic agents simultaneously at a time (combination therapy) targeting the rapidly dividing cancer cells. To overcome the drawbacks of these traditional cancer therapies, researchers have been looking for specific molecular targets for selective elimination of malignant cells. Such targeted therapies would be conceptually more specific than the traditional nontargeted therapies. The availability of human genome map aided in identifying specific cancer causative genes, diagnostic and prognostic markers. This in turn led to explore novel drug targets.

Despite continuous refinement in radio- and chemotherapies, since the last decade, high rate of recurrence and mortality was observed in GI cancers. In view of this, there is an urgent need for better diagnostic and therapeutic approaches for detection and treatment of GI cancers. In the recent decades, cancer therapy has been shifted towards most precise and targeted therapies based on genetic and molecular features of the GI-tumor cells [2]. To reduce the risk of recurrence and for better prognosis, a strategy has been designed to treat the individual patient with most appropriate drug called targeted drug and the therapy is called targeted therapy. Targeted therapy required proper analysis of GI cancer cells at molecular level such as detection of cancer specific genes, genetic mutations, alterations in cell signaling, and molecular target identification. These analysis help in better prognosis of the disease to develop a target-based new drug. Most of the targeted drugs being used for treatment of various types of cancers including GI cancer are either monoclonal antibody-based or synthetic small molecule-based therapeutics.

4.2 Targeted Therapies

The two main approaches of targeted therapy available in clinical practice are (1) monoclonal antibody-based (mAbs) immunotherapy and (2) small molecule-based targeted therapy.

4.2.1 Immunotherapy

Immunotherapy enhances the patient's own immune system's ability to recognize, target, and eliminate cancer cells. Monoclonal antibodies that are used as immunotherapy are usually of higher molecular weight of around 150 kDa. Hence these monoclonal antibodies can only act on molecules that are expressed on the cell

surface. mAbs are mostly administered intravenously and this therapy is more costly as compared to other therapies [3–5].

Other than the cost-effectiveness, immunotherapies also have some limitations like resistance to the drug. Resistance may be acquired because of mutation in their targeted molecules at the genetic level or tumor cells might find an alternate target independent pathway for their survival and progression. Therapy resistance has been reported for all of the below-mentioned immunotherapeutic drugs in the respective targeted cancers [6–8].

4.2.2 Small Molecule-Based Targeted Therapeutics

The transition of research from chemotherapy to targeted therapy for cancer has resulted in development of numerous successful targeted drugs that impacted the lives of a large number of cancer patients. One prime merit of small molecule-based anticancers is their molecular mass which is $\leq$500 Da. Thus, making it easy for these molecules to translocate through plasma membranes. In addition, these are cost-effective compared to immunotherapeutic drugs and are also suitable for oral administration.

Due to their small size, these drugs can easily target intracellular molecules along with extracellular molecules and cell surface receptors that play a key role in cancer cell survival, proliferation, and metastasis. Most of the small molecule-based targeted drugs inhibit critical cancer target molecules such as (a) protein kinases (tyrosine or serine/threonine), (b) proteasome, and (c) matrix metalloproteinases (MMPs), thereby promoting proteasomal degradation, apoptosis, and so on [9].

4.2.2.1 Protein Kinase Inhibitors

More than 500 kinases identified in the human genome are classified into subsets or families based on their sequence and structural similarities [10–12]. Protein kinases catalyze the transfer of the terminal phosphoryl group of high-energy molecules such as ATP or GTP to serine, threonine or tyrosine amino acid residues of protein substrate. These are classified as serine/threonine or tyrosine kinases. MEKs have dual specificity of kinase activity and phosphorylate both serine/threonine and tyrosine residues [13]. Protein phosphorylation regulates several biological processes such as cell survival, cell proliferation, cell differentiation, cell migration, cell adhesion, invasion, and cell apoptosis. A slight change in the kinase's activity may have vast range of disturbance in cellular homeostasis that may lead to tumorgenicity. Figure 4.1 represents the chronological events of discovery of kinase inhibitors. This crucial role has made kinases an extremely important therapeutic target in the field of antitumor drug discovery [14–16].

Protein kinases came into limelight as a drug target in the field of anticancer drug discovery with the approval of small molecule protein kinase inhibitor imatinib by FDA in 2001 [17–19]. Kinases are categorized as cell surface receptor or

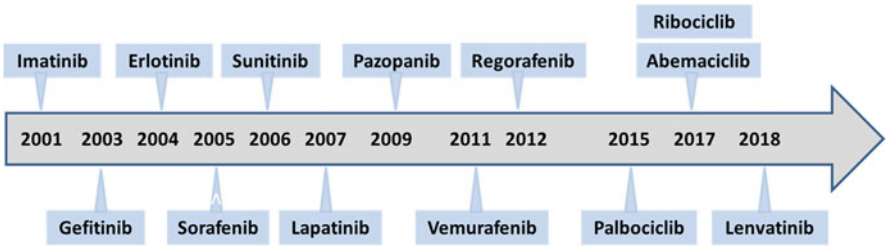

Fig. 4.1 The chronological representation of discovery of synthetic small molecule kinase inhibitors for different cancers

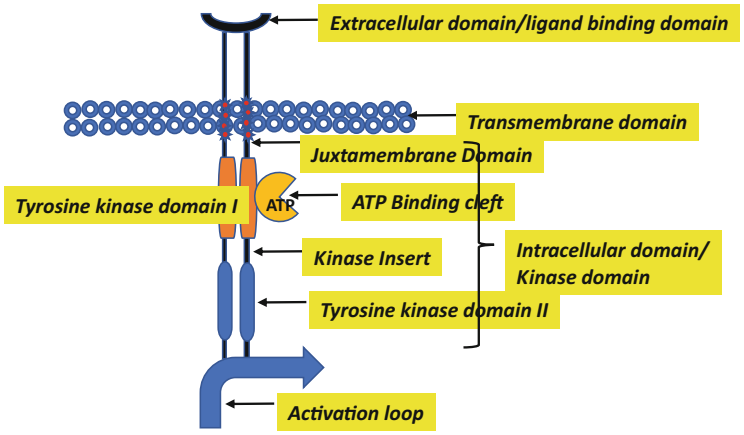

Fig. 4.2 Structural representation of receptor tyrosine kinase

cytoplasmic kinases. Although these are structurally similar, transmembrane domain is absent in the cytoplasmic kinases as shown in Fig. 4.2. Receptor tyrosine kinases are made up of extracellular domain (ligand binding domain), transmembrane domain, and intracellular domain (kinase domain). Intracellular domain has N-terminal lobe and C-terminal lobe and between them ATP binding cleft. Activation loop attached to the end of C-terminal lobe. ATP binding cleft has ATP binding region and hydrophobic region [20]. Based on the binding site for kinase inhibitor, kinase inhibitors, classified as Type I inhibitors, interact directly with the ATP binding pocket. Type II kinase inhibitors bind to hydrophobic pocket directly adjacent to the ATP binding pocket and Type III kinase inhibitors bind to the allosteric site far away from the ATP binding pocket [21]. They do not disturb the binding of ATP, but induce conformational changes. Other types of kinase inhibitors have irreversible (covalent) binding or reversible (hydrophobic) binding. Some of the kinase inhibitor drugs for GI cancer are listed in Table 4.1.

Imatinib is a small synthetic ABL kinase inhibitor molecule regarded as track changer in the field of drug discovery and cancer therapy. This drug most precisely validated the concept of small molecule-based targeted therapy for the defined cancer patients. In case of chronicmyelogenous leukemia (CML) that is induced

Table 4.1 List of synthetic small molecule kinase inhibitors for GI-cancer

Target	Small molecule drugs	Targeted molecules	Cancers targeted	First FDA approval
Tyrosine and serine/ threonine kinases	Imatinib	Bcr-Abl	Philadelphia chromosome-positive chronic myelogenous leukemia Certain types of gastrointestinal stromal tumor (GIST)	2001
Tyrosine and serine/ threonine kinases	Erlotinib	EGFR	Non-small cell lung cancer, pancreatic cancer	2005
Tyrosine and serine/ threonine kinases	Sunitinib	VEGFR2, RET, PDGFR, FLT3, KIT, CSF1	Renal cell carcinoma Imatinib-resistant gastrointestinal stromal tumor (GIST) Unresectable or metastatic, well-differentiated pancreatic neuroendocrine tumor	2006
Tyrosine and serine/ threonine kinases	Sorafenib	B-Raf, VEGFR2, EGFR, PDGFR	Hepatocellular carcinoma	2007
Tyrosine and serine/ threonine kinases	Everolimus	mTOR	Progressive, well-differentiated nonfunctional, neuroendocrine tumors (NET) of gastrointestinal (GI) origin with unresectable, locally advanced or metastatic condition Progressive or metastatic pancreatic neuroendocrine tumors not surgically removable	2016
Tyrosine and serine/ threonine kinases	Vemurafenib	V600E mutated B-RAF inhibition	Solid tumors including colorectal cancer	Early Phase I clinical trial
Tyrosine and serine/ threonine kinases	Regorafenib	VEGFR1–3, c-Kit, TIE2, PDGFR-β, FGFR1, RET, Raf-1, BRAF	Metastatic colorectal cancer Advanced gastrointestinal stromal tumors (GIST) Advanced hepatocellular carcinoma	2012
Tyrosine and serine/ threonine kinases	Lenvatinib	VEGFR1–3	Unresectable hepatocellular carcinoma (HCC)	2018

by translocation of BCR-ABL molecule, it has been observed that targeting ABL by imatinib significantly improved the overall survival of the CML patients [17]. This is the first synthetic small molecule-based targeted drug approved by FDA in 2001 for

the treatment of CML patients having Philadelphia chromosome-positive genotype. Later imatinib has also been used for the treatment of advanced gastrointestinal stromal tumor (GISTs) patient after surgical removal in KIT-positive cancer to prevent recurrence and the same has been approved by FDA.

After success of the imatinib as a targeted therapy, many other synthetic small molecule kinase inhibitors have been studied. Till date, more than 20 kinase inhibitors had been successfully approved by FDA for various critical cancers including GI cancers.

Erlotinib is a small molecule, kinase inhibitor targeting epidermal growth factor receptor (EGFR). This drug is derived from gefitinib that is also an EGFR kinase inhibitor. Erlotinib had been approved as an oral anticancer drug by FDA in 2005 in a combination with Gemcitabine for locally advanced unresectable or metastatic pancreatic cancer [22]. EGFR is a tyrosine kinase, which is highly expressed and sometime mutated in the various cancers including pancreatic cancer. For the signal transduction through EGFR pathway, growth factor binds to the EGFR receptor followed by forming homodimers. This homodimer uses ATP molecules for transphosphorylation of tyrosine residue in the other monomer. This phosphorylation induces downstream signaling cascade to the nucleus resulting in cell survival, cell proliferation, and invasion. Erlotinib binds noncovalently to the ATP-binding site of the EGFR receptor and acts as a competitor for ATP at their binding site and inhibits this signaling cascade [23].

Sunitinib is a small molecule orally administered drug that targets multiple receptor tyrosine kinases (RTK) and was approved by the FDA in 2006 for the treatment of imatinib-resistant GIST [24]. In 2010, sunitinib was approved by the European Commission for the treatment of unresectable or metastatic, well-differentiated pancreatic neuroendocrine tumor. In 2011, this drug was also approved by FDA for the same. Sunitinib was shown to inhibit cellular signaling by targeting multiple RTKs including the platelet-derived growth factor receptors (PDGFR-Rs), vascular endothelial growth factor receptors (VEGFRs), and CD117 (c-KIT). Earlier studies have indicated that PDGFRs and VEGFRs are actively associated with tumor cell proliferation as well as tumor angiogenesis [25]. Inhibition of these signaling molecules by sunitinib reduces tumor angiogenesis, proliferation, and triggers cancer cell apoptosis, thereby shrinking the tumor size. GIST tumors frequently develop mutations in c-KIT that makes GIST tumors resistant to imatinib, sunitinib is used as second line therapy for such GIST patients [26–29].

Lapatinib is a dual tyrosine kinase inhibitor that inhibits HER2/neu and EGFR pathways. It is used in combination therapy for advanced and metastatic breast cancer whose tumor cells overexpress EGFR [30, 31]. In 2013, lapatinib failed to achieve success in combination therapy with chemotherapy for advanced HER-2-positive gastric cancer in Phase III clinical trials [32]. Figure 4.3 shows a schematic representation of different target pathways of kinase inhibitors.

Sorafenib is a synthetic small molecule multikinase inhibitor developed in 1995 for oral administration [33]. This drug was approved by the FDA in 2005 and European Commission granted marketing authorization in July 2006 for the

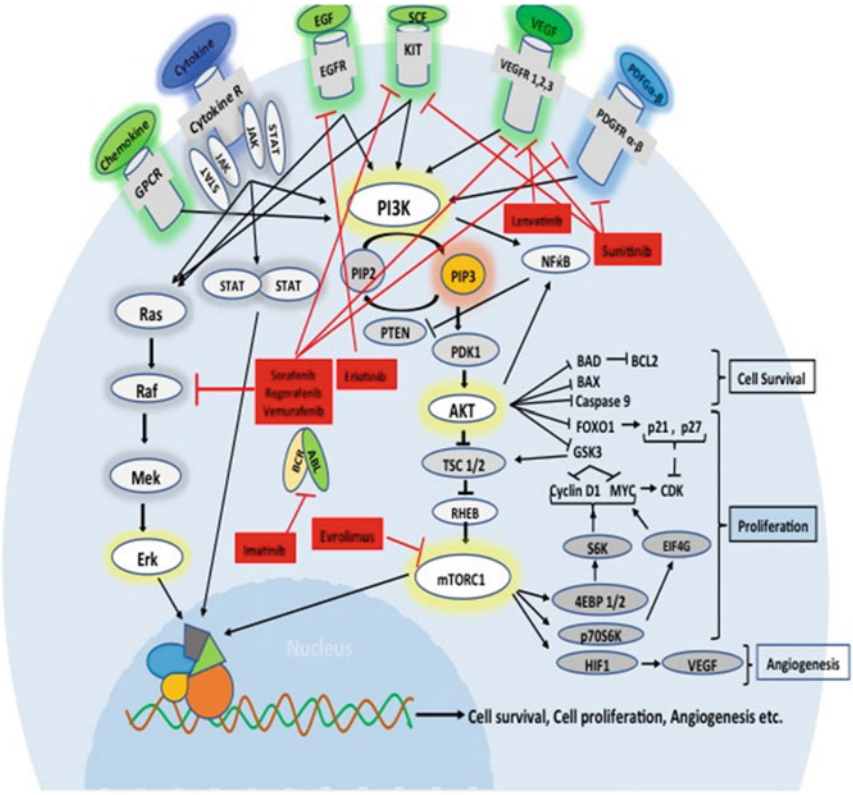

Fig. 4.3 The schematic representation of various signaling pathways in cancer and the mechanism and site of action of different kinase inhibitors

treatment of advanced renal cell carcinoma [34]. The European Commission further approved the marketing authorization in 2007 for the treatment of advanced hepatocellular carcinoma (HCC). Later in the same year, this was also approved by FDA for HCC (National Cancer Institute 2006, FDA approves, 2012). In 2013, sorafenib was approved by FDA for the treatment of locally recurrent or metastatic, progressive differentiated thyroid carcinoma (DTC) resistant to radioactive iodine treatment [35]. This drug is also being used for the treatment of FLT3-ITD positive AML cancer.

Sorafenib targets serine/threonine kinases of the RAF family members A-RAF, B-RAF, and C-RAF/Raf-1. These RAF members play a key role in mitogenic and oncogenic signal transduction through the Raf/mitogen-activated protein (MAP)/extracellular signal-regulated (ERK) kinase (MEK)/ERK signaling pathway resulting in downregulation of cyclin D1 and cell cycle arrest [21, 36, 37]. Sorafenib also inhibits tyrosine kinase receptors VEGFR2, VEGFR3, PDGFR-β, FLT3, and c-KIT, which promote angiogenesis [38–40]. In addition, sorafenib also blocks a broad spectrum of signaling pathways involved in proliferation, angiogenesis, or

apoptosis [33]. Studies have also shown that sorafenib induces cell death through dephosphorylation of signal transducers and activators of transcription Type III (STAT3) and downregulation of myeloid cell leukemia 1 (Mcl-1) and surviving proteins in hepatocellular carcinoma cells. Sorafenib is also able to repress Mcl-1 activity through a MAPK-independent mechanism, which enhances the apoptosis through intrinsic pathway in tumor cells.

Regorafenib is a novel multikinase inhibitor drug approved by FDA in 2012 for metastatic colorectal cancer (mCRC) [41]. In 2013, FDA approved regorafenib for treatment of patients with unresectable advanced GIST. In 2018, the National Institute for Health and Care Excellence (NICE) approved the use of regorafenib in patients with advanced hepatocellular carcinoma who were already treated with sorafenib [42]. Regorafenib was also developed as a RAF1 inhibitor like sorafenib. Regorafenib was the fifteenth RAF1 inhibitor compound developed after sorafenib [43]. Preclinical studies have revealed that like sorafenib it also acts as a multikinase inhibitor. But unlike sorafenib, regorafenib has broad range of therapeutic targets and much more intense effect [41]. Both these molecules bind to hydrophobic space adjacent to ATP binding pocket of kinase domain and hence they are classified as Type II kinase inhibitors [21]. Structurally regorafenib is similar to sorafenib, except for an additional fluorine atom in the central phenyl ring [41].

Regorafenib targets several hallmarks of colorectal cancer progression through its broad kinase inhibition nature such as antiangiogenesis (by targeting VEGFR1–3, TIE2, PDGFR, and FGFR1 and 2), antiproliferation (by blocking c-KIT, RAF1, BRAF, and RET), antimetastasis (by inhibiting VEGFR2 and 3, and PDGFR), and anti-immunosuppression (by targeting CSF1R) effects [41]. Regorafenib has shown significant improvement in the outcome of event in highly aggressive colorectal cancer [44]. Apart from them, because of their broad-spectrum kinase inhibitory property they have wide range of drug sensitivity as well even in RAS and BRAF mutation status [45] mammalian target of rapamycin (mTOR). This is more selective for the mTORC1 complex, with little impact on the mTORC2. Everolimus is being used for the treatment of progressive or metastatic pancreatic neuroendocrine tumors, which are unresectable, and for progressive well-differentiated nonfunctional neuroendocrine tumors of gastrointestinal tract with unresectable, locally advanced, or metastatic cancers. As of 2010, Everolimus was under Phase III trials for gastric and hepatocellular carcinoma.

Lenvatinib is an anticancer drug that acts as a multiple kinase inhibitor against the VEGFR1–3 as well as fibroblast growth factor receptors (FGFR1–4), PDGFR-α, c-KIT, and RET proto-oncogene [46]. By inhibiting these kinases, Lenvatinib inhibits tumor angiogenesis, tumor cell proliferation and induces apoptosis that leads to reduction in the tumor size. In 2018, the FDA approved Lenvatinib for first-line treatment of patients with unresectable HCC [47].

Vemurafenib is a synthetic small molecule, B-RAF V600E mutated kinase inhibitors that interrupt the B-RAF/MEK/ERK signaling pathways. This signaling cascade is associated with tumor cell survival and proliferation [48, 49]. Vemurafenib got approval form US FDA for the treatment of late-stage melanoma in 2011

[50]. This drug was also in the early Phase I clinical trial for various solid tumors including colorectal cancer [49].

4.2.2.2 Proteasome Inhibitors

The ubiquitin proteasome pathway is a regulated protein degradation pathway that helps in degradation of 80% of cellular proteins and aids in maintaining homeostasis and cellular functions. This is a two-step process in which proteins destined for proteolysis are first ubiquitinated by the action of three enzymes E1, E2, and E3, followed by degradation by 26S proteasome. Polyubiquitination at lysine 63 plays an important role in cellular signaling whereas at lysine 48 helps in degradation of the protein. Many proteasome inhibitors were initially synthesized to understand the catalytic activities in vitro. After the basic knowledge of their activity, they were considered as potential therapeutic agents. These compounds exhibited a broad spectrum antiproliferative, antiangiogenic, proapoptotic activities in hematological [51] and solid tumors.

Loss of cell cycle regulation is the first step in oncogenesis. Cell cycle is tightly regulated through the action of cyclins and cyclin dependent kinases. p27 is a tumor suppressor molecule that negatively regulates cyclins D and E thereby blocking the cells in G1 phase of cell cycle [52]. Low level of p27 is reported in colon cancers [53]. Skp-2 a ubiquitin ligase of s-phase kinase protein targets p27 for proteasomal degradation [54]. Proteasome inhibitors were shown to downregulate Skp-2 leading to the accumulation of p27 resulting in cell cycle arrest [55].

Evasion of apoptosis is one of the hallmark features of cancers. Proteasome was shown to control apoptosis by modulating the expression of proapoptotic and antiapoptotic proteins. Proteasome inhibition exhibited an upregulation of proapoptotic proteins such as p53, BAX, NOXA, simultaneously downregulating antiapoptotic proteins like Bcl-2 and IAPs [56]. The lack of p53, a tumor suppressor protein, was often assigned as one of the causes for tumor progression and drug resistance in many cancers. The hyperactivation of MDM2, a E3 ligase, an interacting partner of p53 helps in targeting p53 for proteasomal degradation by downregulating its downstream targets such as p21, Fas ligand, PUMA, and Bax [57]. Proteasome inhibitors were shown to induce p53-dependent apoptosis in colon cancer [58]. Proteasomal inhibitors were also shown to inhibit angiogenesis by decreasing the secretion of VEGF [59].

Bortezomib was also shown to inhibit cell growth of vascular endothelial cells by suppressing G2/M transition [60]. It was approved by FDA in 2003 as third-line treatment for multiple myeloma but was later approved for first-line treatment in 2008. Though it exhibited very good potency as a single agent, its main use was to use it in combination with other chemotherapeutic drugs to overcome resistance and induce sensitivity. Though this drug exhibited very good efficacy in hematological malignancies, it exhibited very poor results in clinical studies in solid tumors [61]. Looking at these results, many next generation proteasomal inhibitors like Carfilzomib [62, 63], CEP-18770 [63], NPI0052 [64], were developed.

4.2.2.3 Matrix Metalloproteinase Inhibitors

Matrix metalloproteinase inhibitors are another group of small molecule inhibitor used for cancer therapy. These are targeted against Matrix metalloproteinases, endopeptidases that degrade the extracellular matrix. Earlier reports indicate their role in tumor invasion, angiogenesis, and metastasis [65]. The two major causes of cancer are relapse and cancer-related mortality. There are around 24 MMP proteins identified till date. MMP-2 and -9 were shown to regulate many signaling pathways and help in cancer progression [66]. Therefore, the use of inhibitors against the MMPs to control cancers gained research interest. Despite strong preclinical data, these molecules showed very poor efficacy in reducing tumor burden and improving overall survival of patients. It was shown that some of the MMPs showed anticancer properties and the inhibition of these further helped in the progression of the disease. These molecules were shown to be nonspecific and hence were found to target all the possible MMPs.

4.3 Limitations of Small Molecule Inhibitor Targeted Therapies

Despite considerable success in the treatment and survival rate of GI cancer patients, there are many limitations associated with the small molecule inhibitor drugs that need to be addressed. Some of the small molecule inhibitors bind multiple targets including cell surface receptors and other intracellular proteins thus increasing the risk of toxicity even in normal cells [67]. Short life span of small molecule inhibitors prompts for frequent dosing [68]. Acquiring resistance to these inhibitors is either due to mutations in their target molecules or progression of disease through an alternate target independent pathway [69–71]. Adverse secondary complications like diarrhea, vomiting, scaly, and itchy skin, and hematological disturbances were reported during the treatment period [72–74]. The heterogeneity of the disease itself limits the drugs only to a certain population of patients with set molecular patterns.

4.4 Conclusion

The increased rate of recurrence and resistance to the traditional chemotherapy has compelled the scientific community to look for alternate approaches for the treatment of GI cancers. With increasing knowledge of molecular pathways in gastrointestinal cancers, one of the new approaches chosen is targeted therapy by small molecule inhibitors. Small molecule inhibitors are designed against pathways that are normally deregulated in GI cancers thereby inhibiting cancer cell proliferation and survival. The major group of small molecule inhibitors that are in use in clinics for

GI cancers are the kinase inhibitors like regorafenib and sorafenib. The overall survival of patients post kinase inhibitor treatment was shown increased. But there are many reports of secondary complications associated with these drugs as well. It was reported that these drugs targeted a variety of kinases that are required for the regular metabolism of other cell types in the body. Through research for ligand-based targeting of these drugs to the specified tissues would enhance the efficacy of the molecule while minimizing the dosage and secondary complications associated with them. Other small molecule inhibitors like the proteasomal inhibitors and matrix metalloproteinase inhibitors are being studied extensively and still need approval for clinical use. Increasing updates of the novel mechanism and pathways, targeted delivery systems and combination therapies of these different classes of small molecule therapeutics would further enhance the treatment efficacy and survival for GI cancer patients.

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Chapter 5
Epigenetic Biomarkers for the Detection of Gastrointestinal Cancers

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Abstract Cancers of the gastrointestinal tract is the fourth common malignancy in humans with gastric cancer and colorectal cancer being most prevalent in both sexes. Despite considerable progress in the therapy of gastrointestinal cancers with an overall improvement in the survival rates, the incidence of both gastric and colorectal cancers is on the rise globally. This emphasizes the need to identify novel potential biomarkers that could be of immense help in the early detection of cancer. Epigenetic changes implicate heritable, but reversible alterations in the genome without any modifications in the DNA sequences. Recent research has shown that understanding these epigenetic changes is important as it has a key role in the onset and development of cancer including gastrointestinal cancers. Hence, tracking such epigenetic alterations during carcinogenic conditions could be a potential strategy in the development of precise, reliable biomarkers that could aid not only in the detection of cancer but also in the evaluation of patient prognosis to therapy. This chapter discusses on the common epigenetic modifications like Histone modification, DNA methylation, chromatin remodelling and the effect of noncoding RNAs especially miRNA in the pathogenesis and progression of gastrointestinal malignancies. The chapter also discusses the significance of these epigenetic alterations in developing cheap but potent markers for the detection of gastric and colorectal cancers in humans.

Keywords Epigenetic modifications · Gastric cancer · Colorectal cancer · Gastrointestinal malignancies · Biomarkers · Cancer detection

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Abbreviations

CIMP	CpG island methylator phenotype
CIN	Chromosomal instability
CRC	Colorectal cancer
DNMTs	DNA methyltransferases
FDA	Food and Drug Administration
GC	Gastric cancer
GI	Gastrointestinal
GIC	Gastrointestinal cancers
HDMs	Histone demethylases
HMTs	Histone methyl transferases
LOH	Loss of heterozygosity
MAPK	Mitogen-activated protein kinases
MBPs	Methyl-binding proteins
MMR	Mismatch repair genes
MSI	Microsatellite instability
QOL	Quality of life

5.1 Introduction

Malignant neoplasms comprise a group of devastating deadly disease with high morbidity and mortality in the developed, developing and under developed world. The disease statistics and survey by GLOBOCAN shows an alarming trend with newly diagnosed cancer cases amounting to 18.1 million and total number of deaths amounting to 9.6 million in the year 2018 owing to cancer [1]. The interesting fact is that this figure is projected and anticipated to rise by at least 70% in 2030 emphasizing the need for understanding the pathogenesis of disease, to identify novel biomarkers for the early detection and framing reliable therapeutic strategies against cancer.

Gastrointestinal (GI) malignancy is the fourth most prevalent malignancy in humans [2], with newly diagnosed cases and deaths accounting to about 4.1 and 3 million, respectively, per year worldwide. Cancers of the gastrointestinal tract majorly include the malignancies occurring at any location in the digestive tract from the oesophagus extending till the rectum with all linked with definite clinical features. Among the GI cancers, colorectal and gastric cancers are the most prevalent malignancy in humans. Liver cancer is the next common malignancy with a high mortality rate. Even though, pancreatic and oesophageal cancers are less common, reports indicate that the survival rate in these two malignancies are very low [3, 4].

Both gastric as well as colorectal cancers are aggressive and invasive due to which they contribute to high rate of mortality globally. These GI cancers were caused due to changes (genetic/epigenetic) that culminate in the transformation of

noncancerous cells to cancerous cells. The frequently observed genetic alterations during tumorigenic conditions could be mutations in tumour suppressor genes or oncogenes that culminate in defects in the functionality of key proteins or dysregulation in gene expression. On the contrary, epigenetic alterations could influence the expression of genes but do not inflict changes in the sequence of the DNA. Epigenetic changes are reversible but heritable. As with gene mutations, epigenetic alterations are major players in the pathogenesis of cancer and contribute to molecular heterogeneity of several types of tumours.

The epigenetic changes that are frequently observed in the two most prevalent gastrointestinal malignancies, viz., the gastric and colorectal malignancies are described in this chapter with an objective to give better insights and to discuss the underlying changes during carcinogenesis. But, the chapter mainly focusses on the ways and means by which these epigenetic alterations can be exploited to be used as reliable tools for the development of clinically relevant novel biomarkers for gastrointestinal cancers (GIC) that could not only aid in the diagnosis and prognosis in cancer patients but also in the assessment of risk in predisposed population.

5.2 Colorectal Carcinoma and Gastric Adenocarcinoma

Colorectal cancer (CRC) has emerged as the fourth common cause of death due to cancer with about 700,000 reported deaths annually exceeded only by lung, liver and stomach cancer. The global male to female incidence statistics on cancer indicates that CRC is the second (9.2%) and third (10%) most common cancer, respectively, in women and in men. Epidemiological studies suggest that CRC is more prevalent in Western countries, with a reported increase in incidence every year. An important feature that underlies CRC is genomic instability and the molecular mechanisms that contribute to the pathogenesis of CRC could be attributed to three major events that include microsatellite instability (MSI), chromosomal instability (CIN) and CpG island methylator phenotype (CIMP) [5].

The CIN pathway, also referred to as classical pathway, is the most prevalent one as it is observed in majority (80–85%) of CRC cases. It is understood that this pathway results in the development of aneuploidy tumours and loss of heterozygosity (LOH) due to imbalances in the number of chromosomes. The factors that contribute to CIN include alterations (DNA damage response, chromosome segregation, telomere dysfunction) that eventually influence the function of critical genes responsible for maintenance of cellular function [6].

Defects in DNA repair mechanisms resulting in a hypermutable phenotype characterize the MSI pathway that leads to a diminished ability to repair short DNA chains or tandem repeats in tumours. Mutations that can affect both noncoding regions and codifying microsatellites tend to accumulate in such regions leading to development of tumours when there are alterations in the reading frames of oncogenes or tumour suppressor genes codified in microsatellites. Spontaneous events like promoter hypermethylation or germinal mutations (as observed in Lynch

syndrome) can cause loss of expression of mismatch repair genes (MMR) that result in tumours that are mainly diploid in nature and harbour less LOH [7].

CIMP pathway is the other common feature in CRC. The classic finding in CIMP tumours is the hypermethylation of oncogene promoters that silences critical genes and thereby culminate in loss of protein expression. Genetic as well as epigenetic events operate in tandem in colorectal cancer, and both contribute to the development of tumours in which more methylation events rather than point mutations are frequently observed. The presence of both BRAF mutations as well as microsatellite instability in many CIMP tumours is the classic example for the synergistic influence of both genetic and epigenetic factors in the development of colorectal cancers [8].

Globally, gastric cancer continues to be the second common cause of mortality owing to cancer, although a major decline has been observed in the past few decades both in terms of the disease incidence and related deaths. The disease incidence is relatively higher in East Asia followed by Eastern Europe, South America and parts of Central America. The prevalence rate is high among men (almost double) as compared to that of women. The disease statistics show a poor prognosis, with only 30% of the patients showing a 5-year relative survival in most of the countries. The most common risk factors for gastric cancer are infection with the bacterium *Helicobacter pylori*, sex factor (being male), smoking and genetic predisposition including a family history of gastric cancer [9]. Almost 90% of all tumours of the stomach are reported to be malignant and gastric adenocarcinoma comprises about 95% of the total number of gastric malignancies.

The common therapeutic strategies include surgical intervention involving complete or partial gastrectomy with lymphadenectomy. Statistics show that only 10–30% of the gastric cancer patients have an surpass the 5-year survival period indicating a poor prognosis for the disease. A striking difference in the overall survival rate is noted between the Asian and Western population indicating that ethnicity could be a potential risk factor. Eastern Asia has the highest incidence (with Japan showing an incidence rate of 40 for every 100,000 individuals) followed by Southern America and Eastern part of Europe. Northern America, Northern Europe and Africa report low incidences. Among countries, Canada (only 10 per 100,000 individuals) and the United States report the lowest incidences of gastric cancer. In a report on the evaluation of ethnicity as a possible risk factor for gastric cancer, The National Cancer Institute has identified three risk groups: a high-risk group that includes Koreans, Japanese, Vietnamese, Hawaiian and native American population. The moderate risk group includes Chinese, Latinos and black population. The low-risk group comprises Filipinos and Caucasians [9] (Fig. 5.1).

5.3 Epigenetic Alterations and Its Relevance to Cancer

Epigenetics is an emerging new arena of molecular biology that has gained research attention over the past two to three decades. During these years, there has been considerable improvement in understanding the role of epigenetic changes in

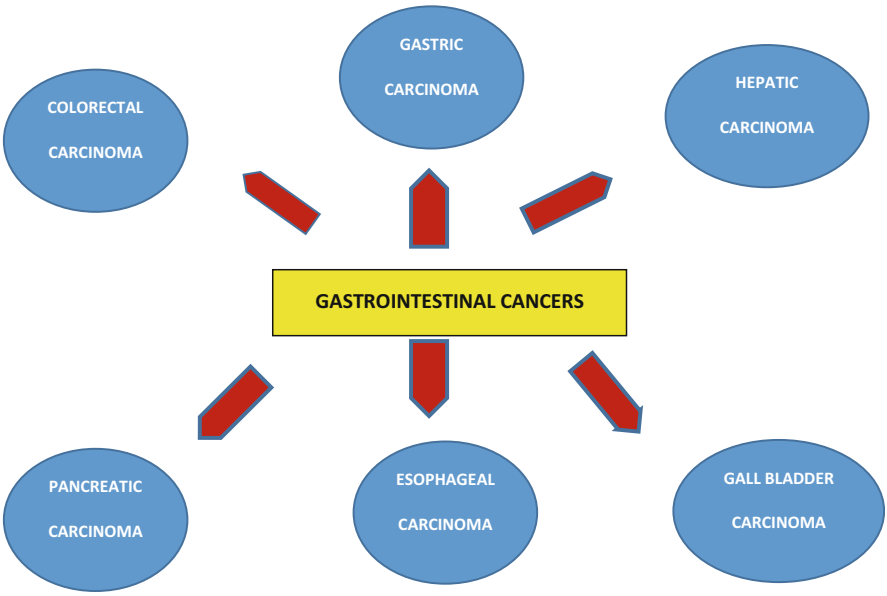

Fig. 5.1 Types of gastrointestinal cancers prevalent in population

contributing to differentiation, aging as well as to disease development. A better understanding of the epigenetic processes has led to a rapid progression in the development of drugs targeting these processes. Looking back into the history of epigenetics, C.H. Waddington in 1942 coined the term '*epigenetics'* when he was trying to understand the relevance between the genotype and the phenotype [10]. It is now understood that epigenetics refers to heritable changes of the genome without any alterations in primary DNA sequences [11]. Epigenetics differs from traditional genetics, with respect to reversibility and position effect. It is reported that similar to genetic abnormalities, epigenetic changes are also significantly responsible for the initiation and progression of cancer. As in the case of genomic biomarkers, the discovery of epigenomic biomarkers via high throughput screening technologies can lead to identification of new molecular targets that can aid in the early diagnosis of cancer. Epigenetic modifications include specific histone modifications, DNA methylation, chromatin remodelling, noncoding RNAs especially altered expression of microRNAs that can modulate the expression of genes by several mechanisms other than variations in genomic DNA sequences [12–15].

The first aberrant epigenetic alterations in human colorectal cancer was discovered by Feinberg and Vogelstein in 1982 [16]. The clinical features of CRC make this disease apt for screening as it has a decipherable clinical manifestation and a defined natural history with effective surgery and high life expectancy during the early stage of the disease [17]. On the other hand, existing screening approaches are not finest while considering expenditure and invasiveness and noninvasive screening methods are preferred. An ideal biomarker for CRC should exhibit precise features

like easily available, low-cost and analysable to detect patients with cancer, so as to upgrade their effect of patient response to exact treatments, that really progress the prognosis and quality of life (QOL) in patients.

Epigenetic alterations take place in a variety of genes such as onco-, tumour suppressor-, mismatch- and cell cycle genes [18]. As described before, three molecular pathways are evident that are distinguished by three different pathways of genomic instability, such as (a) DNA microsatellite instability (MSI) phenotype, characterized by mutations in genes involved in DNA mismatch repair, (b) chromosomal instability (CIN) phenotype by APC and other gene mutations that induce Wnt pathway and (c) CpG island methylator phenotype (CIMP), global genome hypermethylation, advancing into switch off of tumour suppressor genes in CRC. Nearly 65% of CRC develop through the CIN pathway and CIMP is found to be associated with approximately 20% of CRC. Although, differences are there in the patterns of these three pathways, they are not reciprocally exclusive. Reports indicate that a tumour may intermittently exhibit characteristics of multiple pathways [19–21] (Mojarad et al. 2013).

Patient survival is poor in gastric cancer largely due to delayed diagnosis and suboptimal therapeutic strategies. Heterogeneity of the disease is a major obstacle in the therapy, highlighting the necessity for precise treatment strategies. Several studies have reported different subtypes of gastric cancer are characterized by genetic as well as epigenetic hallmarks. It is now clearly understood that epigenetic modifications observed in gastric cancer, although appear to be bystander events, contribute significantly in promoting carcinogenesis through several interlinked mechanisms. Epigenetic alterations, induced by infection with the bacterium *H. pylori*, are early events in the pathogenesis of gastric cancer, probably preceding genetic abnormalities.

5.4 Major Epigenetic Modifications: Significances and Consequences (Fig. 5.2)

5.4.1 Epigenetic Modification Involving Methylation of DNA

One of the well-studied epigenetic modifications is methylation of DNA, during which a group of enzymes known as DNA methyltransferases (DNMTs) covalently add a methyl group to cytosine residues found within CG dinucleotides [22]. CG dinucleotide sequence, shortly referred to as CpG, is the preferred substrate for the DNMTs in mammalian cells and is found nonuniformly distributed throughout the human genome. CpG islands refer to sequences that are more than 200–500 bases in length, have a GC content of more than 50% and a CpG ratio of about 0.6 [23]. They are predominantly present in the gene promoter regions and are found to be extensively methylated in transformed malignant cells [13]. Transcriptional silencing will be observed following CpG island methylation within the promoter region whereas

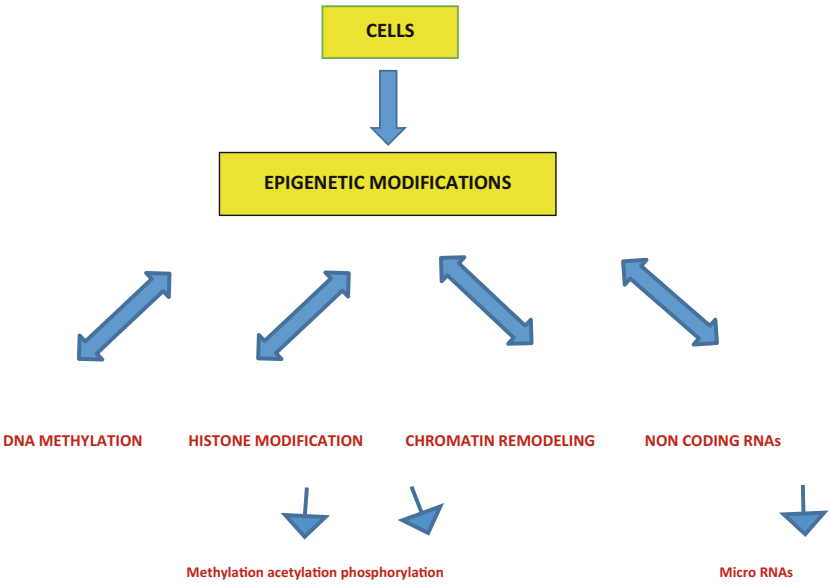

Fig. 5.2 Schematic illustration of the common epigenetic alterations in the genome

transcriptional activation will be witnessed following methylation of CpG sites outside the promoter regions [24]. Simultaneous demethylation (of the entire genome) and hypermethylation (in the CpG islands of gene promoters) has been reported to occur during tumorigenesis [25].

Also, changes in chromatin structure, lowered condensed chromatin and increased genome instability could be induced by wide range of hypomethylation which eventually could lead to occurrence of tumours. Many reports on tumour models have shown that the microsatellite DNA sequences which are hypomethylated are vulnerable for mutations [26]. In addition, hypermethylated CpG islands of gene promoter which silences tumour suppressor genes could also contribute for tumour progression [27]. Interestingly, proteins like methyl-binding proteins (MBPs) that can attach to the methylated DNA with very high affinity can indirectly block the binding of the transcription factors to the promoter regions [28]. Thus, analysing the DNA methylation status can serve as reliable indicator in the detection and screening for cancer.

5.4.1.1 Methylation Status of DNA as a Reliable Marker for Colorectal Cancers (CRC)

Across the average CRC genome, several hundred genes show alterations in DNA methylation. Among these genes, which are detected in various body fluids,

searching for the ones with potential clinical relevance has attracted much research attention. Such genes can be useful and noninvasive markers for gastrointestinal cancers. Few examples for epigenetic biomarkers based on DNA methylation status has been listed below.

- HOP homeobox methylation that has been observed in 84% of hypermethylated samples as against 10% of the matched adjacent tissues has been reported as a relevant biomarker [29].
- Septin 9 encodes for a GTPase involved in dysfunctional cytoskeletal organization. A CRC screening test involving blood-based PCR using the methylated SEPT9 biomarker (Septin 9) aids in the detection of all stages of CRCs anywhere in the colorectal locations. The test that has an overall sensitivity of 90% and a specificity of 88% is approved by Food and Drug Administration (FDA) for use as a blood-based marker for the detection of colon cancer.
- Genes that encode thrombomodulin, runt-related transcription factor 3, secreted frizzled-related protein 2, syndecan-2 are other potential blood-based methylation biomarkers that are currently under investigation [30].
- Sixty-eight per cent of primary colon tumours show promoter hypermethylation-induced inactivation of the novel tumour-suppressor gene, T-box transcription factor 5 (TBX5). Hence, the detection of methylated TBX-5 may serve as an indicator for the occurrence of CRC [31].
- The detection of WIF 1, PENK and NPY in the serum samples of patients has been recently identified as a reliable test with high degree of sensitivity and specificity for the detection of CRC. Hence these can be novel epigenetic markers for CRC and this test could be a cost-effective tool for the screening of asymptomatic CRC patients and help to decide whether or not they should go for further examinations [32].
- A stool-based test to detect the methylation of vimentin gene is commercially available. Combined with colonoscopy, the test has a degree of sensitivity that ranges from 40% to 80% in the detection of CRC [33].
- Tests involving the detection of hypermethylated genes that encode for APC, SFRP1, SFRP2, fibrillin-1, MLH1, MGMT, CDKN2A and NDKG4 in stool samples of patients have been developed for the diagnosis of CRC. These tests are noninvasive and have varied levels of sensitivity that ranges from 60% to 80% [34, 35].
- Ninety-seven per cent of colorectal adenomas and 99% of Stage I to Stage IV CRCs express TFP12. Hence, this can be a marker for colorectal adenoma as well as colorectal carcinoma [36].
- Two FDA approved tests that rely on DNA methylation status, viz., Epi proColon (for the detection of methylated SEPTIN 9 gene in blood samples) and Cologuard (which is a multitarget test and detects two DNA methylation biomarkers in faeces) are currently being used as a preliminary screen for the detection of CRC.

5.4.1.2 Methylation Status of DNA as a Reliable Marker for Gastric Cancers (GC)

Gastric adenocarcinoma, the most prevalent (90–95%) type of gastric cancer, has two histological subtypes. Based on microscopic observation and tumour growth patterns, these are classified as intestinal and diffuse and both types differ widely in molecular pathogenesis [37]. One factor other than the geographic, ethnic and cultural factors that could greatly influence the onset of gastric cancer is chronic *H. pylori* infection that is well known to induce inflammatory changes in the gastric mucosa [38]. However, irrespective of the underlying triggers the common feature that characterizes gastric carcinoma of all types is the involvement of epigenetic alterations. As the predisposing factors for gastric carcinoma are heterogeneous, it is mandatory to assess the alterations observed in each type of gastric cancer so as to enable precision in diagnosis, effectiveness in therapy and to conveniently track the prognosis during the disease.

- Gastric cancer development is reported to be frequently associated with gene promoter methylation. Classic examples for this could be genes like TFPI2, SFRP2, TCF4, CDKN2A, CDK2AP2, MGMT, CDH1, RASSF1, RUNX3, DLC1, ITGA4, PRDM5, ZIC1, PCDH10, hMLH1, SPINT2, BTG4, DKK-3, GRIK2, BNIP3, RAR, CHFR, LRP1B, RASSF1A and SFRP5, the methylation of which has been observed in tissues with gastric cancer but not in the normal gastric tissues [39, 40].
- It has been well established that the promoter hypermethylation of CDH1 (E-cadherin) [41] and MGMT [42, 43] is linked to poor prognosis in patients who underwent surgical intervention for gastric cancer. CDH1 is a cell adhesion molecule and is located at epithelial cell junctions. Loss of activity of CDH1 induces CpG island promoter hypermethylation and hence expression of CDH1 appears to be downregulated in gastric tumours and is linked to poor clinical outcomes in patients. On the contrary, IGF2 hypermethylation in gastric cancer patients was reported to be [44, 45] associated with a better survival rate as compared to those with hypomethylated status [46].
- Methylation status of genes (*DAPK*, *CDH1*, *p15*, *p16*, *GSTP1*, *RASSF1A*, *RARβ*, *TFPI2* and *RUNX3*) in noninvasively obtained body fluids such as serum and gastric washes could be used for the detection of aberrant changes in DNA thereby serve as useful biomarkers for detection of gastric cancer [47].
- It is reported that environmental factors have appreciable influence on DNA methylation. Etiological studies have revealed the close association of two distinct infectious agents, *H. pylori* and *Epstein-Barr* virus (EBV) with gastric carcinogenesis [48, 49]. Promoter methylations were observed in tumour suppressor genes including CDH1, LOX, RUNX3 and p16 following infection [50, 51]. In addition to *H. pylori* infection, *Epstein-Barr* virus infection also predisposes an individual to a high risk of developing gastric carcinoma. Aberrant methylation of p15, p16, p73 and CDH1 is reported in EBV-associated gastric cancer whereas such aberrations were not frequently observed in the surrounding

normal non-neoplastic tissue. This implies that EBV infection is associated with aberrant methylation patterns, which is critical in the onset and progression of EBV-induced gastric tumorigenesis [52–55].

- Significant hypomethylation of long interspersed element-1 (LINE-1) was reported in gastric cancer tissues as compared to nonmalignant gastric mucosa. Hypomethylation of LINE-1 was also found to be frequently associated with a reduction in survival rate of GC patients [56]. Moreover, it is well established that LINE-1 hypomethylation of nonmalignant gastric tissue correlated significantly with *H. pylori* infection in patients with gastric cancer [57].
- Hur et al. reported upregulated expression of sulfatase 1 (SULF1) in gastric cancer tissues and hence SULF1 is an important prognostic factor for assessing the outcomes of therapy in gastric cancer patients [58].
- Aberrant hyper methylation of SLC19A3 (a member of the vitamin transporter family) promoter and methylation of RNF180 (ring finger protein 180) are reported to be good diagnostic markers for GC patients [59]. Promoter methylation of RNF180 in GC patients was observed to be 76%.
- The combined use of four methylation markers including E-cadherin, MHL1, APC and TIMP3 yielded a 55% sensitivity and 86% specificity. It was found that in gastric cancer tissue the catalytic subunit of telomerase hTERT (telomerase reverse transcriptase) has CpG islands, which is aberrantly hypermethylated, whereas this feature is not observed in noncancerous tissue. But it still remains unclear as to whether the hTERT methylation is reliable marker for GC (Table 5.1).

5.4.2 Histone Modification as an Epigenetic Alteration

Modification of the histone tails is another crucial epigenetic alteration wherein modifications like acetylation, methylation, phosphorylation, ubiquitination and sumoylation are frequently observed [60, 61]. Histones are proteins that contain a charged and flexible amino terminus referred to as the histone tails and also a globular domain. The histone tails actively take part in post-translational modifications. The histone proteins (H2A, H2B, H3 and H4) combine together (two subunits unite) resulting in the formation of an octamer, which is covered by DNA to form the basic structural unit of the chromatin—the nucleosome [62]. The active interaction between the histone proteins and DNA will hinder the accession of the enzyme RNA polymerase II and several other transcription factors to the transcription sites on the DNA [63].

Table 5.1 List of epigenetic markers based on DNA methylation for colorectal and gastric carcinoma

S. no.	Methylation status	Sample	Name of the marker	Tumour diagnosis
1.	Hypermethylation	Tissue	HOP homeobox	Colorectal carcinoma
2.	Hypermethylation	Blood	SEPT9	Colorectal carcinoma
3.	Hypermethylation	Blood	Syndecan-2, thrombomodulin, RUNX2	Colorectal carcinoma
4.	Hypermethylation	Blood/ tissue	TBX5	Colorectal carcinoma
5.	Hypermethylation	Blood	NPY, PENK, WIF1	Colorectal carcinoma
6.	Hypermethylation	Stool	Vimentin	Colorectal carcinoma
7.	Hypermethylation	Stool	Fibrillin-1, APC, CDKN2A, SFRP	Colorectal carcinoma
8.	Hypermethylation	Blood/ tissue	TFP12	Colorectal adenoma
9.	Hypermethylation	Tissue	CDKN2A, SFRP2, RUNX3	Gastric carcinoma
10.	Hypermethylation	Tissue/ blood	Cadherin-1 (CDH1)	Gastric carcinoma
11.	Hypermethylation	Tissue/ blood	RUNX3, p16, LOX, CDH1	Gastric carcinoma
12.	Hypomethylation	Tissue	LINE-1	Gastric carcinoma
13.	Hypomethylation	Tissue	SULF-1	Gastric carcinoma
14.	Hypermethylation	Plasma	RNF180 (ring finger protein)	Gastric carcinoma

5.4.2.1 Histone Acetylation

The transcriptional status of genes after active post-translational modifications of the histone tails greatly influences the structure of the chromatin. Histone acetyltransferases (HAT) are a set of enzymes that govern and regulate an important histone modification process referred to as histone acetylation. Histone acetyltransferases (HAT) catalyse the addition of an acetyl group to the histones (at lysine residues), whereas histone deacetylases (HDAC) act to remove an acetyl group. It is understood that HATs can upregulate the transcription and transactivation of specific genes by enabling an open structure of chromatin owing to the neutralization of a positive charge. On the contrary, HDACs can induce chromatin condensation and downregulate the transcription of specific genes [64, 65]. Four catalytic groups of Histone deacetylases have been reported. These include the Class I (comprising 1–3 and 8), the Class II (comprising HDAC 4–7,

HDAC 9 and 10), the Class III (including Sir-2 related protein 1–7) and Class IV (comprising HDAC 11) [66]. Aberrant gene silencing and tumorigenesis is frequently associated with deregulation of HDAC activity that justifies exploiting the HDACs as potential molecular targets in the therapy of cancer and a reliable marker in the diagnosis of cancer [67].

5.4.2.2 Histone Methylation

Methylation of arginine and lysine residues of histone proteins (H3 and H4) are frequently observed. Such methylated histones could influence cellular functions by modulating several DNA regulatory factors. Histone methyl transferases (HMTs) regulate the methylation, whereas histone demethylases (HDMs) regulate the demethylation of the histone tails. Shi et al. [68] reported that LSD 1 (histone demethylase SWIRM1) bring about the demethylation of histones, thereby implicating for the first time that histone methylation is reversible. Mono-, di- and trimethylation of the lysine residues have been reported [69, 70]. The open or closed chromatin structure could be attributed to the residue involved and the degree of methylation. The best examples for the conditions lead to open structure of chromatin could be trimethylation at H3K4 and H3K36, whereas the classic examples for the closed structures are trimethylation (at H3K9, H3K27 and H4K20) and demethylation (H3K9) [71].

5.4.2.3 Histone Phosphorylation

The maintenance of kinase-phosphatase equilibrium at kinetochore by histone phosphorylation prevents chromosomal instability that eventually inhibits the development of cancer. Histone phosphorylation is one of the post-translational modifications that occurs during DNA damage, cell division, chromatin remodelling, apoptosis and activation of transcription and chromatin remodelling/compaction during cell division [72]. For example, H3S10 phosphorylation is induced by the ERK-MAPK (mitogen-activated protein kinases) pathway to induce chromatin condensation and thereby contribute for mitosis progression [73]. Several research groups are working toward identifying potential biomarkers for different malignancies based on histone phosphorylation.

Hence, it is clearly evident that histone modifications at specific regions in a gene could generate either an open or closed structure of the chromatin culminating in modulation (activation or repression) of gene expression [74].

5.4.2.4 Histone Modification Based Biomarkers for Colorectal Cancers

- Upregulation in the expression of several HDACs, such as HDAC 1–3, HDAC 5 and HDAC 7 is reported to be linked to the downregulation of Wnt signalling pathway. This is a common feature that observed in CRC patients.
- Upregulation in the expression HDAC 2 has been reported both in the early stages of colon cancer and also in 62.1% of colorectal adenomas.
- Overexpression of nuclear HDAC 2 was observed in 81.9% of CRC. This was observed to be associated with hypoacetylation (at histones H4K12 and H3K18) during adenoma–carcinoma progression. This indicates that upregulated expression of HDAC 2 and the resultant lack of acetylation are tightly linked to CRC progression.
- Enhancer of zeste homolog 2 (E2H2) that encodes for a H3 methyl transferase induces target gene repression. This implicates poor prognosis and can facilitate promotion of metastasis during CRC.
- Downregulated expression of dual specificity phosphatase 22 (DUSP22) in stage IV colorectal cancer patients was found to be associated with poor survival outcomes [75].
- Upregulated phospho-H2AX expression in colorectal cancer tissues correlate with a poor prognosis [76] (Lee et al. 2015).
- PP1 is a member of phosphoprotein phosphatases superfamily, revealed to reconcile migration and invasion inhibitory protein (MIIP)-S303 dephosphorylation. The downregulated expression of this is linked to increased tumour metastasis.

5.4.2.5 Histone Modifications Based Biomarkers for Gastric Cancers

- Many HATs including p300, CBP and PCAF (p300/CBP associated factor) regulate oncogenesis by inducing acetylation of several proteins (both histones and nonhistones) [77, 78]. During gastric cancer, loss of heterozygosity of p300 was reported [79]. Downregulated expression of PCAF has been implicated during gastric cancer, which correlates well with tumour size, node metastasis and gastric wall invasion, whereas patients exhibiting high-PCAF showed better survival outcomes [80].
- Altered expression of HDACs (HDAC1 or HDAC2) was reported in gastric carcinoma [81, 82]. The class III HDACs influence the deacetylation of several regulatory molecules (including p53 and Rb) controlling cell cycle and apoptosis and thereby indirectly regulate cell survival [83–85]. Several pathological epigenetic alterations in cancers have been attributed to histone acetylation. It is reported that the reduction of p21 is induced by the hypoacetylation of histone H3 [86], whereas hyperacetylation of H3 of the ZNF312b (FEZ family zinc finger 1) facilitate gastric cancer progression [87].

- It is also reported that multiple tumour suppressor genes are inactivated owing to methylation of H3K9, which frequently reported to be linked with advanced carcinoma, metastasis, remission, recurrence and bad prognosis [88].
- Upregulated phosphorylated histone H3 expression and downregulated acetylated histone H4 expression has been shown to correlate with invasion, metastasis and subsequently poor prognosis in gastric cancer patients [89, 90].

All these clearly indicate histone acetylation may have a strong influence on the onset, establishment and progression of gastric carcinomas. Hence, histone acetylation changes could be promising epigenetic biomarkers of gastric cancers [91].

5.4.3 Chromatin Remodelling

Any changes with respect to chromatic location and structure is referred to as chromatin remodelling that culminates in the loss of chromatin structure integrity in nucleosome joint. This leads to the exposure of *cis*-acting factors located in the gene promoter regions that could interact with the *trans*-acting factors [92]. Nucleosome remodelling complex (ATP dependent) and histone covalent modification complex mediate chromatin remodelling through ATP hydrolysis mediated configuration of nucleosome and the covalent modifications on the histone tails, respectively. The two complexes operate in tandem thereby resulting in the activation of chromatin-modifying enzymes.

The enzymes that modify chromatin can be classified into two different families. The ISWI family (which mobilizes nucleosomes on DNA) and the SWI/SNF (switch/sucrose nonfermentable family that modifies the structure of the nucleosome transiently resulting in exposure of DNA) [93, 94]. Active chromatin remodelling is mandatory for several key biological processes including DNA replication, DNA damage/repair and gene transcription. Hence, any alterations in these processes can be directly or indirectly linked to the incidence and development of tumours.

5.4.4 Noncoding RNAs (miRNAs) and Role in Epigenetic Changes

The discovery of the regulatory noncoding RNAs is one of the most remarkable and spectacular discoveries in the field of molecular biology. This discovery has contrasted and challenged the basic principle of central dogma in molecular biology that projects the RNA as an intermediate between genes and protein. The noncoding RNAs are classified as following:

(a) long noncoding RNAs (lncRNAs) that are longer than 200 nucleotides
(b) small regulatory RNAs such as microRNAs (miRNAs)

(c) short interfering RNAs (siRNAs)
(d) piwi-interacting RNAs (piRNAs)
(e) small nucleolar RNAs (snoRNAs) and other short RNAs

miRNA is the extensively investigated type of ncRNAs. These ncRNAs are about 22 nucleotides in size. They regulate the silencing (posttranscriptional) of several protein-coding genes by exerting a tight control on the translation of mRNA into proteins [95, 96]. miRNAs are known to exert their functions by influencing mRNA cleavage or by translational inactivation during pairing with the untranslated 3′-UTR location of target genes [97, 98]. miRNAs regulate several crucial biological processes including cell proliferation, differentiation and cell death [99, 100].

Altered expressions of miRNAs have been reported during tumorigenic conditions. miRNAs currently have immense applications in the classification of human cancers [101]. Downregulated expression of miRNAs is commonly noted during carcinogenic conditions, whereas the onco-miRNAs exhibit upregulated expression. Thus, the small size, higher stability and significant control on translational regulation project miRNAs as powerful biomarkers for gastrointestinal malignancies in comparison with mRNA and proteins [102]. miRNAs have the advantage that tumour-specific miRNAs can be detected in biological samples like serum and faeces at appreciable levels. Also, miRNAs are significantly protected from endogenous ribonuclease activity thereby placing these RNAs as potential candidates to serve as markers for detection of gastrointestinal cancers.

5.4.4.1 miRNAs as Biomarkers for Colorectal Cancers

- Ng et al. [103] demonstrated significant elevation of miR-92 in CRC patients. This test was found to have 89% sensitivity and 70% specificity and can be extremely helpful in discriminating CRC patients from normal population. This suggests the usage of miR-92 as a potential, noninvasive molecular marker for cancer screening.
- miR-21 and miR-31 that are well-known oncogenic miRNAs are involved in the negative regulation of key tumour suppressor genes including TPM1 and PTEN. Overexpression of these miRNAs has been reported in diverse human tumours. The serum miR-21 levels can distinctly distinguish CRC patients from controls and high levels of miR-21 expression in serum and tissue samples correlate with tumour size, metastasis and poor survival outcomes. Hence, miR-21 can be a promising molecular marker for the early diagnosis of CRC [104, 105].
- Detection of miR-194 was reported in patients with advanced colorectal adenoma following polypectomy. This can be a useful marker for the screening of individuals who are highly predisposed for developing CRC in future [106].
- Elevated levels of miR-17 and miR-106a in cancer cells were reported previously [107].
- The levels of miR-21 and miR-92a (oncogenic miRNAs) in stool were significantly elevated in CRC patients as compared to samples from control.

- A recent report reveals that many of the well-studied miRNAs (miR-92a, miR-21, miR-29a and their combinations with other miRNAs) have overall diagnostic sensitivity and specificity of 81% and 79%, respectively. This implicates the usefulness of miRNAs as novel molecular markers for CRC [107, 108].
- Emerging evidences clearly highlight the diagnostic significance of noncoding RNAs other than miRNAs in GIC. High levels of the long intergenic noncoding (lncRNA) HOTAIR were reported to be present in the serum and tumour tissues of colorectal cancer patients. Interestingly, such a finding is reported to correlate with poor prognosis [109].
- Downregulated expression of ncRAN was reported in CRC patients with liver metastases. The finding predicted poor survival outcomes in cancer patients [110].
- Lnc RNA called CCAT-L is detected specifically in human CRCs [111].

However, the tests for noncoding RNA may be used in combination with other conventional screening tests for confirming the presence of GIC [107].

5.4.4.2 miRNAs as Biomarkers for Gastric Cancers

- Downregulation of miR-218 was observed in gastric carcinoma and it was found that it blocks its molecular target Robol, resulting in activation of slit/Robol signalling pathway. This is reported to induce invasion and metastasis in gastric carcinoma patients. Hence miR-218 can be a marker for metastasis in GC patients [112].
- miR-9 was reported to inhibit gastric cancer cell growth by targeting NF-κB. This suggest that miR-9 could suppress gastric carcinogenesis and hence can be a marker for gastric cancer [113].
- Elevated levels of miR-378 was observed in the serum sample of GC patients [114].
- Interestingly, the detection of miR-31 in serum of cancer patients is significantly higher than that of serum carcinoembryonic antigen. This implicates that miR-31 could serve as a precise indicator for GC as compared to CEA [115].
- Several miRNAs are reported to be deregulated in gastric cancer. Silencing of miR-129-2 was reported in gastric cancer and it was observed that reversal of this condition could trigger apoptosis through a coordinated regulation of Bcl-2 family members [116].
- Increased expression of E-cadherin mediated through miR-141 was reported in primary gastric cancer [117–119].
- Downregulated miR-452 expression in GC patients correlates with poor response to cancer therapy. Interestingly, miR-451 overexpression inhibits cellular proliferation and increases the sensitivity to chemotherapy. Report implicates the usefulness of miR-451 as a target in the therapy of GC [120].
- Overexpression of miR-15b or miR-16 was reported to sensitize SGC7901/VCR cells for Vincristine at least partially via inhibiting antiapoptotic Bcl-2 thereby

Table 5.2 List of miRNAs and lncRNA as markers for the detection of colorectal and gastric carcinoma

S. no.	Sample	Name of the marker	Expression status	Tumour diagnosis
1.	Plasma	miR-92	Elevated expression	Colorectal carcinoma
2.	Serum	miR-21	Elevated expression	Colorectal carcinoma
3.	Blood	miR-194	Elevated expression	Colorectal carcinoma
4.	Stool	miR-21 and miR-92a	Elevated expression	Colorectal carcinoma
5.	Serum/ tissue	HOTAIR	Elevated expression	Colorectal carcinoma
6.	Blood/ tissue	miR-218	Downregulated expression	Gastric carcinoma
7.	Blood/ tissue	miR-9	Downregulated expression	Gastric carcinoma
8.	Serum	miR-378	Elevated expression	Gastric carcinoma
9.	Tissue	miR-129	Downregulated expression	Gastric carcinoma

increasing apoptosis in cancer cells [121]. This indicates the usefulness of miR-15b and miR-16 in adjunct therapy.

- Cell-free circulating miRNAs (other than those present in primary and metastatic tumours) can be detected in plasma and serum and are resistant to RNase [122, 123]. For example, miR-378 showed elevated levels in serum of gastric cancer patients as compared to normal individuals [114]. This difference in miR-378 levels could be detected at early stages of gastric cancer and hence can be a useful biomarker in the screening of high-risk population (Table 5.2).

5.5 Future Perspectives

Hence, understanding the epigenetic modifications and their impact on gene expression, drug sensitivity and resistance is mandatory in the development of precision medicine-based therapeutic strategies for gastrointestinal cancers. Understanding these epigenetic changes could pave the way for exploiting these changes as reliable markers for the screening, detection, therapy and response monitoring for the therapy of gastrointestinal cancers. Such biochemical markers are not only reliable tools, but they are also probable precise indicators that can be cost-effective and noninvasive. Although high end imaging techniques and histopathological findings can be valuable in the detection of cancers, these techniques have their own merits and demerits. Imaging techniques although powerful tools can provide inconclusive data, have the hazards of radiation exposure and are not cost-effective. Biopsy

followed by histopathological analysis, although is indispensable gold standard in the diagnosis of cancer, has the disadvantage that the technique is invasive that can be stressful in patients and is time consuming. Techniques like colonoscopy or endoscopy although can provide a real time data have the disadvantage that the techniques are invasive and cannot provide an idea about the staging of the disease. Hence, a reliable potential biomarker that can be analysed noninvasively in the serum or stool sample but provides precise data about the status of the disease, then, invasive procedures like biopsy, colonoscopy, or endoscopy can be limited only to such cases where it is deemed mandatory to make the final diagnosis. Currently, used tumour markers for GIC like CEA or AFP although can provide some idea about the status, therapy and prognosis of the disease they are unlikely or insufficient to give a conclusive opinion about the disease as the results could be misleading and variable, based on various factors like smoking habits and age groups Hence, epigenetic biomarkers have the advantage that they are reliable, precise, cost-effective and most importantly noninvasive. Undoubtedly, exploiting these markers can be a potential strategy in the therapy of gastrointestinal malignancies in the future. Epigenetic modifications and the related changes described in this chapter have immense potential to be exploited as both diagnostic and therapeutic targets. These changes can be useful in classification of cancer subtypes. Also, proteins/complexes that modify epigenetic mechanisms may be excellent targets for drug development and such drugs are currently in clinical trials or already approved for therapy.

5.6 Conclusion

Understanding epigenetic changes and identifying epigenetic markers could mark a new era in the diagnosis and therapy of cancer. It is possible that based on these reliable data patient-specific personalized therapeutic strategies can be designed and developed in future, which could be a horizon in man's fight against cancer.

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Chapter 6
CD151: A Lateral Organizer and Modulator of Tumor Microenvironment in Gastrointestinal Cancers

Rama Rao Malla

Abstract The gastric, hepatic, pancreatic, and colon cancers are major gastrointestinal (GI) tract cancers account for 50% of all types of cancers with poor 5-year survival rate and high mortality rate due to extremely high metastatic ability. Even though GI tract cancers differ in histology, they share primary events of metastasis and master regulators of tumor microenvironment (TME), which initiate differentiation programs during ontogeny and tumor progression. The tetraspanin family members present on the surface of TME cells, respond to TME signals, and control proliferation, migration, invasion, apoptosis, and angiogenesis. CD151 is one of the oncogenic tetraspanins, cluster the membrane receptors, signaling proteins and other tetraspanins by lateral interactions in tetraspanin-enriched microdomains (TEMs) on the cell membranes or exosomes. CD151 regulates various events of TME by serving as a lateral organizer and modulator as well as signaling platform. This chapter illustrates how CD151 modulates organization of web and regulation of signaling molecules at molecular level in tumor microenvironment of GI tract cancers.

Keywords CD151 · GI cancers · Metastasis · Tumor microenvironment · Tetraspanin

Abbreviations

CAFs	Cancer associated fibroblasts
CC	Colon cancer
CD151	Cluster of differentiation 151
CD9	Cluster of differentiation 9

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ceRNA	Competing endogenous RNA
DSF	Disease free survival
EMT	Epithelial–mesenchymal transition
FAK	Focal adhesion kinase
GC	Gastric cancer
GI	Gastrointestinal
GM3	Monosialodihexosyl ganglioside 3
GPCR	G protein coupled receptor
HGC	Human gastric cancer
HIFα	Hypoxia inducing factor α
HSP27	Heat shock protein 27
IL-2	Interleukin-2
LAMC1	Laminin subunit gamma-1
LEL	Large loops at extracellular side
MMP-7	Matrix metalloproteinase-7
OS	Overall survival
PC	Pancreatic cancer
PDC	Pancreatic ductal carcinoma
PI3K	Phosphoinositide 3-kinases
RDS	Retinal degeneration slow
ROM-1	Rod outer segment membrane protein-1
RTK	Receptor tyrosine kinase
SIL	Small intracellular loop
SNHG3	SMALL nucleolar RNA host gene 3
T5EM	TM4SF5-enriched microdomain
TEM	Tetraspanin enriched microdomain
TILs	Tumor infiltrating lymphocytes
TME	Tumor microenvironment
UTR	Untranslated region
VCAM-1	Vascular cell adhesion protein 1

6.1 Introduction

6.1.1 Gastrointestinal Cancers

Globally, the major gastrointestinal (GI) tract cancers are gastric, hepatic, pancreatic, and colon cancers account for 50% of all types of cancers. The 5-year survival rate of GI tract cancers is ranging from $>50\%$ (colon cancer) to $<1\%$ (pancreatic cancer). The high rate of mortality cancer death is extremely relating to high metastatic ability of GI cancers. The prognosis of GI cancer is poor. The incidence of gastric cancer (GC) is marginally falling in the past decades, but its prevalence is quite high. Colon cancer is very common in the developed countries with better prognosis. The death rate of pancreatic carcinoma is accounted for approximately 50% of the prevalence

rate. The hepatic cancer is rare in developed countries but common in underdeveloped and developing countries with survival rate of 1–2%. The benign tumors exhibit failure of control overgrowth, contact-independent growth, and a loss of growth factor requirements, and malignant tumors exhibit invasiveness as well as metastasis that frequently marks the cornerstone of curative therapy. The GI cancers share "pathways" of tumor metastasis with different histology, provided support by wealth of experimental evidences. Further, recent concept of the tumor and the tumor stroma has provided a key information about microenvironment as well as master regulators, which control differentiation at the time of ontogeny and tumor progression [1]. The molecular mechanisms by the micro-ecosystem associated with GI cancer cells are poorly understood. In addition, the master regulatory genes which differentially intricate with regular development and differentiation as well as in carcinogenesis are unknown to great extent. Therefore, to develop novel therapeutic approaches it is vital to comprehend the mechanism of actions of microenvironment and master regulators in tumor progression.

6.1.2 Tetraspanins

The members of tetraspanin superfamily (34 proteins) mainly associate with diverse functions including activation of B- and T-cells, platelet aggregation, and progression of tumors to metastatic phenotype. The single polypeptide chain of tetraspanins passes four times across the membrane and form two large loops at extracellular side (LEL) and one small intracellular loop (SIL) along with N- and C-terminal tails at cytosolic side [2]. The tetraspanins are characterized by the presence of palmitoylation sites in SIL as well as N- and the C-terminal domains. The structure and the conformation of the LEL are stabilized owing to presence of polar amino acids in the transmembrane regions. The LEL domain is organized into three constant and one variable regions. These regions comprise of vital sites for protein–protein interactions [3] (Fig. 6.1).

The tetraspanins connected to each other as well as with other proteins due to their hydrophobic character [4]. The tetraspanins facilitate a signaling platform for

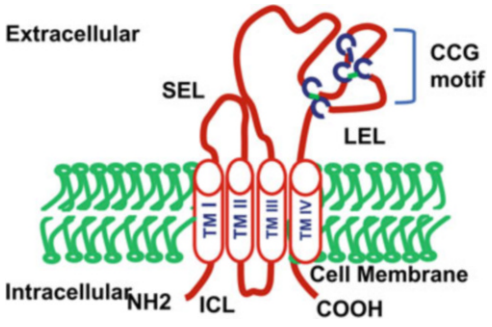

Fig. 6.1 Typical structure of tetraspanins

establishing tetraspanin-enriched microdomains (TEMs) [5]. The TEM holds membrane receptors, integrins, and other tetraspanins, which serve as signaling platform [5]. In TEM, tetraspanins function as adaptors by congregating variety of proteins into clusters and assist the specific signal transduction. The functions of proteins in TEM depend on the combination of tetraspanins as well as expression level of individual tetraspanins. From TME, tetraspanins regulate functions of the associated proteins by providing proximity, along with intracellular trafficking, vesicular budding, and exosomes formation as well as metastatic phenotype. The integrins are one of the outstanding partners of tetraspanin. The association of tetraspanins with integrins leads to alterations in adhesiveness versus motility [6–8].

6.2 CD151

CD151 [Tetraspanin 24 (Tspan24)] is an oncogenic protein cluster at the cell membrane in TEM. It is widely reported in epithelial, endothelial, muscle, and hematopoietic cells and acts as a lateral organizer and modulator of activities of transmembrane proteins [9]. The LEL of CD151 is receiving much attention in the recent past, due to the presence of functionally important sites [10]. In addition, CD151 contributes to the adhesion of leukocytes to human umbilical vein endothelial cells [9]. In tetraspanin webs, CD151 and putative partners held by various levels of interactions. Type I interactions or direct protein–protein interactions form in early stage of biosynthesis, for example, the association of CD151 with a3b1. However, type II interactions establish during later stage of biosynthesis in Golgi or post-Golgi stage, facilitated by palmitoylation [11–13], for example, CD151 interacts with other tetraspanins. Further, type III interactions develop during the formation of tetraspanin complexes, which are disrupted by very mild detergents, for example, signal transducing proteins [14] (Fig. 6.2).

The most imperative concept is that CD151 contributes to metastasis of solid tumors, by unraveled mechanism, but most likely by organizing tetraspanin clusters in lipid-enriched membrane microdomains of membranes. Remarkably, CD151 in association with integrins facilitates integrin-dependent cell motility. For instance,

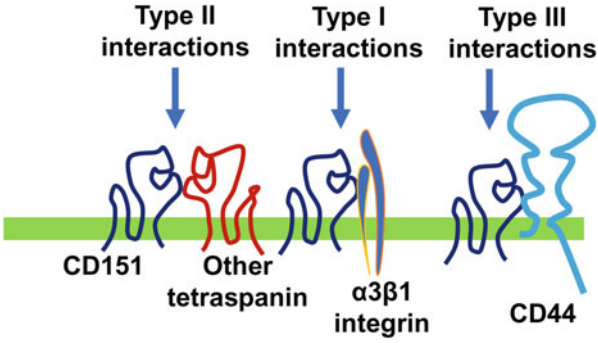

Fig. 6.2 Lateral interactions of CD151 with other proteins

mutations at primary interaction site of CD151 cause loss of integrin associations and integrin-mediated migration [6, 8]. However, mutation at C-terminal region distinctly changes integrin-mediated cell migration, cable formation, and adhesive strength. The C-terminal region of CD151 maintains integrin conformation, which promotes phosphorylation of a3 integrin tail and subsequent recruitment of PI4K and PKCs [15] for regulating downstream signaling of Rac and Cdc42 [16]. In addition, CD151 is also associated with cell migration by internalization, membrane traffic or endocytosis and recycling [14], and redistribution of integrins to filopodia and lamellipodia [17]. CD151 modulates cell adhesion strengthening integrin-ligand binding [18]. For example, CD151 controls post-ligand-binding events such as retraction of platelet clots [19] by recognizing type III or type I PDZ domains via C-terminal residues [20]. Further, CD151 mediates a6β1 integrin-dependent network formation [10, 21]. It is also essential for coagulation process mainly for controlling bleeding time by converting the constitutively expressed inactive aIIbβ3 conformation to high affinity state by inside-out signaling through G-protein-coupled or tyrosine kinase-linked signaling. The activated β3 attached fibrinogen and ensuring outside-in signals cause reorganization of cytoskeletal reorganization, activation of platelets, retraction, as well as spreading of clot [19]. The expression of CD151 has been increased with disease progresses especially with metastatic stages of colon cancer [22], hepatocellular carcinoma [23], and prostate cancer [24].

CD151 is mostly associated with tumor progression and metastasis [22, 25, 26]. The overexpression of CD151 has been coupled to poor prognosis of GI tumors [27, 28]. The CD151-mediated motility and invasiveness of cancer cells [29] are blocked by specific monoclonal CD151 antibodies [30] and recombinant adenoviral vectors with anti-sense CD151 [31]. For example, CD151 controls migration in association with laminin receptors by enhancing Rac and Cdc42 activation [16] and also via FAK activation [29]. Besides, CD151 activates MMP-7 by physical association capturing at the cell membrane and thus admits for pericellular lysis [32]. The CD151 promotes metastasis in association with pro-growth factors [26] and growth factor receptors [33] or MMPs [34, 35]. The miR-506 inhibits EMT by reducing CD151 along with other metastatic proteins in gastric [36, 37] and colon cancers [38]. Nonetheless, it is tempting to speculate that CD151 has considerable contribution to tumor progression (Fig. 6.3).

6.2.1 CD151 and Tumor Microenvironment

CD151 also known as PETA-3 or SFA-1 involves in uncontrolled behavior of cells intercommunication as well as with neighboring cells in tumor microenvironment (TME) [39]. The TME controls the release and intercellular trafficking of exosomes containing pro-invasive proteins including CD44 and CD151 in EGFR over expressing glioma [40]. The TME also shapes the antitumor immunity of immune cells either by releasing soluble factors or through cell–cell contacts via central building blocks of the plasma membrane, including broadly expressed tetraspanins

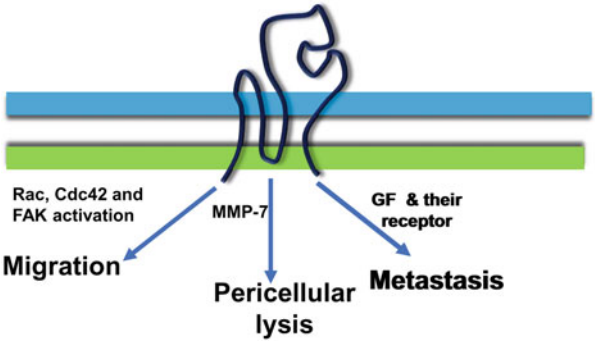

Fig. 6.3 CD151-dependent signaling in metastasis

CD81, CD151, and CD9 [41]. The interplay between tumor cells and cancer associated fibroblasts (CAFs) in TME is critical for metastasis. The signaling cascade promoted by CD151 communicates CAFs, to release activated pro-migratory kinases such as FAK, Src, and HSP27, in addition to MMP-13 [42]. The CD151 present in tumor and endothelial cells connect to tumor induce angiogenesis in TME [43]. CD151 is also a regulator of tumor cell communication with adjacent cells in TME [44]. In addition, CD151 is also a partner of TM4SF5-enriched microdomain (T5EM) on hepatic cell surface, which controls initiation and maintenance of tumors in primary as well as metastatic regions [45, 46]. Further, TM4SF5-CD151 interactome promotes internalization of cell surface CD63 in hepatic cells and causes termination of tumor-suppressor activity of CD63 [47]. FAK promotes tumor progression by controlling the TME by means of lateral interactions with binding partners CD151 and CD9 via RhoGEF, Src family, talin, cortactin, and paxillin [48]. These studies highlight the role of CD151 in lateral interactions among the TME cells as means of communication. In T-cells, CD151 actively changes the cell cycle control and cell death motifs of T-cells in response to IL-2 and induces an antigen-independent and hyperresponsive proliferation phenotype in T-cells [49].

6.2.2 CD151 and Exosomes

Exosomes are microvesicles with nanosize, released extracellularly by every cell. Recently, exosomes are identified as an essential cellular communicator due their horizontal transfer ability of proteins, DNA, mRNAs, as well as mi-RNAs. They essentially also involve in tumor progression, growth and metastasis, angiogenesis, immune escape, and therapeutic resistance. The recent documents display that exosomes are useful in the diagnosis and required for development as well as progression of GI tract cancers [1, 50–52]. Tetraspanin-associated exosomal proteases play an important role in the processes of cell motility, migration, invasion, and formation of metastases [53]. CD151 is one of the tetraspanins found in the

tumor exosomes. It essentially targets exosomes to lung and lymph node or stroma [54]. Exosomal CD151 and Tspan8 promote angiogenesis by associating with GPCR and RTK in EC and tumor cells [55]. The TEM-linked CD151 and Tspan8 in exosomes associate with multiple biological processes [56]. Exosomal CD151 and other tetraspanins are demonstrated as prospective biomarkers [57]. CD151 and Tspan8 are the major components of exosomes for crosstalk of cancer initiating cells with surrounding cells in PDC [58]. The tetraspanins, Tspan8-CD9-CD151, form membrane complex in exosomes and bind and promote migration of tumor cells by reducing matrix and cell adhesion [59]. These studies describe that lateral interactions in tetraspanin-web have robust functional emanations for selection of exosomes in exosomes-dependent drug delivery. This insight will be fundamental for development of humanized exosome-based therapies.

6.2.3 CD151 in Gastric Cancer

Globally, gastric cancer (GC) has been the most prominent among GI tract common cancers. The carcinogenic process of GC is complex due to deregulation of various oncogenic and tumor suppressor genes [60]. CD151 expression has in prognostic significance in patients with advanced GC. Further, the overexpression of CD151 is an independent prognostic marker of worse overall survival as well as disease-free survival, and its prognostic applications are similar to T and N stages [61]. Despite great advances, the diagnosis and treatment of GC are remaining the second most common cancer worldwide.

CD151 is highly expressed in GC cells as well as in tumor tissues [61–63] and forms functional cluster with integrin α3. In addition, increased CD151 expression correlates with the enhanced invasion and metastasis of HGC cells. CD151 expression and lymphatic metastasis of GCs are increased by exosomes indicating that exosome CD151 is mediating premetastatic niche formation in GCs [64]. The CD151 antibody inhibits migration of GC cells without changing the adhesive and proliferative capacity [65]. Furthermore, elevated CD151 expression linked to enhanced tumor size, but poor differentiation of GC. CD151 upregulation was more often noticed in young GC patients. However, the rate of CD151 expression was increased constantly based on the depth of invasion, that is, T stage, N stage, and pathologic stage [63]. It indicates that CD151 was found to be an independent prognostic factor for patients with advanced GC.

The CD151 and α3 integrin associate with enhanced metastatic ability of GC cells. Clinically, CD151 and integrin a3 overexpression is considerably associated with higher TNM stage, invasion depth, as well as involvement of lymph node. Further, the postoperative 5-year OS of patients with CD151 and low levels a3 is higher than that of patients with CD151 and high integrin α3 [62]. As CD151 and α3 integrin are positively associated with the invasiveness, they may be considered as novel markers for the prognosis of GC as well as prominent therapeutic targets.

Recent, CD151 gene silencing studies have shown impairment of TEM formation and inhibition of functions of associated proteins, which contribute to TEM formation as well as its function. Besides, blockade of CD151 distinctly impaired the metastatic ability potential of cancer cells. Therefore, targeting the CD151 or TEMs is the most favorable therapeutic strategy [5]. Co-overexpression of CD151 and MET was observed more frequent in advanced pN stages of GCs. Moreover, the co-overexpression of CD151 and MET was a strong independent prognostic factor for OS and DSF [61]. Therefore, CD151/MET overexpression is an autonomous prognostic marker as well as promising alternative molecular therapeutic targets of advanced GC patients.

miRNAs are small noncoding RNAs, which regulate gene expression by binding to the complimentary sequences in the 3′-UTR of target mRNA and promote target mRNA degradation or translational suppression [66]. Recent studies proposed miRNAs as a major regulator of diverse target genes, which associated with carcinogenesis of GC. Many studies explored the aberrant expression of miRNAs in GC [67, 68] and their involvement in the GC development as well as progression [69, 70]. miRNAs are promising prognostic factors of GCs [71]. miR-22 significantly suppressed of GC cells and reduced the expression of CD151. Likewise, CD151 overexpression markedly reduced the tumor suppressing activity of miR-22 [72]. These results suggest that miR-22 can reduce GC growth as well as motility partially by inhibiting CD151. Similarly, miR-152 impedes both the proliferation of GC cells and overexpression of CD151 in GC cells [73]. These results highlight CD151 role in the regulation of proliferation and suggest a potential application in GC treatment. The functions, particularly in metastasis suggesting CD151 as a master regulator of gastric cancer.

6.2.4 CD151 and Hepatocellular Cancer (HCC)

CD151 is a critical regulator of metastasis of HCC, by forming of functional complexes [23, 34, 74]. The high-throughput proteomic studies mapped the "interactome" network with CD151 at center. This study further identifies CD151-mediated "a tetraspanin web organization" with various partners which serve as a facilitator or adaptor for signaling of HCC cells [75]. The integrins are common partners CD151-mediated tetraspanin web [18, 76, 77]. The stable lateral CD151/integrin b1 axis helps in integrin b1-mediated remodeling of matrix as well as cell spreading, and metastasis [43]. A monoclonal antibody specific to α6β1 binding site on extracellular domain of CD151 inhibits metastatic mechanisms of HCC, indicating the importance of lateral binding sites in tumor progression [78]. The CD151-integrin complex regulates chemokine-mediated migration of T-cells [79]. The migratory and invasive capacity of HCC are controlled by CD151 via transmembrane 4 L6 family member 5 (TM4SF5) [47]. CD151 along with partner β1 integrin promotes migration, invasiveness, as well as metastasis of HCC cells probably through MMP-9 [75]. CD151 promotes invasiveness of HCC cells by amplifying

EMT in response to laminin-5 in a α6-dependent hyperactivation of PI3K/Akt-Snail-PTEN homolog feedback pathway [74]. CD151 disseminates neovascularization in HCC by controlling MMP-9 expression [34]. The CD151 promotes liver cancer cell metastasis through SP1-mediated transcriptional regulation [80].

Mortalin, one of the functional partners of CD151, stabilizes the CD151-dependent TEM and promotes HCC progression [81]. A recent report shows that the miR-128/CD151 pathway mediates small nucleolar RNA host gene 3 (SNHG3)-dependent invasion, EMT, and metastasis of HCC cells [82]. CD151 elevates malignant phenotype of HCC cells via miR-124-dependent negative regulation of LAMC1 in HCC cells [83]. CD151 overexpresses by discrete microenvironmental signals in primary liver cancer and assists VCAM-1-mediated recruitment of lymphocyte in HCC [9]. CD151 is negatively regulated by miR-199a-3p and promotes metastatic development of HCC [84]. CD151 in collaboration with PIK3C2A, a candidate ceRNA of CD151, enhances HCC malignancy in a ceRNA mechanism [85].

6.2.5 CD151 and Colon Cancer

Colon cancer (CC) is the third most deadly and fourth most commonly diagnosed cancer worldwide [86]. After potential curative surgery and adjuvant chemotherapy, the patients with CC have 50% relapse and eventually die due to metastasis [87]. The overexpression of CD151 is also correlated with bad prognosis of CC [22]. In fact, CD151 exhibits various oncogenic features in distinct CRC. The expression of CD151 in colon cancer ranged from 55% to 77%, and CD151-positive patients have lower OS within 5 years [88]. The expression of CD151 is basal as well as lateral sites of normal colon cells plasma membrane. On the other hand, colon cancer cells exhibit least staining on the membrane but mostly in the cytoplasm. Highly ordered with glandular morphology this study confesses two specific functions of CD151 in CC cancer. First, as CD151 associates with cell–cell and cell–matrix adhesion, however, decreased expression or mislocalization causes disorganization and decreased loosen of contacts with ECM and neighboring cells, and eventually leads migration of tumor cells from highly hypoxic primary site. Second, CD151 facilitates in integrin-dependent migration of tumor cells. In highly advanced-stage, HIFα induces CD151 expression to ensure the ability of colon cancer cell motility. Interestingly, hypoxia-induced reduction of CD151 abolishes motility adhesion of colon cancer cells [88]. CD151-mediated migration and metastasis require FAK in human colon cancer cell line RPMI4788 [29]. HIF-1 directly binds to the promoter region of CD151 gene and represses its expression in CC cells [88]. CD151 along with other cell surface proteins involves in communication between colon cancer cells and tumor infiltrating lymphocytes (TILs) for systemic relapse [89]. CD151 is identified in CD133+ cells obtained from fresh biopsy of human colon cancer patients, suggesting that CD151 may be associated with stemness and tumorigenesis of CC [90]. In CC cells, CD151 captures and activates proMMP-7 on the cell surface

after interaction and control the pericellular activation mechanism, a proteolysis mechanism prerequisite for cancer invasion and metastasis [32].

The CD151 expression is observed to be high in colon cancer patients with early-stage compared to patients with metastasis [91], indicating the dynamic changes in the expression with advancement of colon cancer. The CD151 expressing in exosomes derived from colorectal cancer specifically target to lung, lymph node, and stroma cells [54]. Hypoxia enhances invasion as well as metastasis of colon cells by inhibiting the expression of tethering protein CD151 via HIF-1 [92]. Inhibition of CD151 expression by hypoxia instigated the removal of tumor cells from the adjacent matrix and cells [88].

6.2.6 CD151 and Pancreatic Cancer

Pancreatic cancer (PC) is another GI tract cancer with high mortality worldwide. It is highly difficult to diagnose at early-stage disease due to poor understanding of the mediators, which associate with tumor progression [93]. The CD151 as well as c-Met, and integrin α3/α6 are reported to overexpressed in pancreatic cancer. The CD151 overexpression along with c-Met cardinally associated with TNM stage, lymph node, invasion as well as poor survival of PC patients [94]. In PC, CD151 colocalizes with α3β1 or α6β4 along with CO-029 via protein kinase C, helps in integrin–tetraspanin complex internalization and migration by decreasing laminin 5 adhesion [28]. CD151 along with Tspan8 is essentially required for exosome binding/uptake to support matrix degradation, reprogram stroma, and hematopoietic cells, and to transform nonmetastatic to metastatic pancreatic cancer cells [58]. CD151 promotes metastasis coordinately with Tspan8 by the recruitment of integrins out of adhesion site and activation of MMP9 and MMP13 in pancreatic cancer [95] (Table 6.1).

6.3 Conclusion

In conclusion, CD151 is regarded as a prognosticator of poor outcome in patients with GI tract cancers. CD151 alone or the CD151-integrins α3 could be potential targets for the treatment of GI tract cancers. In addition, the CD151 is an autonomous prognostic indicator of worse OS and DFS in patients with GI cancers. However, its prognostic impact is similar to the T and N stage of tumors. Thus, as a key regulator of various malignancies, in which it can modulate or interact with other oncogenic proteins in TME, CD151 could be an interesting target for therapeutics in patients with GI tract cancers.

Table 6.1 Summary of CD151-dependent cellular and signaling mechanisms in GI tract cancers

Type of GI cancer	CD151	CD151 and integrins	Signaling pathways
Gastric cancer	• Enhances invasion and metastasis of GC cells. • Exosome CD151 increases lymphatic metastasis of GC cells. • Elevated CD151 expression linked to enhanced tumor size and poor differentiation of GC cells. • CD151 expression correlates with T stage, N stage and pathologic stage. • CD151 is mediates premetastatic niche formation in GCs.	• CD151 and α3 integrin associate with enhanced metastatic ability of GC cells. • CD151 and integrin a3 overexpression associate with higher TNM stage, invasion depth, and lymph node involvement. • CD151 and α3 integrin are positively associated with the invasiveness.	• miR-22 reduces GC growth as well as motility partially by inhibiting CD151. • miR-152 impedes both the proliferation of GC cells and CD151 overexpression.
HCC	• Migratory and invasive capacity of HCC are controlled by CD151 via TM4SF5. • Disseminates neovascularization in HCC by controlling MMP-9 expression. • Promotes metastasis through SP1-mediated transcriptional regulation. • CD151 overexpression assists VCAM-1-mediated recruitment of lymphocyte in HCC. • CD151 in collaboration with PIK3C2A enhances HCC malignancy.	• CD151/integrin b1 axis mediates remodeling of matrix, cell spreading, and metastasis. • By lateral interaction with α6β1 through LEL, CD151 controls tumor progression. • CD151-integrin complex regulates chemokine-mediated migration of T-cells. • CD151 and β1 integrin promote migration, invasiveness, as well as metastasis of HCC cells through MMP-9.	• CD151 promotes invasiveness of HCC cells by amplifying EMT in via PI3K/Akt-snail-PTEN homolog feedback pathway. • miR-128/CD151 pathway mediates small nucleolar RNA host gene 3 (SNHG3)-dependent invasion, EMT and metastasis. • CD151 elevates malignant phenotype of HCC cells via miR-124-dependent negative regulation of LAMC1. • CD151 is negatively regulated by miR-199a-3p and promotes metastatic development of HCC.
PC	• CD151 overexpression along with c-Met associates with TNM stage, lymph node, invasion as well as poor survival of PC patients.	• CD151 colocalizes with α3β1 or α6β4 along with CO-029 via protein kinase C, helps in integrin–tetraspanin complex internalization and migration.	• CD151 promotes metastasis coordinately with Tspan8 by activating MMP9 and MMP13.
CC	• CD151 overexpression correlates with bad prognosis of CC. • CD151 associates with cell–cell as well as cell–matrix adhesion. • Hypoxia-induced reduction of CD151 abolishes motility adhesion of CC cells.	• CD151 facilitates in integrin-dependent migration of tumor cells. • CD151 along with other cell surface proteins involves in communication between colon cancer cells and tumor infiltrating lymphocytes (TILs).	• CD151 captures and activates proMMP-7 on the cell surface after interaction and control the pericellular activation mechanism. • CD151-mediated migration and metastasis require FAK in human colon cancer cell line.

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Chapter 7
Identification of Potential Key Genes Involved in Progression of Gastric Cancer Using Bioinformatics Analysis

Vigneshwar Suriya Prakash Sinnarasan, Dahrii Paul, Leimarembi Devi Naorem, Mathavan Muthaiyan, Dinakara Rao Ampasala, and Amouda Venkatesan

Abstract *Background:* Despite the extensive effort on gastric cancer (GC) research and its achievement over the last decades, GC continues to remain the third leading cause of cancer mortality in the world. Detection of key genes involved in gastric cancer progression and prognosis leads to efficient approach to treat cancer.

Methods: Two datasets (PRJNA506381 and PRJNA438844) from Sequence Read Archive (SRA) database were analysed using available bioinformatics tools and differently expressed genes (DEGs) were identified. The enrichment, protein–protein interaction (PPI) network and survival analysis were carried out to unaware the potential genes responsible for GC progression.

Results: Totally, 227 upregulated and 247 downregulated genes were obtained, out of that overlapping 45 DEGs were selected for further analysis. Protein digestion and absorption and gastric acid secretion were the most enriched pathways. The PPI network was constructed by GeneMANIA and visualized in Cytoscape having 55 nodes and 689 interactions. Subsequently, NetworkAnalyzer plugin in Cytoscape was used and found 13 hub genes (MT1X, MT1E, MT1H, MT1F, MT1G, MT2A, MT1M, MT1A, MT1B, ATP4A, MT1HL1, PGC and CA9) based on high degree of connectivity $\geq$30. Further, eight highly connecting genes (KCNE2, CPA2, GIF, DRD5, CTSE, CLIC6, CHIA and LIPF) from the highly enriched modules were selected. Also, the prognostic value of the key genes was checked using Kaplan–Meier plotter, in that MT1X, MT1H, MT1E, ATP4A, KCNE2, CPA2, DRD5, CLIC6 and CHIA were associated with survival in overall survival of GC.

Conclusion: These results reveal that 13 hub genes and 8 highly connecting genes might contribute a major role in GC progression. Further, study of CAP2 could be utilised as potential prognostic biomarker.

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Keywords Gastric cancer · Next-generation sequencing (NGS) · Bioinformatics analysis · Differential expressed gene

Abbreviations

ACRG	Asian Cancer Research Group
ATP4A	ATPase H+/K+ transporting subunit alpha
CA9	Carbonic anhydrase 9
CBLIF	Cobalamin binding intrinsic factor
CHIA	Chitinase acidic
CLIC6	Chloride intracellular channel 6
CNE-2	CNE-2 enhancer upstream of SHOX
CPA2	Carboxypeptidase A2
CTSE	Cathepsin E
DEGs	Differently expressed genes
DRD5	Dopamine receptor D5
GC	Gastric cancer
GO	Gene ontology
HTS	High throughput sequencing
KCNE2	Potassium voltage-gated channel subfamily E member 2
KEGG	Kyoto Encyclopedia of Genes and Genomes
LIPF	Lipase F, gastric type
MCODE	Molecular complex detection
MT	Metallothionein
NCBI	National Centre for Biotechnology Information
NGS	Next generation sequencing
PGC	Progastrics in (pepsinogen C)
PPI	Protein–protein interaction
SRA	Sequence read archive

7.1 Introduction

Gastric cancer (GC) also known as stomach cancer is a disease in which malignant cells start forming in the stomach lining which causes different symptoms [1] and is classified histologically as intestinal and diffuse types. Intestinal types are often linked with atrophic gastritis, intestinal metaplasia, dysplasia and risk of *Helicobacter pylori* infection which commonly manifest in elderly patients. Whereas, diffuse types exhibit loss of cell cohesion and form signet-ring cell carcinoma with negative *H. pylori* infection and occur mostly in younger age patients [2, 3]. In 2018 GLOBOCAN reported that GC is third leading cause of cancer mortality with 1 out of 12 of all cancer death globally and estimated ~1 million new cases each year [4]. The aging population growth increase may reflect

the high prevalence of the disease [4]. GC has been often associated with different factors, like *H. pylori*, genetics, lifestyle, food habits and socioeconomic status [5].

Over the last decades, the prevalence of GC worldwide has reduced and survival rates have also been improved. It reflects the improvement of GC management and human development index (HDI) [5–7]. Despite the improvement, it continues to have a very low survivability rates, that is, 5-year survival rate with case fatality as high as 74.5% [5, 6]. Various methods have been developed to counter GC, yet prognosis, early diagnosis and treatment still remain unfavourable due to lack of sensitivity or specificity of biomarkers, heterogeneity of the disease, non-specificity of symptoms and limited treatment choices [8].

In recent years, researchers and physicians around the world have put tremendous efforts on omics, epidemiology and clinical trial studies thereby generating enormous amounts of data on GC [9]. These data hold great promises in better understanding of the GC and its treatment. With the advancement of high throughput sequencing (HTS) technology, it continues to shape the genetic landscape of GC and elucidation of its novel genes [10]. Genomic data have been used by TCGA and Asian Cancer Research Group (ACRG) for GC classification [11, 12]. Before the HTS era, TP53 and CDH1 genes were considered to be the sole driver of GC, but with the emergence of HTS technology similar to such genes like BRCA2 and CTNNA1 were discovered with similar function and pathogenesis [10]. Genetic heredity studies have also revealed that mutation of CDH1 gene is linked to diffuse GC and is also linked to risk of getting other cancers like colorectal, thyroid and ovarian cancers [13]. At present epigenetics is also gaining momentum in GC research and successful in restricting GC pathogenesis by methylation and histone modifications of tumour-related genes [13, 14].

Although, significant progress has been made in GC from diagnosis to treatment, all the patients do not respond equally to the existing therapy or biomarkers. Therefore, identification of novel biomarker in terms of its biological complexity remains the outmost necessity [15]. HTS technologies have been used in various GC characterisation and have been very promising [10, 15]. In this study, taking the advantage of publicly available GC samples and its potential into accounts, DEGs were identified. Functional enrichment analysis was further conducted on DEGs. Subsequently, key genes affected during GC were identified using PPI network and prognosis analysis.

7.2 Materials and Methods

7.2.1 Data Collection

GC transcriptome datasets (PRJNA506381, and PRJNA438844) were downloaded from sequence read archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra) of

the National Centre for Biotechnology Information (NCBI) [16]. The PRJNA506381 series contain 6 samples (3 normal and 3 tumour cases) and PRJNA438844 with 12 samples (6 normal and 6 tumour cases).

7.2.2 *Data Processing and Identification of Differently Expressed Genes (DEGs)*

Transcriptome data were analysed using R (v3.6.1) and RStudio (v1.2) to find DEGs [17]. First, the quality of the data was checked using fastqcr (v0.1.2) package in R [18]. The adapter, overexpressed sequences and low-quality bases were removed using trimFastq in seqTools (v3.6) package [19]. In addition, the University of California Santa Cruz (http://genome.ucsc.edu) human reference genome (hg38) was downloaded and indexed to make alignment faster using build index of Rsubread (v2.0) package. The pre-processed data were aligned with human reference genome (hg38) using align of Rsubread (v2.0) package, in turn generated binary alignment map (BAM) formatted data [20]. The count matrix was constructed from BAM files using summarize Overlaps of Genomic Alignments (v1.8.4) and DESeq2 was used to identify DEGs between the tumour tissues and adjacent normal tissue samples [21]. A $|\log2\text{-fold change}| \geq \pm 1.5$ and $P < 0.05$ were considered as threshold values for DEG identification. Further, DEGs of two different datasets were processed to Venn diagram using online resources (https://bioinfogp.cnb.csic.es/tools/venny/) to find the overlapping DEGs.

7.2.3 *Enrichment Analysis of DEGs*

Gene Ontology (GO) analysis is a familiar technique for describing genes/gene products to identify biological process, cellular component and molecular function of high-throughput data [22, 23]. Kyoto Encyclopedia of Genes and Genomes (KEGG), a renowned database was performed to find the pathways that are closely associated to gastric cancer [23]. The appropriate biological annotation associated with DEGs was found using the Enrichr online tool [24]. Enrichr is an online tool for high-throughput gene functional analysis, it uses set of genes as input to enumerate the enrichment as a result of pathways, ontologies, diseases/drugs, cell types and so on [24]. $P < 0.05$ was taken as a statistically significant difference for enrichment analysis.

7.2.4 Network and Module Analysis

DEGs were used to acquire PPI network information through the GeneMANIA online tool (https://genemania.org/), and visualized in Cytoscape (Version 3.7.1) software [25, 26]. Moreover, PPI network was analysed using NetworkAnalyzer plug-in in Cytoscape and genes with degree of connectivity $\geq$30 were selected as hub genes. Subsequently, cluster analysis of PPI network was performed to find the highly interconnected regions using the molecular complex detection (MCODE) of Cytoscape and KEGG pathway analysis of hub genes using Enrichr tool.

7.2.5 Survival Analysis

The prognosis of key genes was analysed by Kaplan–Meier plotter. Kaplan–Meier Plotter is a publicly available database that combines gene expression data along with their clinical data. The correlation between key genes and overall survival was estimated in GC patients [27].

7.3 Results

7.3.1 Identification of DEGs

The datasets (PRJNA506381 and PRJNA438844) were analysed to detect the DEGs in stomach normal tissue and tumour tissue. There were 237 DEGs (161 downregulated and 112 upregulated) in PRJNA506381 and 201 DEGs (86 downregulated and 115 upregulated) in PRJNA438844. Further, the analysis of the DEGs using Venn diagram showed that there were 45 DEGs together with 28 downregulated and 17 upregulated genes detected in both datasets (Fig. 7.1) and overlapping 45 DEGs are tabulated in Table 7.1.

7.3.2 Functional Analysis

Further, to examine the biological functions of 45 DEGs, GO analysis was carried out using Enrichr. The DEGs were mostly enriched in the iron ion transmembrane transporter activity, aspartic-type peptidase activity and cellular transition metal ion homeostasis activity which maintain the internal steady state of transition metal ions at the level of a cell (Fig. 7.2). Furthermore, by KEGG pathway analysis, DEGs are mainly enriched in protein digestion and absorption, gastric acid secretion, signalling pathways regulating pluripotency of stem cells, mineral absorption and PPAR

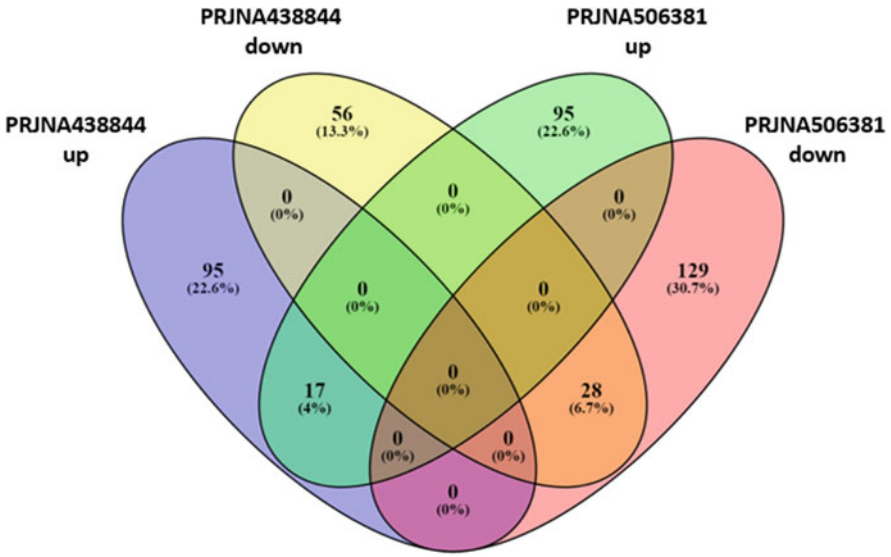

Fig. 7.1 Venn diagram of 45 DEGs (17 upregulated and 28 downregulated) out of two datasets of Sequence Read Archive (SRA)

Table 7.1 Overlapped DEGs from two datasets

Expression	DEGs (gene symbol)
Upregulated	SLC11A1, OR13H1, POTEF, CHI3L1, CST2, DIO2, CELSR3, CST1, INHBA, EFNA3, MELTF, SDS, CENPF, CLDN1, TTYH3, FNDC1, FAM81A
Downregulated	SLC5A5, ATP4A, ATP4B, CCKAR, PLIN5, PGA3, PGA4, PSAPL1, LIFR, PTGR1, CA9, ESRRG, HIST2H3PS2, SMIM38, FABP4, ID4, CPA2, CBLIF, MT1X, LYVE1, PGA5, ADHFE1, SLC2A12, KCNE2, RNF152, SCARA5, MT1M, MFSD4A

signalling pathway (Fig. 7.3). Proteins like pepsinogen-I (PGA), gastrin-17 and pepsinogen-II (PGC) are considered to be specific markers of gastric cancer due to their gastric specific gene expression [28]. Significantly, these PGA3, PGA4, PGA5 are downregulated in both datasets and enriched in protein digestion and absorption pathway, macromolecule catabolic process and aspartic-type peptidase activity. Metallothioneins (MTs) are cysteine-rich proteins that play a major role in DNA damage and oxidative stress. Functional isoforms of MT1 were MT1A, MT1B, MT1E, MT1F, MT1G, MT1H, MT1M and MT1X [29]. MT1X is found to be downregulated in both datasets and is enriched in mineral absorption pathway, cellular response to zinc ion and copper ion activity.

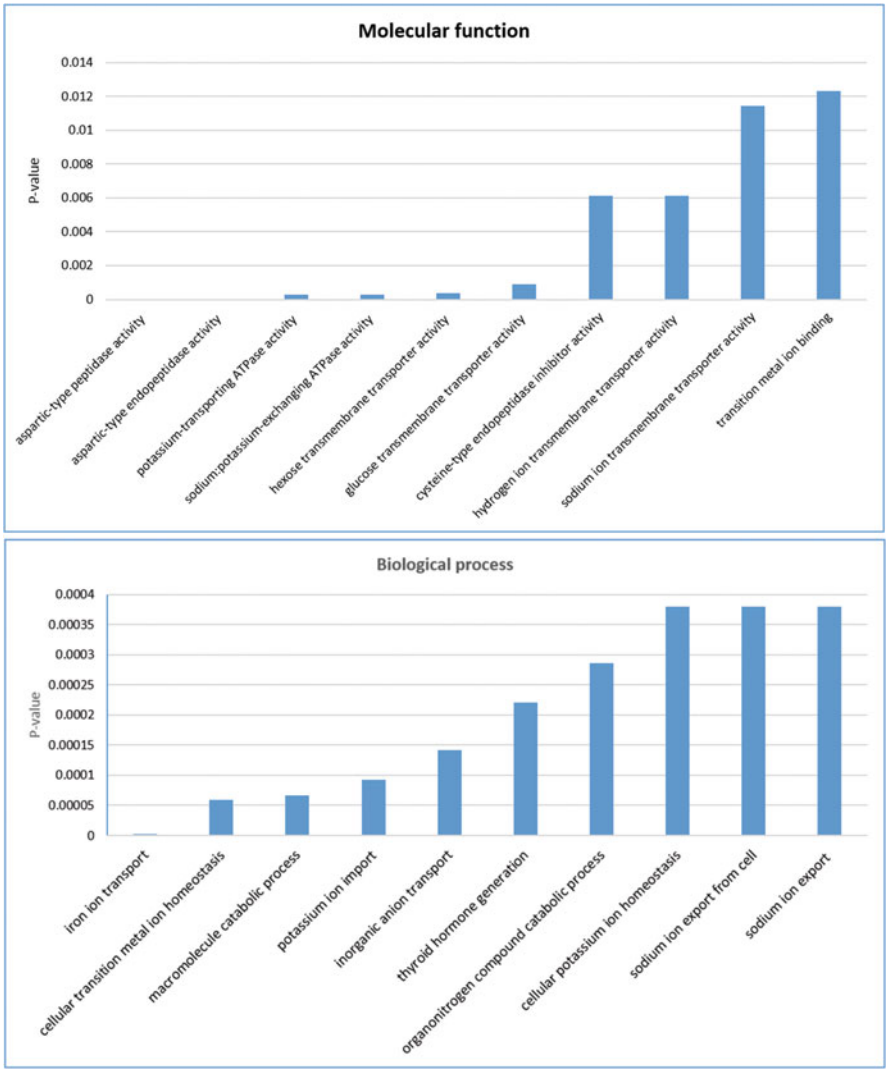

Fig. 7.2 Illustration of significant enrichment of top ten elements in GO categories: Molecular function and biological process for overlapping DEGs

7.3.3 PPI Network and Module Analysis

There were 55 nodes and 689 interactions (Fig. 7.4). They are found by performing PPI network analysis of overlapping DEGs using Cytoscape. These proteins were selected based on the result of GeneMANIA which is used to find the interaction relationship between genes. Through the analysis of PPI network, MT1X, MT1E,

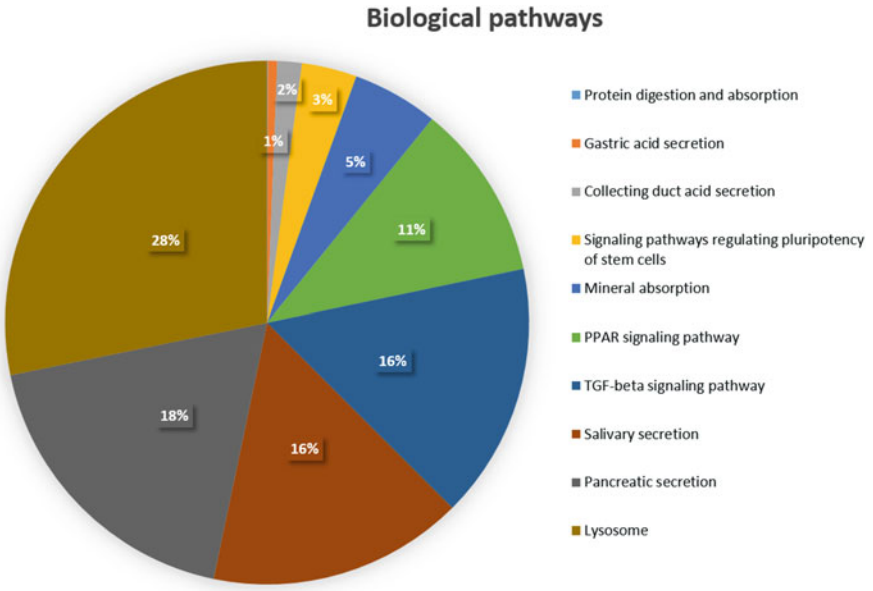

Fig. 7.3 Illustration of top ten functional pathways associated with DEGs with the value of $P < 0.05$ by KEGG pathway analysis

MT1H, MT1F, MT1G, MT2A, MT1M, MT1A, MT1B, ATP4A, MT1HL1, PGC and CA9 were identified as hub genes having higher degree of connectivity (Fig. 7.4). Other than hub genes, some genes like KCNE2, CPA2, GIF, DRD5, CTSE, CLIC6, CHIA and LIPF were found to be highly interconnected with hub genes when module score is ≥ 5. Also, the pathway analysis of hub genes revealed that more genes were enriched in mineral absorption pathway.

7.3.4 Survival Analysis of Key Genes

The prognosis of key genes and highly connected genes was analysed for overall survival (OS) using Kaplan–Meier plotter. Genes with $P < 0.05$ value were considered as prognostic biomarkers. Overexpression of MT1X (P value: 6.4e−5), MT1H (P value: 0.0368), MT1E (P value: 0.0229), KCNE2 (P value: 1.6e−9), CPA2 (P value: <1e−16), DRD5 (P value: 2.4e−9), CLIC6 (P value: 8.9e−16) and CHIA (P value: 3.1e−8) is associated with good prognosis and ATP4A (P value: 4.0e−6) shows worse prognosis for overall survival in GC patients (Fig. 7.5).

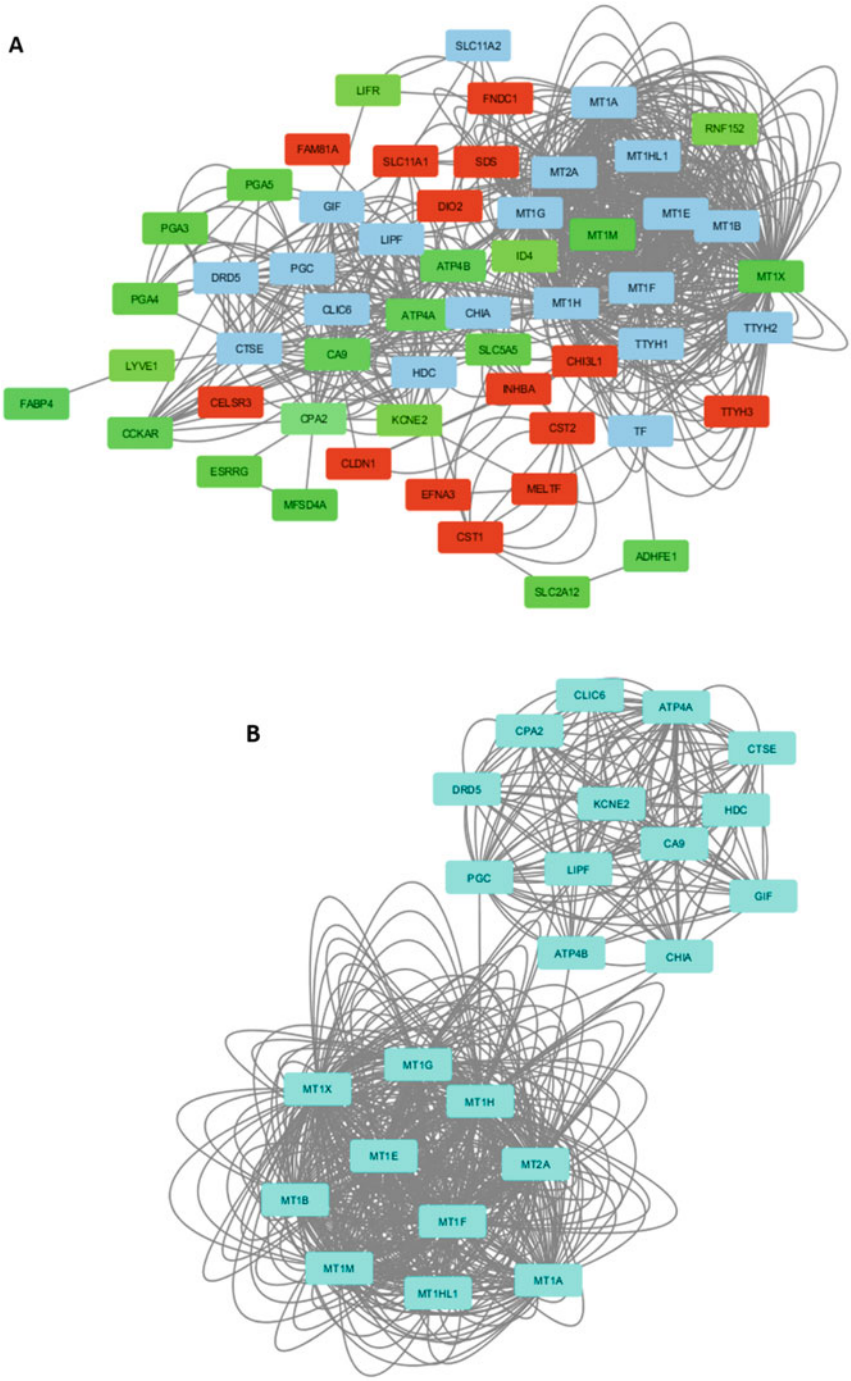

Fig. 7.4 (**a**) PPI network constructions for overlapping DEGs. Red nodes for upregulated genes, green nodes for downregulated genes and blue nodes for genes from GeneMANIA. (**b**) Illustration of significant module identified using molecular complex detection (MCODE) having score of $\geq$5.0

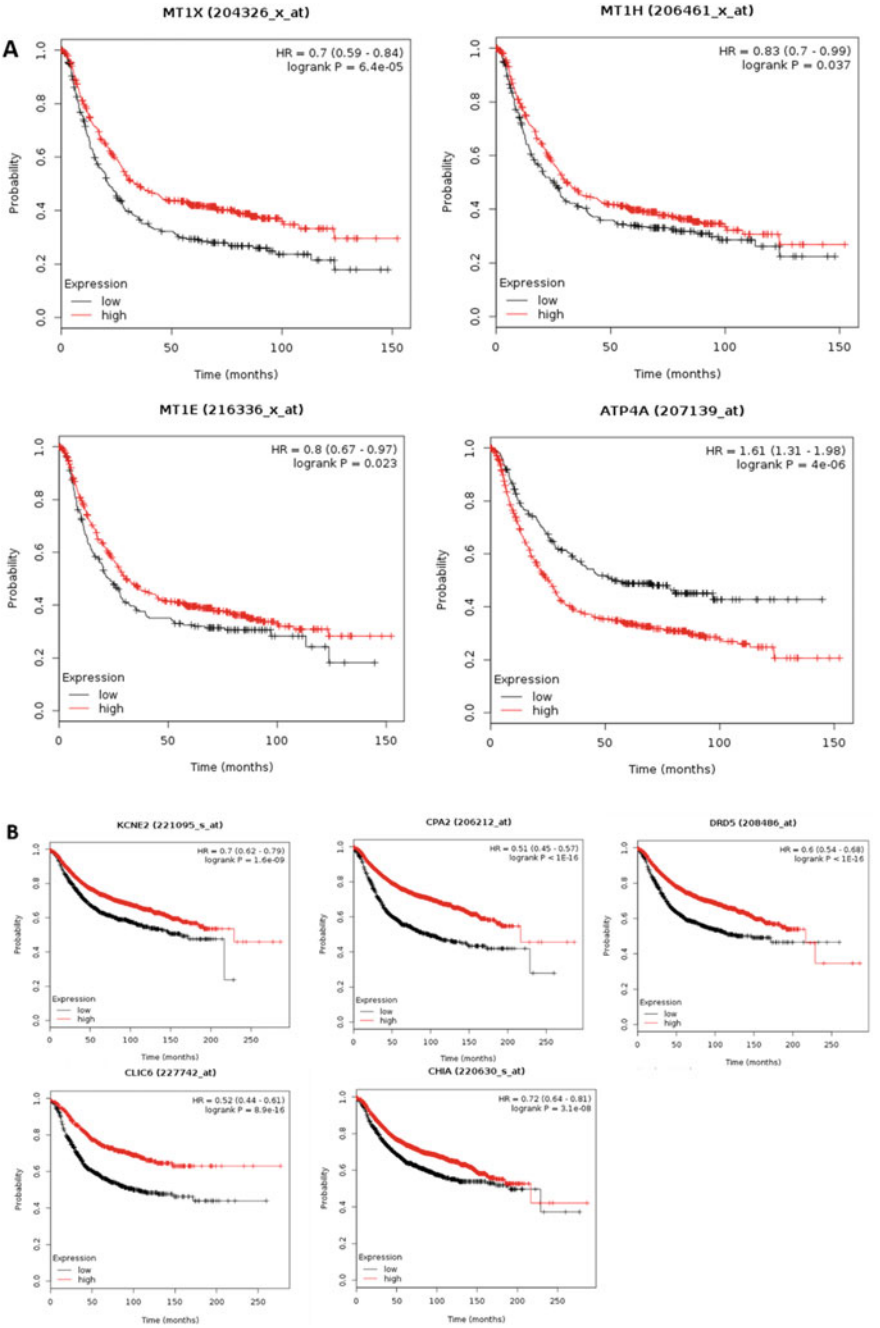

Fig. 7.5 Kaplan–Meier survival curves of four hub genes (**a**) and highly connected genes (**b**) in GC patients

7.4 Discussion

In this study, normal and tumorous tissues were analysed using several bioinformatics techniques to detect the key genes involved in GC progression. As a result, 474 dysregulated genes (227 upregulated and 247 downregulated genes) from two datasets and 45 overlapping genes (17 upregulated and 28 downregulated) were considered for further analysis. Protein–protein network analysis was carried out to find the relationship between DEGs and from the constructed modules of network hub genes were identified based on a high degree of connectivity. Survival analysis was performed to find the prognostic value of the DEGs. MT1X, MT1E, MT1H, MT1F, MT1G, MT2A, MT1M, MT1A, MT1B, ATP4A, MT1HL1, PGC and CA9 hub genes were found to be responsible for the progression of gastric cancer and KCNE2, CPA2, GIF, DRD5, CTSE, CLIC6, CHIA and LIPF were more interconnected with the hub genes.

For more understanding of 45 overlapping dysregulated genes, GO and KEGG pathway analysis was carried out. The analysis showed that these genes were more enriched in aspartic-type peptidase activity, potassium-transporting ATPase activity and cellular transition metal ion homeostasis activity. Furthermore, pathway analysis revealed that the DEGs are enriched in pathways like protein digestion and absorption, gastric acid secretion, collecting duct acid secretion and PPAR signalling pathway. Out of 45 genes, 13 hub genes were also subjected to pathway analysis revealed that they are enriched in mineral absorption pathway.

Interestingly, most of the hub genes (MT1X, MT1E, MT1H, MT1F, MT1G, MT2A, MT1M, MT1A, MT1B and MT1HL1) are isoforms of Metallothioneins (MTs). Metallothioneins are small, highly conserved, cysteine-rich metal-binding proteins involved in zinc/copper homeostasis. Cellular homeostasis of zinc is required for cellular proliferation, differentiation and acting as antioxidants for the protection of cells oxidative stress produced by mutagens, antineoplastic drugs and radiation [29]. Previous studies showed that MTs overexpression contributes an important role in carcinogenesis and tumour progression. MTs overexpression is reported as in ductal breast cancers, squamous cell carcinoma, colorectal cancers, ovarian cancer and bladder cancer [30–34]. Similarly, overexpression of MTs in gastric cancer is evidenced [35, 36]. Prognostic value of MTs varies according to the cancer types and in gastric cancer overexpression of MTs is associated with poor survival rate [36, 37]. Once again this study proved that the MTs overexpressed in gastric cancer by the evidence of downregulation of MT1X and MT1M genes.

PGC is an aspartic protease family protein and is produced by gastric chief cells. PGC digests polypeptides and amino acids by activating pepsin C. Expression of PGC is in three forms (gastric mucosal in situ, serum and ectopic expression) [38]. PGC is involved in tumour progression and reported as a likely biomarker for GC [39, 40]. Carbonic anhydrase 9 (CA9) is a transmembrane protein. They are involved in respiration, calcification, acid–base balance and formation of gastric acid, aqueous humour and cerebrospinal fluid. CA9 expression is involved in many types of cancer and it is related to the prognosis of the clinical outcome [41]. The

expression of CA9 and prognostic value of CA9 in GC was reported that patients with high expression of CA9 have more survival time and less survival time for low expression of CA9 [42]. Further, the genes CNE2, GIF, DRD5, CTSE, CLIC6, CHIA and LIPF [43–48] found in the module also validated that the expression of all the genes contributes to the gastric cancer progression and their studies are reported. Interestingly, in this study, Carboxypeptidase A2 (CPA2) is downregulated as well as noted in the module that highly connected to the hub genes. Already CPA2 expression is also reported in most of the gastric cancer study [49] and suggested as a candidate biomarker for GC by differential correlation network [43]. However, the study of CPA2 towards gastric cancer remains unclear. This research study proposes that CPA2 involves in gastric cancer progression and might be a potential prognostic biomarker as well as a therapeutic target for gastric cancer.

7.5 Conclusion

In summary, 13 hub genes and 8 highly connected genes were obtained. Out of that nine genes MT1X, MT1H, MT1E, KCNE2, CPA2, DRD5, CLIC6, CHIA and ATP4A are associated with overall survival of GC. Thus, the obtained hub genes can act as a prognostic as well as a therapeutic target for GC. However experimental studies are required for further validation. Further study of CAP2 could be utilised as a potential prognostic biomarker.

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Chapter 8
Recent Development in the Biomarkers for the Gastric Cancer

Dhananjay Shukla, Saurabh Saxena, and Pranav Kumar Prabhakar

Abstract Gastrointestinal or gastric cancer is a prominent cause of mortality in several parts of the world and it ranks at fourth in the death caused due to cancer-related disease. The preliminary detection may give a better medical result, but the major issue is the asymptomatic nature of the disease in early stages, patients get medical attention only in the late phase of the disease. The gastric cancer biomarkers can help in the early diagnosis of the disease and it may also replace the endoscopic and histological invasive diagnostic technology for gastric cancer. Here we are going to elaborate the presently available therapeutic strategy for gastrointestinal cancer and also the biomarkers that can help in diagnosis and detection. Some of the important biomarkers are vascular endothelial growth factor (VEGF) and their family, HER and its family, E-cadherin, programmed death ligands (PD-L1 and PD-L2), fibroblast growth factor receptor (FGFR), and mTOR. There are some newly diagnosed biomarkers as well such as instability of microsatellite, mesenchymal–epithelial transition (MET), and differences in microRNA. Detection, identification, and validation of diagnostic, predictive, and pharmacological markers will help in the drug development process as well as improve the already existing medicines.

Keywords Gastrointestinal cancer · Biomarker · microRNA · PD'L1 · VEGF · E-cadherin

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Abbreviations

CA	Carbohydrate antigens
CEA	Carcinoembryonic antigen
CT scan	Computed tomography scan
EGF	Human epidermal growth factor
EGFR	Epidermal growth factor receptor
FAK	Focal adhesion kinase
GC	Gastric cancer
hCG	Human chorionic gonadotropins
HDGC	Hereditary diffuse gastric cancer
HER	Human epidermal growth factor receptors
HGC	Hereditary gastric cancer
HGF	Hepatocyte growth factor
lncRNAs	Long noncoding RNA
LOH	Loss of heterozygosis
MAPK or MAP kinase	Mitogen-activated protein kinase
MSI	Microsatellite instability
PIGF	Placental growth factor
PKC	Protein kinase C
ROCK	Rho-associated protein kinase
RT-PCR	Real-time polymerase chain reaction
SLE	Systemic lupus erythematosus
SNPs	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
TGF	Transforming growth factor
TNM	Tumor size, presence of lymph node, and metastasis
VEGF	Vascular endothelial growth factor

8.1 Introduction

Gastric disease is one of the most prevalent malignancies with the fourth rank and second leading cause of death due to cancer-related abnormalities [1]. Biologically as well as genetically, the gastric cancer (GC) is multifactorial in nature with very less understood molecular carcinogenic pathogenicity. Recent times, the incidence rate for gastric cancer is decreased but the outcome remains same. In any case, the rate and mortality of viscus malignancy do not equally occur round the world. Gastric cancer is more prevalent in middle Asia, Central and middle Europe, and Central and South America, especially Japan and China [2]. The prevalence of GC in men is almost twice that of women and in case of some specific ethnic groups of human the risk for getting is more than that of others [3, 4]. According to one estimate, more than 1 million people get detected for the gastric cancer every year in

Eastern Asian countries and Western Europe. The disease is undiagnosed in the early stage of its pathogenesis due to the asymptomatic nature and the nonspecific symptoms. The most common nonspecific symptoms are abdominal pain, vomiting tendency, weight loss, and anorexia, unable to swallow food. So, the GC is normally diagnosed in its late stage or advanced stage of pathophysiology, which has a negative health outcome. Gastrointestinal cancer has been broadly divided into two categories on the basis of its histology:

(a) *Intestinal stomach/gastric cancer*: This cancer is more predominant and prevalently affects men compared with women older than 50 years. This is also linked with the intestinal metaplasia in which the epithelial layer of cell is transformed into another form of epithelium. The common location for this cancer is the gastric cavity.
(b) *Diffuse-type cancer*: This cancer is less common and occurs equally in both the genders. This cancer preferentially occurs in the age group of 45 years. The cancer starts from the mucosal layer of stomach [5, 6].

There are a number of reasons that can ultimately lead to gastric cancer and some of these are infection of *Helicobacter pylori*, dietary components, and family history. All of these factors cumulatively caused gastric cancer [7]. Laparoscopic techniques and gastroscopy techniques are the most common diagnostic tool for the spotting of gastrointestinal cancer and it provides both the status of mucosal gastrointestinal lining as well the sample for biopsy. Once a patient gets detected and goes for surgery, it is also required to detect any kind of remote spreading of tumor and this can be performed through computed tomography (CT) scan or sometimes ultrasound or echoendoscope [8]. Even today when we have so many tools and techniques available for surgery and adjuvant-associated treatment, the gastric cancer remains a worldwide public health burden. This chapter focuses on the early evaluation of gastrointestinal cancer through different biomarkers for the various stages of the gastric cancer development.

8.2 Predictive Biomarkers for Gastric Cancer

Biomarkers are explained as "a characteristics that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [9]. These play an important role at various stages of the diseases, from diagnosis to the evaluation of risk and disease management, for the reduction of the burden of disease in both mortality and morbidity. On the basis of some common clinical diagnostic parameters such as the size of the tumor, occurrence of lymph node and metastasis (TNM) production, the place of tumor occurrence, gender of patients, and histological subgrouping are unable to differentiate between respondents and nonrespondents. The use of unconventional methodology for the treatment of GC on the basis of the tumor origin site is putting some additional intricacy in the management. Rather the treatment strategy

should be chosen with the help of biochemical properties of the tumor, which will provide a better outcome. The different types of abnormal cellular pathways got activated in the case of cancer, which also results in the different types of responses for chemotherapy [10, 11].

A predictive biomarker is the objectively measurable properties of tumor such as the soluble dispersing proteins, mobilizing cells, or modified and mutated/modified genetic material, which can differentiate between the healthy normal and cancerous abnormal physiological condition and also help in spotting a patient's specific unhealthy condition (called diagnosis) or to evaluate the risk for the developing cancer in specific tissue in near future linked with the reoccurrence, death, or other outcome (called prognosis) and lastly to evaluate the response to a chemotherapy or any other targeted therapeutic strategy and estimate which patients will be getting benefitted by specific type of treatment (called predictive) [12–15]. A predictive marker for a cancer is a specific DNA fragment (gene) or its product (protein) that provides the information about the sensitivity and resistivity for a specific type of therapy in a specific tissue. Recently the use of predictive markers is rising specifically in case of cancer as it gives a positive response for chemotherapy in a specific patient as the response varies person to person because of their genetic variability and dietary factors. A preferred idealistic prognostic tumor marker should be dependable, authentic, easily accessible, and perceptible through the normal laboratory protocol. Marker should be very particular and provide a quantitative information of tumor size along with a very small false +ve rate and a sensible small false –ve rate [10]. There are a number of reports available for the role of predictive biomarker in case of different types of solid tumors such as brain tumor [16], breast cancer [17], colorectal cancer [18], chronic myeloid leukemia [19], and lung cancer [20, 21].

8.2.1 Conventional Biomarkers for Gastric Cancer

There are a large number of biomarkers available and some of these are carcinoembryonic antigen (CEA), carbohydrate antigens (CA) 72-4, 19-9, 12-5, alpha fetoprotein, systemic lupus erythematosus (SLE), glycoprotein BCA-225, human chorionic gonadotropins (hCG), and human pepsinogens I (PGI) and II (PGII). Among these biomarkers carcinoembryonic antigen and carbohydrate antigens 19-9 are among the most commonly and widely used biomarker for gastric cancer.

8.2.1.1 Carcinoembryonic Antigen (CEA)

CEA is a protein normally present during the pregnancy in the maternal blood. CEA is present in the normal adult blood in a very low amount but its level rises during certain specific type of cancers or some noncancerous condition also. For the detection of colorectal cancer and gastric cancer, the carcinoembryonic antigen is

a most preferred biomarker in the current clinical practices. CEA is treated as a separate risk factor to diagnose hepatic metastasis relapse [22]. In the advanced stage of gastric cancer patient's blood, the increased level of CEA has been found and hence it is not a very efficient tool for the screening purpose. The occurrence of CEA level in the peritoneal fluid gives more accurate result for the curative resection of GC [23]. Estimation of CEA mRNA with the help of RT-PCR is very much helpful in the detection of micrometastasis in the peritoneal cavity [24].

8.2.1.2 Carbohydrate Antigen (CA) 19-9

CA 19-9 is a tetrasaccharide Sialyl Lewis that is linked with the O-glycan on the plasma membrane of cell. This antigen plays a significant role in the communication between cells and is also helpful to diagnose tumor as a tumor marker. It is an antigen that is released and secreted by pancreatic tumor cells. It works as a tumor marker for colorectal cancer, pancreatic tumor, gastric cancer, and so on, and also works as the ligand for the glycoprotein E-selectin of endothelial cell surface [22]. Recently, CA19-9 has been one of the commonly used markers for GIT malignant growth such as pancreatic cancer and gastrointestinal cancers. Gastrointestinal cancers having CA19-9, exhibited particular clinical and pathological qualities, for example, cavum area, isolated histology, noticeable lymphatic attack and venous attack, increased extent of lymph hub metastasis, and progressed phase [25, 26]. In addition, the combination of other tumor markers along with CA19-9 gave increasingly valuable data to expectation of recurrence [27]. The combination of CEA and CA 19-9 has shown the increament in their efficacy 87%.

8.2.2 The Protein Biomarkers

The developing area of gastrointestinal biomarker has been concentrated on the analysis of specific process associated with the macroscopic responses such as angiogenesis. These factors have been evaluated that predictive biomarker at the genetic level like polymorphism, transcription level like expression of mRNA, or the level of protein synthesis may play an important role in evaluating responses of different developed drugs or therapeutic strategies for GC [27].

8.2.2.1 Vascular Endothelial Growth Factor (VEGF)

The development of tumor cell requires continuous blood supply and hence new blood vessel has to be formed in the tumor mass. The angiogenesis process, formation, development, and growth of new blood capillaries are highly regulated through different stimulatory and inhibitory factors, as this is very much important for normal development of an organism and also for the healing of any kind of

wounds [28]. Sprouting and intussusceptive are two different types of angiogenesis process. In the case of sprouting angiogenesis, a blood vessel is sprouted or branched off from the main blood vessels, whereas in the case of the old main blood vessels split and give rise to two branches. The amount of angiogenesis is affected because of the imbalance in the release of pro- and antiangiogenesis factors [29, 30]. The formation of abnormal blood vessel is linked with the growth of tumor, enlargement of tumor, and metastasis of tumor [31–35]. When there is not enough vascular support, the growth of tumor is halted and grows up to 1–2 mm^3 in diameter and sometime it leads to necrotic or apoptotic tumor [36]. There is a large group of proteins which works as an angiogenic activator and the most important and very well-known are VEGF-mediated pathway and its ligands. The well-analyzed ligands for VEGF family are VEGF-A to E, and placental growth factor (PIGF-1 and 2). These ligands work through the binding to a receptor present in plasma membrane VEGFR1 and VEGFR2. These nature of these receptors like insulin receptor are tyrosine kinase which transduce signal intracellularly after binding of the ligand [32–36]. All the family members of VEGF give cellular response after association with the present extracellular portion of receptor and dimerizes (either homo or hetero) and later transphosphorylation occurs. In the cascade, after phosphorylation, a series of events starts such as Ras/MAPK pathway (for regulating the expression of genes), FAK/paxillin pathway (for modulation of cytoskeleton), the PI3K/AKT pathway (for regulating the survival of cell), or the RhoA/ROCK pathway (for the regulation of cell growth and proliferation, plasma membrane permeability, cell survival, cell movement) (Fig. 8.1). These pathways regulate different cellular process such as induction of endothelial cell growth, their migration and maturation, other cell development, growth and development of cytoskeleton, the cell survival strategies,

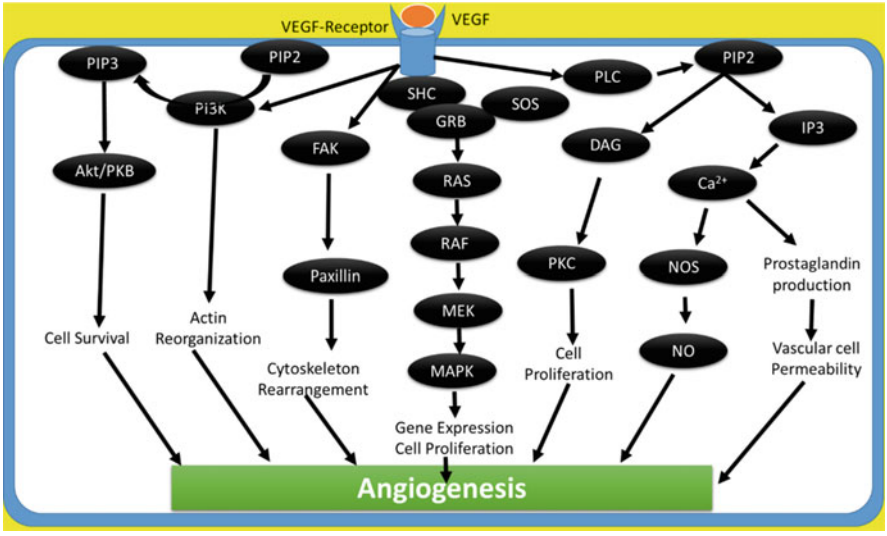

Fig. 8.1 Vascular endothelial growth factor signaling cascade

and the cell proliferations. All these pathways merge together and result in the induction of angiogenesis [37–40].

Scientific studies on the circulatory VEGF and the factors associated with angiogenesis have shown mixed kind of results [41, 42]. Further biomarker analysis has shown that an increase in the VEGF-A concentration is linked with the shorter survival in a number of cancers [43, 44] and at the same time, decreased level of neuropilin-1, a kind of transmembrane glycoprotein complex in the process of angiogenesis in the form of coreceptor for the ligand for VEGF, is linked with low degree of prognostic features [44]. The VEGF and their receptors have also been studied as a marker for GC. Roughly in 42–49% of gastric cancer cases, the VEGF has been highly expressed and hence it is one of the most significant therapeutic targets for the treatment of gastrointestinal cancer [45, 46]. The two different phase II clinical trials for the evaluation of the efficacy and safety of the combination of humanized anti-VEGF-A monoclonal antibody (mab) bevacizumab along with the semisynthetic agents like "docetaxel" and "oxaliplatin" [47] and "irinotecan" and "cisplatin" [48] and a positive result were achieved. The mechanism of action of bevacizumab is that it binds and nullifies all the human VEGF-A isoforms. Biomarker subgroup analysis has been studied, which also includes VEGF and their receptors, and neuropilin-1 [44], of cancer cells to distinguish progressively responsive to monoclonal antibodies bevacizumab. The denotation scope of a molecular biomarker is associated with the particular ethnicity of the test subject. Patients from the non-Asian countries have shown an increased value of blood VEGF-A, a weak diagnostic marker, whereas Asian patients have shown in high value of neuropilin-1 levels, a good diagnostic marker [49]. Subject with high value to VEGF-A (non-Asian group) are thought to get a better result and benefit when bevacizumab is given along with chemotherapy. A reduced neuropilin-1 concentration were also associated with the intake of bevacizumab along with chemotherapy [49]. Ramucirumab, which is a perfect monoclonal antibody against VEGFR-2, is well tolerated and develops survival in the case of refractory gastric cancer and esophageal cancers [50].

8.2.3 The Genetic or Noninvasive Biomarker for Gastric Cancer

8.2.3.1 Chemotherapeutic Drugs and Their Targets

The normal mechanism of action of chemotherapeutic agent that works by causing cell death or inhibit the cell growth are works by the inhibition of microtubule formation, dysregulation the cytoskeletal, inhibit translation process, or nucleic acid synthesis, damage DNA and cause mutation, or topoisomerase degradation. All these target components are involved in cell cycle and stopping these processes stops cell cycle at some stage and affects the normal cellular growth [21, 51–55]. The efficacy of anticancer drug also gets affected and blocked by various ways of chemo resistant gene expressed in different cells. Hence, the best therapeutic strategy is the

combination therapeutic strategy where more than one drugs are combined together to get a synergistic effect commonly known as polychemotherapy [56–59]. The two main mechanisms by which cytotoxic drugs work are through the directly interaction with DNA such as alkylating agent [52, 60] and affect the biochemical process that generates the precursor of DNA or RNA such as antimetabolites [61, 62]. Some of the more commonly used cytotoxic drugs for the management of gastric or esophagogastric cancer are platinum containing anticancer agents such as cisplatin and carboplatin [53], 5-fluorouracil [63, 64], capecitabine [65], anthracycline [66, 67], and ionizing type of radiation [68] (Fig. 8.2).

In our cell we have a very effective and highly conserved DNA damage sensor process that senses for any kind of DNA damage. In case of any DNA damage, the cell cycle progression will be suspended and terminated till the DNA repair mechanism repairs the damage of DNA. If cell is unable to repair the DNA damage, the apoptotic process will be initiated, which removes the cell with damaged DNA. The DNA repair process that allows cancer cell to repair the cytotoxic compound-induced DNA damage induces resistance against therapeutic agents. Rather, problematic DNA reclamation of typical tissue may adversely impact on ordinary tissue resistance. The important quality of human cancerous cells is their genomic unbalance. Some cytotoxic components have a capacity to induce single-strand break and it leads to single nucleotide polymorphism (SNPs) and results in the variation in the genetic differences. Generally, SNP doesn't have any effect on our health, but sometime these mutations result in the defects in amino acid sequence and ultimately protein sequence or also affect the splicing process of RNA [28, 50]. Notwithstanding SNPs, short couple rehashes [69], microRNAs [70], and other genomic varieties, for example, auxiliary varieties have been accounted for to be related with gastric disease [71]. In addition, transformation could adjust medication digestion or medication targets, actuate endurance flagging pathways, or inactivate downstream demise flagging pathways prompting drug opposition [72, 73].

8.2.3.2 Instability of Microsatellite

The "microsatellite markers," or "short tandem repeats," are two to seven nucleotides repeats on to the chromosome locus and hence polymorphic in nature. In a very specific locus, the number of repeated nucleotides may differ and hence the alleles also differ in length. Microsatellite instability (MSI) is a genetic condition of hypermutability that is the result of defective mismatch repair system. When this repeated DNA locus replicated, the DNA polymerase slips and results in the abnormality in nucleotide pairing and these may be corrected by the mismatch repair mechanism. Any kind of abnormality in the repair system of this misalignment results in mutation. These days the instability in the microsatellite is used as an effective way to detect gastric and colorectal cancer [74–77]. If any tumor has 10–29% of microsatellite instability, it is known MSI-low and at the same time tumor with more than 30% microsatellite instability is known as MSI-high. In the case of gastric cancer, 15–30% of tumor shows MSI.

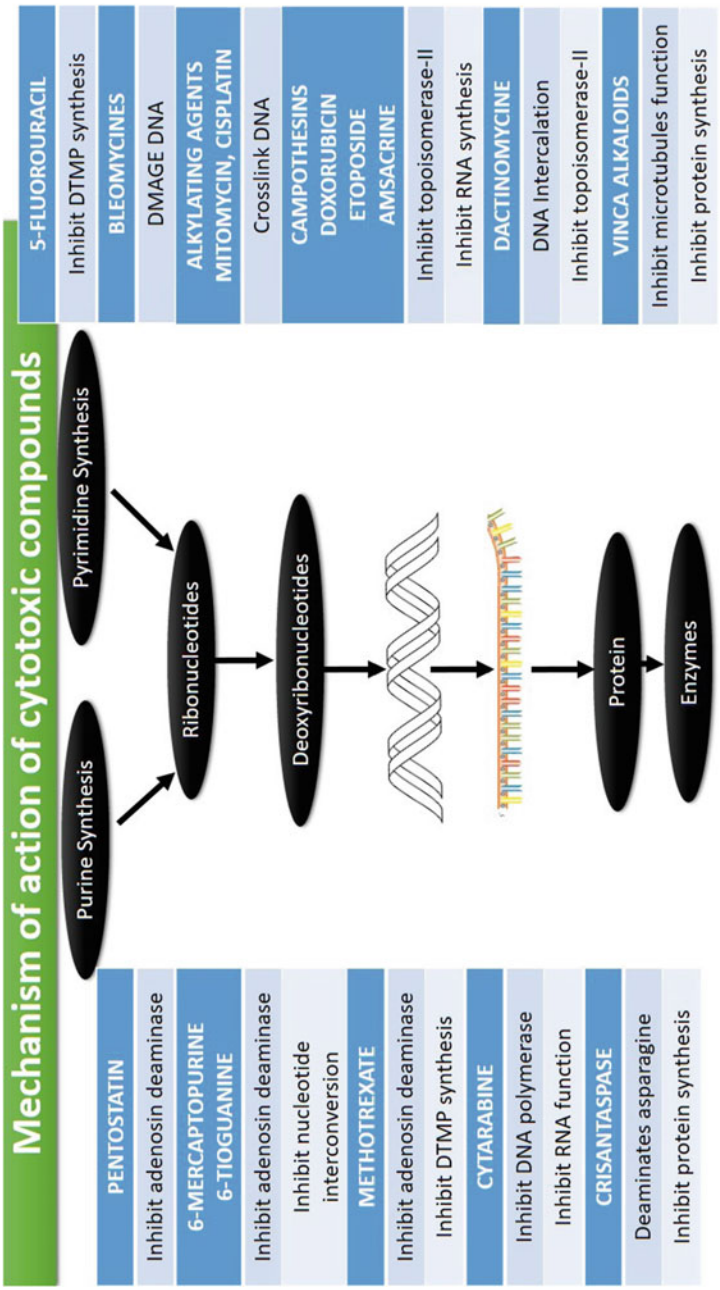

Fig. 8.2 Mechanism of action of cytotoxic compounds

8.2.3.3 microRNA

A microRNA is a small noncoding polyribonucleotide that is present in animals, plants, and viruses. The major function of microRNA is in RNS silencing and also plays a role in posttranslational regulation of expression of a gene. It works via the complementary base pairing with the mRNA at 3′ untranslated portion of the target messenger RNAs. MicroRNA regulates a number of physiological processes within cells such as apoptotic cell death, proliferation of cells, cell division and differentiation, and embryogenesis. Recently studies have analyzed differentially expressed miRNAs, including "miR-17-5p/20a, miR-100, miR-125b, miR-133b, miR-145, miR-148a, miR-196a/-196b, miR-199a-5p, miR-302, miR-506, miR-940, miR-1182, miR-1207-5p/miR-1266, miR-29a/c, miR-29b/c" [78, 79]. These microRNAs have shown its significant role in the diagnostic of gastric cancer. Clinical examinations are continuous to dissect the articulation level of microRNAs utilizing cutting-edge sequencing such as next-generation sequencing, in gastrointestinal cancer tissue and blood through chemotherapy. Likewise, a stage II concentrate to explain whether reaction to pralatrexate can be anticipated by miR-215-5p is right now in progress. At the point when these preliminaries will finish with persuading proof, miRNAs can be an effective marker or new remedial focuses for tranquilizing reaction expectation and control just as adjustment of ordinary adjuvant treatment.

8.2.3.4 Long Noncoding RNA (lncRNAs)

These are more than 300 nucleotide long DNA sequences that can function as proto-oncogene or tumor suppressing gene [80]. These long noncoding RNA (lncRNAs) involved in many molecular functions as the regulator for transcription, regulator for splicing, processor for posttranscriptional, enhancer, and remodeler of chromatin. As these lncRNAs are expressed mostly in the abnormal disease condition and thus play a role as a biomarker also [81]. Till today roughly around 56,000 lncRNAs have been detected and sequences whereas around 135 lncRNA have shown their association with the gastric cancer, and hence it can be understood that these are associated with the cancer and works as a tumor marker [80, 82]. Defective gene expression of ncRuPAR is significantly linked with the lymph node metastatic cancer, and TNM stage of gastrointestinal cancer patients [83]. A reduction in the expression of AI364715, gastric cancer-associated transcript 1 (GACAT1), and gastric cancer-associated transcript 2 (GACAT2) in gastric cancer tissue also functions as a tumor marker for the GC [84]. Long noncoding RNA (lncRNA) PVT1 was especially highly expressed in gastric cancer tissues contrasted and it can be an effective diagnostic marker [85, 86]. The uses of lncRNAs in the clinical need to be further investigated.

8.2.3.5 Exosomes

These are membrane enclosed extracellular small vesicles derived from cell and protect RNA and miRNAs from being degraded [87–91]. The RNAs present in the Exosomes are protected from the action of RNAs whereas the cellular RNAs are degraded by the same RNAs [90]. The Exosomes have a huge potential to be used for both prognosis and diagnosis and are also very useful as tumor biomarker [92]. In the gastric patients, miR-19b and miR-106a were found to be highly expressed in the serum-circulating exosomes when compared to the healthy human [93]. Expanded articulations of micro RNA miR-21 and miR-1225-5p present in exosomes, secluded peritoneal lavage liquid, were shown in patients with T4-organize disease contrasted with that in T1- with T3-arrange patients [94]. All these results explain that exosomes can work as an efficient and novel biomarker for gastric cancer in terms of its detection and therapeutic status measurement.

8.2.4 Human Epidermal Growth Factor Receptors (HER)

Human epidermal growth factor (EGF) is significantly involved in the growth and development of cells through its association with their receptor. HER is a 6-kDa protein of 53 amino acid. The human epidermal growth factor receptor (HER) is a transmembrane receptor for EGF family of extracellular ligand. There are four members in the HER family and these are HER1 (also known as ErbB1, epidermal growth factor receptor [EGFR]), HER2 (also known as ErbB2), HER3 (also known as ErbB3), and then HER4 (also known as ErbB4).” The main function of ErbBs receptors is associated with various types of cellular functions such as growth and development of cells, cellular survival, transportation of cells, and variation [95–98]. The signaling cascade of HERs is of two different types. When a ligand binds to the HER1, HER3, and HER4, the receptor gets dimerized and cytoplasmic domain of receptor tyrosine kinases autophosphorylation and initiates the downstream signaling cascade [99, 100]. The HER2 is physiologically different from the rest of the three in the manner it gets heterodimerized once ligand binds to the extracellular portion of receptor [95]. After the autophosphorylation there is divergence of the signaling cascade through various pathways like Ras/MAPK, phospholipase (PLC)-γ1/protein kinase (PKC), PI-3 K/Akt, and STAT pathways.

8.2.4.1 Epidermal Growth Factor (EGF) and Its Receptor (EGFR)

EGFR is an important member of human epidermal growth factor family and different from HER2 and is activated by EGF and transforming growth factor (TGF)-α. Roughly in more than 33% of gastric cancer cases, EGFR is expressed of cell surface [101, 102], and there is an indication that rise in the expressed EGFR is linked with the weak diagnosis in GC [102–104]. There is some contradictory

report also that says that the expression of EGFR works as a good prognostic factor [105] or not at all significant in the prognostic [106]. The prognostic role of EGFR is not very much understood and it is still controversial. Cetuximab is a monoclonal antibody (mab) produced as an agonist of EGFR for the management of various cancers such as neck and head cancer and lung cancer. This is a chimeric antibody made up of mutable portion of rodent EGFR mab. It competitively blocks the binding of EGF and TGF-α and stopping the phosphorylation of receptor tyrosine and further stops the signaling cascade. This blockage leads to the suppression of growth and development of cells and starts apoptosis, diminished matrix metalloproteinase outflow, and, finally, a decreased VEGF synthesis [107, 108]. In the period of 2008–2010, a big group of 904 homogeneous patients. From 25 different countries, who already had metastatic malignant growth has been given two different types of treatment: one group received only capecitabine and cisplatin but not cetuximab and other group received capecitabine and cisplatin with cetuximab. The result says that the inclusion of cetuximab in the capecitabine and cisplatin does not have any significant positive evitable benefit on the given chemotherapy in the case of advanced stage of gastric cancer [109].

8.2.4.2 HER2

EGFR family is having four important members HER1 to HER4, and HER2 is one important member in this family with tyrosine kinase receptor (RTKs). The gene for HER2 is present on the chromosome 17, which is type of protooncogene ERBB2. This also plays a significant role in the growth and development of cell and cellular survival [110]. Similar to other RTKs, it also has three domains, extracellular, transmembrane, and intracellular domain. The extracellular portion is easily cleaved by metalloproteases [111]. As soon as the ligand binds to the HER2, it got heterodimeriose with other family members mainly with the EGFR [112]. The overexpression of HER2 gene results in the survival of cancer cells, their growth, proliferation, and differentiation through the PI3K-AKT and MAP kinase-mediated pathways [113, 114]. The tumors where HER2 is overexpressed are differentiated tumors. The overexpression of HER2 works as a diagnostic as well as prognostic biomarker for gastric cancer [115–118]. Its prevalence is almost 9–32% of gastro-esophageal cancers [118]. The higher expression of HER2, which occurs because of the mutation in the ERBB2 gene, takes place in the primitive phases of tumorigenesis [116–118]. In the clinical practice, HER2 was the first molecular diagnostic biomarker that was available to detect. Trastuzumab, a HER2-targeted agent, prevents the cleavage of extracellular domain of HER2 and stops the HER2-associated cellular pathways [119]. Trastuzumab was the first targeted monoclonal antibody that was approved for the management for the gastric cancer. A phage III randomized controlled trial showed that the combination of trastuzumab to capecitabine or 5-FU and cisplatin has shown a clinical health benefit compared to the chemotherapy single for tumor response and now it is considered as the standard care for the HER2 positive gastric cancer [119]. There are a few other HER2 focused on specialists, for

example, pertuzumab, lapatinib, and trastuzumab emtansine being researched in randomized clinical preliminaries in patients with HER2-positive GC [120–122]. In any case, no critical proof was found at this point. A few snags, for example, deciding the reasonable portion of trastuzumab, distinguishing a prescient biomarker, exist for the progression of HER2 focused on treatment in GC [123]. Some of the scientific communities have demonstrated the handiness and adequacy of trastuzumab alone or combined chemotherapy, for example, $p27^{Kip1}$ and HER2 extracellular domain [124, 125]. Protection from trastuzumab is additionally these days subject to HER2-positive GCs. One of the most significant components, basic trastuzumab obstruction, is dysregulation of phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR pathway. It is notable that PIK3CA transformations and phosphate and tensin homolog (PTEN) inactivation may influence the viability of HER2 focused on therapy [126]. Consequently, mix treatment of trastuzumab with PI3K inhibitors may furnish significant advantage in patients with HER2-positive GC. CCNE1 enhancement, one of the most well-known cohappening duplicate number change, is adversely related with the reaction to HER2-coordinated treatment, proposing its potential job as a biomarker of obstruction in patients with ERBB2-intensified GC [127]. The job of HER2 as a prognostic biomarker is as yet dubious; in fact, prior examinations show a relationship of HER2 with a more terrible visualization and an increasingly forceful ailment; others contrariwise don't locate a noteworthy distinction in guess between HER2 +ve and −ve tumors [118–123]. Still the association between the higher expression of HER2 and the clinical pathophysiology of gastrointestinal cancer is doubtful. But, there are a number of reports that show the link between the expansion of ERBB2 with tumor volume, metastasis of lymph node, and stages of tumorigenesis.

8.2.4.3 E-Cadherin

Cadherins are one type of calcium-mediated cell adhesion transmembrane molecules. E-cadherin is also known as epithelial cadherins, which is present on the surface of cell and associated with the similar type of molecule on other cells through the formation of bridge. It has been understood that the loss of E-cadherins is linked with the carcinoma formation (epithelial cell cancer) [128, 129]. E-cadherin has a significant role in the cellular attachment, adhesion, and also in the proliferation and differentiation of epithelial cells of gastric cells and also in the cessation of cancer occurrence [129]. CDH1 is a very important tumor suppressor gene present in the gastrointestinal cancers and the suppression of CDH1 result in the tumorigenesis process of gastric cancer such as the proliferation and invasion and lastly the metastasis of tumor cells [124, 130–134]. There are a number of processes at present, which results in the loss of E-cadherin function and these processes are CDH1 gene mutation, loss of heterozygosis allelic nature, by using suppressors, and lastly through the microRNAs for the regulation of E-cadherin gene expression [129]. Germline transformations in the CDH1 quality are recognized in hereditary diffuse gastric cancer (HDGC), prompting the histological attributes like diffuse-type GC. The combined danger of GC by 80-year-old in male CDH1 transformation

bearers is 83% for cutting-edge GC [135]. Tragically, metastatic HDGC patients show lower endurance contrasted and other sporadic GC. An ongoing report portrayed that E-cadherin/catenin-EGFR crosstalk is firmly connected with HDGC. Improved affectability to EGFR and PI3K kinase restraint was instigated by loss of E-cadherin/catenin-EGFR association in HDGC families with CDH1 germline changes, recommending that these inhibitors would be an alluring device for the focus on treatment in hereditary gastric cancer (HGC) patients sooner rather than later. Patients with GC indicating substantial CDH1 epigenetic and auxiliary adjustments have a more awful, generally speaking, endurance than patients with tumors negative for CDH1 modifications. This discovery demonstrates that the nearness of CDH1 epigenetic and auxiliary adjustments in a symptomatic/preoperative biopsy may fill in as a clinically helpful biomarker [134]. An ongoing report inspected the indicative job of advertiser methylation status of CDH1 in blood tests of patients with GC [136]. Strikingly, the huge assistance of advertiser methylation of CDH1 appeared in blood tests, recommending that advertiser methylation of CDH1 might be a decent competitor of biomarkers in patients with GC.

E-cadherin may be an effective diagnostic biomarker to access the efficacy of specific ongoing treatment as its loss of capacity lessens the reaction to both traditional and focused-on treatment [133, 137]. Distinguishing CDH1 changes right now of the analysis can foresee if malignant growth will be receptive to a treatment; thus, it could help in picking the more reasonable treatment for a particular patient [134]. Highlight that a high level of families with HDGC should have a transformation of E-cadherin quality; this clearly suggests that there must be other atomic changes that lead to the inclination to gastric malignant growth that still has not been distinguished [134, 138, 139].

8.2.4.4 PI3K/Akt/mTOR

This is a signaling process that occurs intracellularly and mainly regulates cell division process and hence involved in the cell survival, longevity, cell growth, and proliferation. The activated PI3K activates the plasma membrane-associated Akt protein through phosphorylation that has a number of downregulating proteins and signaling components [35]. The PI3K has two components, p110 (catalytic) and p85. PIK3CA gene is responsible for the catalytic isoform p110α, which is the second most commonly mutated oncogene, and PTEN gene is responsible for the major phosphatidylinositol phosphatase, which is one of the maximum mutated tumor silencer genes [140, 141]. Hereditary deregulations in the PI3K/Akt/mTOR pathway have been distinguished often in GC. PI3K/Akt/mTOR articulation has been related with the lymph hub status and poor survival [142]. The PI3KCA gene has been accounted for to be recognized in 4–25% of the gastrointestinal cancer patients. Despite the fact that PIK3CA transformation has a basic job in the protection from antitumor medications and securing of metastatic potential, its changes did not liable to have an established efficient on prognosis. It has been accounted for that no ethnic contrasts in PIK3CA transformation frequencies exist, while the PIK3CA changes

are overwhelmingly found in 80% of Epstein–Barr infection (EBV)-positive subgroups [143]. An ongoing report pointed that p-Akt −ve neoplasm is more harmful than p-Akt +ve neoplasm and however is protected by the adjuvant chemotherapy for GC patients experiencing gastrectomy paying little heed to the PIK3CA transformation status [144].

8.2.4.5 Mesenchymal–Epithelial Transition Factor (c-MET)

Mesenchymal–epithelial transition factor (c-MET) is a member of liver cells growth factor receptor and an RTKs-type transmembrane receptor. The gene for this factor is present on the 7q21-31. These transition factors have a number of regulatory functions for cellular physiologies such as cell growth and proliferation, cellular differentiation, cellular mobility, cell cycle regulation, and programmed cell death. Initiation of MET cascade phosphorylates a few signaling cascade components, prompting disease cell development, angiogenesis, relocation, and metastases and leads to gastrointestinal cancer [145–147]. The estimation and evaluation of hepatocyte growth factor (HGF) play a critical job in assessing the tumor microenvironment, which induces the metastasis process also the resistance toward drug [145]. The currently immunostaining test has revealed that the expression of c-MET is involved in the invasion of lymphatic vessel and less survival, inferring that the gene expression of HGF/c-MET cascade may fill in as a planned prescient factor in patients with GC [148, 149]. Surprisingly, gastric cancer patients with a low pre-treated HGF amount demonstrated a positive reaction to the management of trastuzumab. The blood value of HGF also rose in those patients in which there were no effects of trastuzumab when compared to the pretreated level [150]. In the interim, MET might be a helpful prescient evaluator for chemotherapy, in light of the fact that MET flagging decidedly related with chemoresistance of GC treatment by means of expanding UGT1A1 level [151].

8.3 Conclusion

Our cell contains a very high conserved DNA damage sensor that detects the DNA damage and halts the cell cycle progression. Meanwhile the DNA repair mechanism works to repair the DNA damage. Aggressive cancer cell modifies their transcriptome and, because of DNA repair, mechanism gets resistance toward damage. Some of the chemotherapeutic cytotoxic agents induce single- or double-strand break in the DNA and leads to single nucleotide polymorphism and finally a genetic variation. Most of the time the SNP does not have any significant effect on the physiology of human.

Definitively, gastric malignant growth is as yet a critical danger to worldwide well-being and unexpectedly there are no institutionalized proposals for preventive screening and restorative intercession against this destructive ailment. There is a

noteworthy opportunity to get better of revelations in the area of biomarkers in giving exact indicative, diagnostic, and prescient examination. Notwithstanding that, through the improvement of present-day innovations, including the complete gene sequencing, microsatellite and microarrays investigations can likewise be involved and affirmed for efficient and early identification. Furthermore, large sample size multichannel and global examinations are obligatory to intensify the information on inclining hazard elements, biomarkers, and viability of medications that can importantly affect endurance without poisonous quality.

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Chapter 9
Gastric Cancer and Its Remedy

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Abstract Gastric cancer or stomach cancer is a very harmful disease in which malignant cells grow in the tissues of the stomach at an uncontrolled rate. It is one of the most prevalent malignant diseases with one of the highest mortality rates around the world. It is the fifth most common cancer and the third leading cause of cancer-associated death in the globe. Approximately 1 million of new incidences of gastric cancer happen each year and near 750,000 people die from this disease annually. The cases of gastric cancer have been reduced significantly during the last 50 years in the United States of America and Western Europe without taking preventive actions for gastric cancer. The rate of occurrence of gastric cancer was also declined in the population in Japan and other high-income nations. The significant decline in the case of gastric cancer is considered because of the development in socioeconomic status of people. For example, widespread availability and general uses of refrigeration as standards food preservation method, availability of enough vegetables and fresh fruits, a common improvement of potable water and nutritional status during the last century, and decline trends in the prevalence of *Helicobacter pylori* infection attributed to decline in incidence of gastric cancer worldwide. When cells in the body commence to grow out of control, this condition is called cancer. Cells in any area of the body can become cancer and can disseminate to other parts of the body. The most common causes of gastric cancer are *H. pylori* infection and account for about 60% of incidences, preserved vegetables, smoking, alcohol intake, and genetic factors. Gastric cancer is generally asymptomatic or nonspecific symptoms. Initial symptoms include indigestion, abdominal discomfort, loss of appetite,

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constipation, nausea, vomiting, diarrhea, blood in the stool, and heartburn. Gastric cancer can be diagnosed by taking patient's history and endoscopic test. The treatment of gastric cancer includes surgery, chemotherapy and/or radiotherapy, and immunotherapy.

Keywords Gastric cancer · Carcinogenesis · Epidemiology · Risk factor · *Helicobacter pylori* · Immunotherapy · Chemotherapy

Abbreviations

AJCC	American Joint Committee on Cancer
CA	Carbohydrate antigen
CDK	Cyclin-dependent kinase
CEA	Carcinoembryonic antigen
CT scan	Computed tomography scan
EGC	Early gastric cancer
EGFR	Epidermal growth factor receptor
ESD	Endoscopic submucosal dissection
EUS	Endoscopic ultrasound
GE	Gastroesophagus
GERD	Gastroesophageal reflux disease
GIST	Gastrointestinal stromal tumor
H. pylori	*Helicobacter pylori*
HDGC	Hereditary diffuse gastric cancer
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
PD1	Programmed cell death protein-1
PD-L1	Programmed death-ligand 1 (PD-L1)
PET scan	Positron emission tomography scan
VEGF	Vascular epithelial growth factor
WHO	World Health Organization

9.1 Introduction

Gastric cancer is nowadays the fifth most common cancer worldwide, after lung, breast, colorectal, and prostate cancer [1, 2]. In the year 2012, about 952,000 new occurrences of gastric cancer were reported globally, estimated for 7% of all new occurrences of cancer. Men are two times greater vulnerable than women to develop gastric cancer and it is more common in older adults. For instance, in the United States, the average age of patients at diagnosis of cancer is 72 years. Gastric cancer is the third most common reason of fatality from the cancer diseases. Symptoms of gastric cancer are generally manifested at later stage. That's why it is difficult to

perform prognosis of gastric cancer at early stage. Nevertheless, the survival rates are very lower in less developed countries where gastric cancer is generally identified at more advanced phase. Major incidence of gastric cancer occurs mainly in less developed countries with about 50% of all cases in Easter Asia. The decrease in *Helicobacter pylori* infection and the use of modern refrigerator to preserve food rather than using salts led to decline in the overall incidence rates of gastric cancer worldwide. Based on the location of the tumor, the gastric cancer can be classified into various types. At upper portion of the stomach nearest to the esophagus, gastric cardia occurs whereas gastric noncardia happens in all other parts of the stomach. Globally, gastric noncardia cancer is more common than the gastric cardia and is most widespread in Asia. On the other hand, gastric cardia cancer is more general rather than noncardia cancer in more rich countries like the United Kingdom and the United States, and is increasing the rates in all countries day by day [2, 3]. The most common causes of gastric cancer are *H. pylori* infection and account for about 60% of incidences, preserved vegetables, smoking, alcohol intake, and genetic factors. Gastric cancer is generally asymptomatic or nonspecific symptoms. Initial symptoms include indigestion, loss of appetite, nausea, vomiting, diarrhea, constipation, blood in the stool, abdominal discomfort, and heartburn. Gastric cancer can be diagnosed by taking the patient's history and endoscopic examination (biopsy) followed by medical imaging. The treatment of gastric cancer includes surgery, chemotherapy and/or radiotherapy, and immunotherapy [3]. The decline in gastric cancer rates has mainly been attributed to a number of factors associated with the advance of living standards of population, for instance, the increase in the intake of fresh fruits and vegetables and decline in consumption of salted food and salts [4].

9.2 Discussion

9.2.1 The Structure and Functions of the Stomach

When we chew and swallow food, it enters into the esophagus, which is a tube that passes food items through the throat and chest to the stomach (Fig. 9.1). The esophagus is connected with the stomach at the gastroesophagus (GE) junction. The GE junction is located under the diaphragm, the thin sheet of breathing muscle below the lungs. The stomach is a sack-like organ that retains food and begins to digest it after releasing gastric juice. The food and gastric juice are blended with each other and transfer to the first portion of the small intestine named the duodenum. Gastric cancer cannot be obfuscated with other types of cancers that generally happen in the abdomen, for example, colon cancer in large intestine, small intestine, liver, or pancreas. Different cancers can have different sorts of symptoms, different outlooks, and varieties of remedies [3].

Parts of the stomach (Fig. 9.2):

The stomach is one of the vital parts of the digestive system and it has five parts:

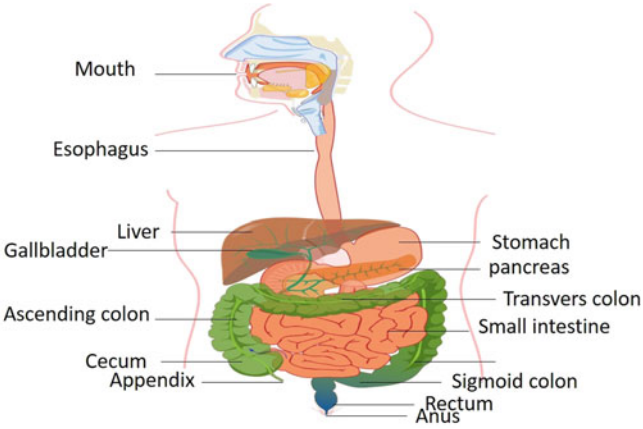

Fig. 9.1 Human digestive system

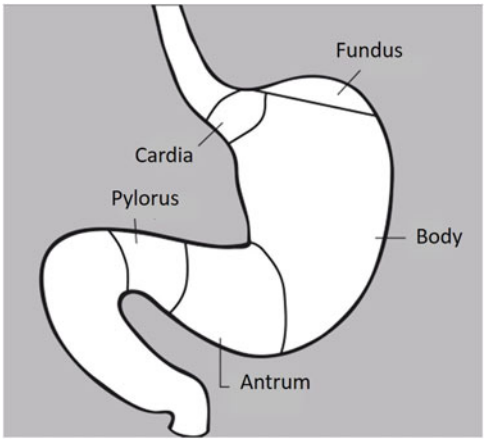

Fig. 9.2 Different parts of stomach

1. Cardia: This is the first part of the stomach directly connected with esophagus.
2. Fundus: Fundus is the upper portion of the stomach and located near the cardia.
3. Body: This is the main portion of the stomach and located between the upper and lower portions of the stomach.
4. Antrum: The lower part near the intestine and here the food is blended with gastric juice.
5. Pylorus: Pylorus is the lower portion of the stomach and it functions as a valve to control transferring the stomach contents into the small intestine.

The first three portions (cardia, fundus, and body) of the stomach are occasionally called the proximal stomach. A number of cells located in these portions of the

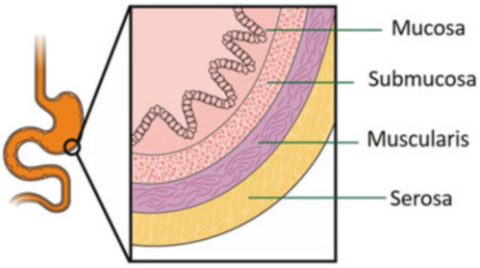

Fig. 9.3 Different layers of stomach wall

stomach produce acid and pepsin, a digestive enzyme helps to digest food. They also produce a protein called intrinsic factor that helps to absorb vitamin B_{12} in the body.

The stomach has two curves called the lesser curvature (inner boarder) and greater curvature (outer boarder). The neighboring organs of the stomach are the colon, liver, small intestine, spleen, and pancreas.

The stomach wall comprises five layers (Fig. 9.3):

(a) Mucosa: The innermost layer where gastric acid as well as digestive enzymes are produced. Most of the gastric cancers begin in this layer.
(b) Submucosa: This is the supporting layer for mucosa.
(c) Muscularis propria: The thick layer of the muscle and responsible for movement and mix the stomach content properly.
(d) Subserosa: This layer covers the stomach.
(e) Serosa: The outermost layer that wraps up the stomach. Subserosa and serosa are significant to estimate the content/stage of cancer and to determine the patient's outlook/prognosis [3].

9.2.2 Epidemiology of Gastric Cancer

Gastric cancer is positioned as the fourth incidence (after lung, breast, and colorectal) and second cause of mortality (after lung cancer) among all cancers globally [5]. There is a significant variation in the incidence of gastric cancer worldwide. The widespread occurrence of gastric cancer has been reported from Southeast Asia, notably from Japan, China, and South Korea. The reason of high incidence of gastric cancer is demonstrated because of the consumption of preserved food containing carcinogenic nitrates [6]. Regionally, about half of the incidences of gastric cancer happen in Eastern Asia and it is considered as second prevalent cause of cancer death globally [7]. In the year 2008, there were 989,600 new cases and 738,000 deaths from gastric cancer worldwide and about 70% of both new cases and deaths happened in the developing countries. The danger of developing gastric adenocarcinoma increases with respect to age, the most vulnerable patients are between 55 and 80 years of age. The gastric cancer is rare in patients under 30 years [5]. In the year 2012, about 952,000 new occurrences of gastric cancer were reported

globally, estimated for 7% of all new occurrences of cancer. Men are two times greater vulnerable than women to develop gastric cancer and it is more common in older adults [3, 8, 9]. The highest rate of occurrence in males is reported in Eastern Asia, mainly Korea, Mongolia, Japan, and China, with rates between 40 and 60 per 100,000 population; Eastern Europe, about 35 per 100,000 population and in some Latin American countries, mainly in central America and the Andean region, with rates between 20 and 30 per 100,000 population. Lowest incidence rates are reported in some African countries and North America [6]. Male gender (two times vulnerable than female), *H. pylori* infection, salt and salted food, tobacco use, atrophic gastritis, partial gastrectomy, and Menetrier's disease are dominant risk factors for gastric cancer worldwide. The most common anatomical subsites of disease are influenced by the regional variation in gastric cancer. Distal or antral stomach cancer due to *H. pylori* infection, excess consumption of alcohol, highly salted diet, processed meat, and low fruits and vegetables eating are widespread in East Asia. Tumors located in the proximal stomach (cardia) are responsible for the patient's obesity, and tumors of the gastroesophageal connection are related to reflux and Barrett's esophagus and these conditions are more common in different parts of the world other than Asian countries [8].

A significance difference in the risk of gastric cancer is observed among diverse ethnic groups within a specific geographic location. For instance, in the United States of America, Hispanics, African-Americans, and Native Indians are more vulnerable to gastric cancer than Caucasians. However, these variations cannot be generalized as simple racial differences, because lower socioeconomic status is also connected with elevated gastric cancer risk. During the last five decades, the occurrence rates of gastric cancer have been declined steadily in a number of regions of the globe. It is considered that the use of refrigerated food, the accessibility to fresh fruits and vegetables, and the decrease in the use of salts for preserving food, a decrease in the prevalence of *Helicobacter pylori* infection in many countries, and reduction in smoking in some industrial counties contributed to the decline in risk of gastric cancer [5]. Gastric cancer pathology is varied significantly between East and West. Generally, consideration starts with anatomic localization that guides treatment and outcomes. According to the epidemiologic studies, gastric cancer in the West is generally located in the proximal part of the stomach and appears at a more advanced stage and exhibits worse prognosis than the East [10].

9.2.3 Development of Gastric Cancer

Gastric cancers generally develop very slowly over many years. Precancerous variations often happen in the inner layer called mucosa of the stomach. The early changes do not reveal symptoms and it is very difficult to detect the cancer. Cancers originated in different locations of the stomach may express different symptoms and tend to show various consequences. The location of gastric cancers may also interfere the treatment choices. For instance, gastric cancers that begin at the

gastroesophagus junction are staged and treated the same as cancers of the esophagus. On the other hand, a cancer that generates at the cardia of the stomach but then begins into the gastroesophagus junction is also staged and provide treatment considering a cancer of the esophagus [3].

9.2.4 Types of Gastric Cancer

(a) Adenocarcinoma: Most of the gastric cancers are adenocarcinomas and adenocarcinomas are developed from the stomach mucosal cells.
(b) Lymphoma: Lymphoma are the cancers of the immune system tissues that are sometimes obtained in the gastric wall.
(c) Gastrointestinal stromal tumor (GIST): GIST generates in the wall of the stomach in the very early stage of gastric cancer. Although some of these tumors are benign (noncancerous), but others are detrimental (cancerous).
(d) Carcinoid tumor: These cancers are developed in hormone-producing cells of the stomach and they do not generally spread to the other parts of the body.
(e) Other cancers: Other sorts of cancer, for example, squamous cell carcinoma, small cell carcinoma, and leiomyosarcoma, may also be generated in the gastric, but these cancers are very rare in the world [3].

9.2.5 Risk Factors of Gastric Cancer

Gastric cancer happens as a consequence of many contributory factors or causes and it occurs two times more in males than in females [11]. Environmental and genetic factors play a vital role in the etiology of gastric cancer. Among the environmental risk factors, diet and *H. pylori* infection are most common risk factors of the gastric cancer. Genetic factors also play a significant role in gastric carcinogenesis by either abnormal genes over expression or inappropriate expression of normal genes [12].

(a) *Dietary factor*: Dietary risk factors include sodium-rich food, salts and salty diets, spicy food, pickled vegetables and foodstuff, fried food, meat (red, smoked, processed, and salty), irregular food habits, dairy food, starchy food and sweets, salted and smoked fish fermented with salts, hot food, hot tea, lack of access of safe drinking water, moldy and leftover bread and food, N-nitroso compounds, diet with limited vitamin C (vitamin C deficiency), inadequate intake of fresh fruits and vegetables, rich food, fats and oil, refined grains, and fermented food [13]. Diet and dietary habits are one of the most prominent factors in developing the gastric cancer. Research has shown that long-time preserved meats, fruits, and vegetables increase the risk of gastric cancer. Nitrates and nitrites in cured meats can be transformed to harmful compounds by certain bacteria, including *H. pylori*, and have been identified to cause stomach cancer in animals. Intake of fresh fruits and vegetables, citrus fruits,

and antioxidants is linked with the lower risk of gastric cancer [2, 3, 11, 14, 15]. Consumption of sodium-rich food and salts is connected to the high risk of gastric cancer. Excessive consumption of salt may stimulate gastric mucosa, leading to atrophic gastritis, increased DNA synthesis and cell proliferation, and finally the incidence of gastric cancer. Studies have shown that the risk of gastric cancer is higher in people who consume excessive amount of salt than in the people who have less intake of dietary salts. Excessive dietary intake of red meat, smoked meat, processed meat, and salty meat is also a possible risk factor of gastric cancer. The production of carcinogenic compounds, for example, heterolytic amines, N-nitroso compounds, and polycyclic aromatic hydrocarbons, may result from cooking or due to endogenous reactions [13]. Adverse effects due to a number of dietary causes, especially cured or salted meat and fish, are considered to be connected to the N-nitroso model of gastric carcinogenesis. N-nitroso compounds are shown to be a potent carcinogenic entity made in vivo during the nitration of amides or amine compound in the stomach by nitrites. Nitrites are chiefly derived in the stomach from different food items and water sources [16].

(b) *Helicobacter pylori infection*: *Helicobacter pylori* is a gram-negative bacterium and is responsible for gastric cancer. Infection rate of *H. pylori* tends to be highest in lower socioeconomic environment. The incidence rate of gastric cancer has been decreased in the developed country due to the declining in *H. pylori* infection rates [16, 17]. Current research has demonstrated that the specific sorts of *Helicobacter pylori*, especially the cagA strains, are directly associated with the gastric cancer. Recent study has also found the interaction of the *H. pylori* infection with other possible risk factors. For instance, the researchers have shown that a healthy diet is very important for decreasing the gastric cancer risk for patients infected with *H. pylori* [1–3, 14]. In 1982, *Helicobacter pylori* was identified as a causative factor for ulcer by two Australian scientists—Robin Warren and Barry J. Marshal. In developing countries, the rate of gastric cancer is high among the children aged about 10 years and infected with *H. pylori*. *H. pylori* can survive and multiply in gastric environment, and interfere with the growth of other beneficial bacteria. Epidemiological data show that gastric cancer happens more frequently among the population with a higher rate of infection by *H. pylori*. The World Health Organization (WHO) has categorized the bacteria as Class 1 carcinogen for gastric cancer [11].

(c) *Smoking*: Smoking is one of the causes of gastric cancer and about 11% of all cases globally are due to chronic tobacco use [2]. It has devastating and irreversible effects on the stomach tissues that result in increased risk of malignancy. It elevates the risk of generating gastric cancer significantly among the smokers. Gastric cancers caused by smoking mainly happen in the upper portion of the stomach, next to the esophagus. Cigarette smoking results in the reduction of circulating epithelial growth factor and elevates free radical generation in gastric mucosa [11, 13, 14].

(d) *Industrial chemical exposure*: Professional exposure to dusty and high-temperature atmospheres, for example, wood-processing and food-machine operators, has been related to elevated risk of gastric cancer. Rubber manufacturing, coal mining, metal processing, and chromium production industries have also been related to an increased risk of gastric cancer [2, 14]. Epidemiological studies have shown that occupational exposure of dusts, nitrogen oxides, N-nitroso compounds, and ionizing radiation has increased the risk of gastric cancer. Occupational groups that contribute to the greater risk of gastric cancer are miners and quarryman, farmers, masonry and concrete workers, machine operators, nurses, food industries workers, cooks, launderers, and dry cleaners [16].

(e) *Obesity*: The overweight or obesity increases the risk of gastric cardia cancers. Research has shown that obesity is a physical risk factor that elevates the risk of gastric adenocarcinoma by facilitating the development of gastroesophageal reflux disease (GERD). The exact mechanism of development of GERD due to obesity is not completely known to scientists yet. Studies hypothesized that increased pressure created by dietary fat on the stomach and esophageal sphincter may play a vital role in generating gastric cancer. A relationship has been found between iodine deficiency and gastric cancer [3, 11, 15].

(f) *Alcohol*: Consuming about three or more alcoholic drinks per day also elevates the risk of gastric cancer. Consumption of alcohol is a great factor that contributes to the development of gastric cancer. Chronic alcohol consumption interferes the gastric mucosal barrier by obstructing COX receptor enzymes, which results in reduced production of cytoprotective prostaglandin. Studies have shown that consumption of alcohol by patients with *H. pylori* infection increases the risk of gastric cancer manifolds [2, 3, 11, 14, 15].

(g) *Genetic factors*: Family history of tumor and stomach cancer is one of the risk factors of gastric cancer. A genetic defect of the CDHI gene known as hereditary diffuse gastric cancer (HDGC) has been found as a genetic risk factor of stomach cancer. The main components of the genetic field are mutations and polymorphisms that interfere with the functions of protein. When the gene causes particular mutation, gastric cancer develops by a mechanism that is not completely known to researchers [11, 13].

(h) *Treatments and medical conditions*: A number of medical conditions have been shown to elevate the risk of gastric cancer [16]. Treatments and medical conditions that may contribute to the development of gastric cancer are history of gastrectomy and gastric surgery, history of esophageal cancer, blood type, reflex, personal history of stomach ulcer, and menstrual and reproductive factors. A history of gastrectomy and gastric surgery, even after 30 years of surgery, could elevate the danger of gastric cancer due to the decrease of gastric acid after surgery and increase in its sensitivity to *Helicobacter pylori* [13].

(i) *Demographic factors*: Demographic characteristics include age, economic status and income level, level of education and awareness, sex, race, and residential status [13, 17]. Risk of gastric cancer in indigenous people are greater than that

of nonindigenous inhabitants in a number of countries, and results in occurrence and death up to fivefold than that of nonindigenous inhabitants [18].

(j) *Ionizing radiation*: Ionizing radiation is one of the possible risk factors of gastric cancer. Gamma radiation is very detrimental to and can play a significant role in the development of gastric cancer [13].

(k) *Miscellaneous risk factors*: Diabetes, pernicious anemia, chronic atrophic gastritis, Menetrier's disease, and intestinal metaplasia are also possible risk factors of gastric cancer [11].

9.2.6 Clinical Manifestations of Gastric Cancers

Gastric cancer may often be either asymptomatic or it may reveal nonspecific symptoms. When the cancer has reached its advanced stage and may have metastasized, symptoms are identified. For this reason, the prognosis of gastric cancer is difficult. Initial symptoms of gastric cancer may include the following:

(a) Early stage of cancers may be connected with indigestion or heart burning sensation. However, less than 1 in 50 people suffered from indigestion and was advised for endoscopy had gastric cancer.

(b) Patients may suffer from abdominal distress and loss of appetite, particularly for meat.

(c) Patients who have distended and invaded normal tissue can experience weakness, fatigue, bloating of stomach after meals, nausea and occasional vomiting, diarrhea, constipation, and abdominal pain in the upper abdomen.

(d) Further enlargement of tissue may result in weight loss, weakness or fatigue associated with mild anemia, discomfort in the upper and lower parts of the abdomen, pain or bloating in the stomach after eating, bleeding with vomiting, and blood in the stool [11, 19–21].

9.2.7 Diagnosis of Gastric Cancer

Gastric cancer can be diagnosed by taking the patient's history and gastroscopic examination. Upper gastrointestinal series and computed tomographic (CT) scanning can detect gastric cancer. A biopsy with subsequent histological analysis can confirm the existence of cancer cells. Gastroscopic modalities, for example, optical coherence tomography, are analyzed and monitored for similar and effective applications. Various cutaneous conditions related to the gastric cancer include a darkening hyperplasia of the skin of palms called tripe palms. To identify tumor markers, for instance, CEA (carcinoembryonic antigen) and CA (carbohydrate antigen), blood tests are suggested [11]. Early identification and treatment of gastric cancer contribute to a decline in mortality rates. With the augmented use of endoscopic treatment in early gastric cancer (EGC), the natural background of EGC

must be considered to evaluate the efficacy of endoscopic resection and to advance treatment decision-making. This is especially pertinent for elderly gastric cancer patients [22].

In general, cancer starts when a mutation happens in the DNA of a cell in the body. The cell grows due to the mutation and divides at a rapid rate and continues living when normal body cells would die. The aggregated cancerous cells form a tumor and invade in the neighboring structures. Cancer cells detach from the tumor and disseminate the whole body. Apart from the physical examination, the following tests can be performed to identify gastric cancer [20]:

Biopsy: A biopsy is the process of collecting a small amount of affected tissue for microscopic examination. Then the tissue sample is analyzed in the laboratory by a pathologist to ensure the cancer.

Endoscopy: Endoscopy allows the doctor to observe the inner parts of the body. Inserting a gastroscope or endoscope through the mouth of the patient, the doctor can collect a sample of tissues from the gastric tumor and analyze it for evidence of gastric cancer.

Endoscopic ultrasound: This test method is analogous to an endoscopic test; nevertheless, the gastroscope has a small ultrasound probe on the edge that creates a complete image of the stomach wall. The ultrasound image assists the doctor to estimate how far the cancer has disseminated into the stomach and adjacent lymph nodes, tissues, and organ.

X-ray: X-ray is one of the diagnosis methods to identify gastric cancer. It makes a picture of the structure inside of the body with the help of little amount of radiation.

Computed tomography (CT) scan: A CT scan makes a three-dimensional image of inner part of the body with an X-ray machine. Then, a computer amalgamates these pictures into a complete, cross-sectional view that expresses any aberrations or tumors present inside the body. Sometimes, a contrast medium, a special dye, is administered into the patients to get detailed information.

Magnetic resonance imaging (MRI): Magnetic fields, not X-ray, are used in magnetic resonance imaging technique to provide detailed pictures of the body. A contrast medium, a special dye, can be injected into the patient's vein to get a better-quality image.

Positron emission tomography (PET) scan: It is one of the methods to make an image of organs and tissues inside the body. A small quantity of a radioactive material is injected inside the body of the patient and the radioactive material is absorbed by organs and tissues that utilize maximum energy. A scanner is used to detect the radioactive material and create pictures of the inner parts of the body.

Laparoscopy: It is a minimal invasive surgery and the surgeon inserts a scoop (device) into the abdominal cavity of the patient to assess the spreading of the gastric cancer. It has the potential benefits of less postoperative morbidity and shorter recovery time as well.

Gastric cancer staging: The clinical staging is very important to take therapeutic decision because surgery with curative purpose cannot be prescribed during the

presence of metastatic disease [23]. The clinical staging of gastric cancer consists of:

Stage 0: Confined to the inner layer of the stomach and curable by mucosal resection, gastrectomy, and lymphadenectomy, without the use of chemotherapy or radiotherapy.

Stage I: Cancer cells enter into second or third lining of stomach (stage IA) or into the second layer and nearby lymph nodes (stage IB). Stage IA and stage IB can be treated by surgery and chemotherapy, respectively. Stage IB can also be treated with chemotherapy and radiation therapy.

Stage II: Cancer cells penetrate into the second layer and other distant lymph nodes. Treatment is similar to stage I and sometimes additional neoadjuvant chemotherapy is prescribed.

Stage III: Enter into the third layer and more remote lymph nodes or enter into the fourth lining or more remote lymph nodes. Treatment is like stage II and a cure is still expected for some of the cases.

Stage IV: Cancer cells disseminate to nearby tissues and more remote lymph nodes or metastasize to other parts of the body. A rare cure history of this stage has been found [11] (Table 9.1).

Patients in Asian countries are often diagnosed with gastric cancer at relatively in early phase rather than in non-Asian countries around the globe. The incidences of gastric cancer are higher in Japan and Korea compared with Western countries and the screening for gastric cancer is regular task for medical technicians. Most of the gastric cancers are adenocarcinomas and these are subclassified as per the histological appearances into diffuse and intestinal categories. If a diagnosis of gastric cancer is distrusted, diagnosis should be performed from a gastroscopic biopsy observed by a well-experienced pathologist, and histology should be described as per the World Health Organization (WHO) standard [8] (Table 9.2).

Early staging and risk assessment may comprise physical examination, blood count and differential, liver and renal function tests, endoscopy and contrast-enhanced computed tomography (CT) scan of the thorax, abdomen, and pelvis. Laparoscopy is prescribed for patients with respective gastric cancer, and multidisciplinary treatment plan is required before any treatment [8] (Table 9.3).

9.2.8 Prevention of Gastric Cancers

The sufficient consumption of fresh fruits and vegetables, the escaping of excessive amount of salt intake, and deterrence of exposure to tobacco smoke are the common measures to prevent gastric cancer. Treatment and annihilation of *H. pylori* are advised in patients with gastritis. A number of studies have shown that anti-*H. pylori* treatment helps in lowering the progression of precancerous lesions and the danger of gastric cancer as well. However, large-scale *H. pylori* eradication as a

Table 9.1 TNM staging of gastric cancer according to American Joint Committee on Cancer (AJCC), 7th edition

Primary tumor (T)	Regional lymph nodes (N)	Distant metastasis (M)
TX: Primary tumor cannot be evaluated.	NX: Regional lymph nodes cannot be evaluated	M0: No distant metastasis
T0: No evidence of primary tumor.	N0: No regional lymph node metastasis	M1: Distant metastasis or positive peritoneal cytology
Tis: Carcinoma in situ, intraepithelial tumor without the attack of the lamia propria. T1a: Tumor attacks the lamia propria or the muscularis mucosa. T1b: Tumor attacks the submucosa.	N1: Metastasis in (1–32) regional lymph nodes. N2: Metastasis in (3–6) regional lymph nodes	–
T2: Tumor attacks the muscularis propria. T3: Tumor enters the submucosal connective tissue without invasion of the visceral peritoneum or connected structures.	N3: Metastasis in 7/more regional lymph nodes N3a: Metastasis in (7–15) regional lymph nodes N3b: Metastasis in 16/more regional lymph nodes	–
T4: Tumor attacks the serosa or connected structures. T4a: Tumor attacks the serosa. T4b: Tumor attacks connected structures.	–	–

preventive treatment for gastric cancer has been noticed as the matter of debate due to the risk of the presence of antimicrobial resistant strains [5, 19]. Declined prevalence of *H. pylori* infection and improved diet, for example, diet variety and food preservation, are mainly attributed to the decline in gastric cancer mortality rates worldwide. A diet containing high fruits and vegetables and low in starchy and salty food may have a defensive role against the gastric cancer [4, 24].

Chemo-prevention is the process of using natural or human-made chemicals to reduce the danger of developing cancer. There are a number of chemicals that can be useful in assisting to forestall the gastric cancer.

(a) Antioxidants: The cells of the body forms free radical due to the effect of carcinogenic factors and the free radicals can cause the damage of significant parts of the cells, for example, the genes. The damage cells finally may die or they may turn to cancerous. Antioxidants are a bunch of nutrient and other chemicals that can annihilate free radicals or forestall them from generating. Examples of antioxidants include vitamin C, vitamin E, beta-carotene, and the mineral selenium [3].

Table 9.2 Anatomic stage/prognostic groups according to AJCC, 7th edition [8]

Stage grouping	T stage	N stage	M stage
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
	T1	N1	M0
Stage IIA	T3	N0	M0
	T2	N1	M0
	T1	N2	M0
Stage IIB	T4a	N0	M0
	T3	N1	M0
	T2	N2	M0
	T1	N3	M0
Stage IIIA	T4a	N1	M0
	T3	N2	M0
	T2	N3	M0
Stage IIIB	T4b	N1–0	M0
	T4a	N2	M0
	T3a	N3	M0
Stage IIIC	T4b	N2–3	M0
	T4a	N3	M0
Stage IV	Any T	Any N	M1

Table 9.3 Diagnostic and staging investigations in gastric cancer [8]

Procedure	Purpose
Full blood cell count	To identify iron deficiency anemia
Renal and liver function	To evaluate renal and liver functions and to select the appropriate therapeutic options
Endoscopy and biopsy	To collect tissues for diagnosis, histological classification, and molecular biomarkers, for example, HER2 status
CT thorax + abdomen ± pelvis	To perform staging of tumor, to identify local or remote lymphadenopathy and metastatic disease
Endoscopic ultrasound (EUS)	To assess T and N stage accurately mainly in operable tumors. To estimate the proximal and distal quantity of tumor
Laparoscopy ± washing	To remove occult metastatic disease with peritoneum or diaphragm
Positron emission tomography (PET)	To improve, in some cases, the detection of occult of metastatic disease

(b) Antibiotics: Studies have shown that antibiotic treatment of individuals chronically infected by *H. pylori* will support to prevent the incidence of gastric cancer. Some studies have shown that treating the infection caused by *H. pylori* may forestall the precancerous stomach aberrations; nevertheless, further scientific study is required [3].

(c) Nonsteroidal anti-inflammatory drugs (including aspirin): A number of studies have shown that individuals who take nonsteroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen) may have lesser risk of developing gastric cancer.

Further research is required to show support of using nonsteroidal anti-inflammatory drugs to prevent gastric cancer [3].

9.2.9 Treatment of Gastric Cancer

If the stomach cancer is not identified at early stage, it is very hard to treat. The gastric cancer reaches its peak level when the diagnostic response is found. It can be treated by a number of ways, for example, surgery, chemotherapy, radiotherapy, and immunotherapy [11]. Because of the improvement in surgical method and the decrease in postoperative problems, long-term survival in patients with stomach cancer has found a gradual evolution in recent years [23]. Multidisciplinary treatment plan is very important before starting relevant treatment and the core member of the multidisciplinary team must include surgeons, medical and radiation oncologists, radiologists and pathologists, and other skilled persons as required [8]. The surgical operation is the favorite treatment option for advanced gastric cancer. For some patients with no possibility to have surgical treatment, the final objective of the comprehensive treatment is to extend survival and advance the quality of patient's life [25]. Trastuzumab has been recognized as the first targeted living agent that shows a survival advantages in advanced gastric cancer patients or esophagogastric cancer. Combination of trastuzumab with chemotherapy can be considered as a novel standard treatment of patients with HER2 positive advanced gastric cancer or esophagogastric connection cancer [26].

9.2.9.1 Surgery

Gastric surgery is effective curative remedy for gastric cancer [8]. Complete surgical resection is the potentially curative treatment of gastric cancer [12]. Endoscopic mucosal resection (EMR) is used to treat early cancer. In this methodology, the tumor along with the inner layer of the stomach (mucosa) is detached from the wall of the stomach by an electrical wire loop via the endoscope. The positive side of this method is due to smaller operation rather than removing the stomach. Endoscopic submucosal dissection (ESD) is a method analogous to EMR and used in Japan to resect a large part of mucosa. If the pathological analysis of resected sample evades imperfect resection or deep invasion by tumor, the patient requires a formal stomach resection [11]. Endoscopic resection is very suitable for specific and very early tumors [8]. Surgical treatment of initial gastric cancer is a commonly executed methodology in the eastern countries of the world due to the feasibility of early gastric cancer diagnosis [27]. Palliative gastrectomy has two purposes, one is symptom removal and other is survival advantage. Surgical resection is considered as a prompt strategy to lessen gastric cancer-related symptoms [28].

9.2.9.2 Chemotherapy Drugs and Combinations

A number of chemotherapy drugs have been permitted for the treatment of gastric cancer. Some drugs are used alone and others are used in combination. New types of chemotherapy drugs are also being researched for the treatment of gastric cancer. For instance, S-1 is an oral usage chemotherapy drug associated with 5-FU. New way of administering chemotherapy is to give chemotherapy drug infusion directly into the patient's abdomen. This is called intraperitoneal chemotherapy [3, 11]. Anticancer (cytotoxic) drugs are administered to annihilate cancer cells. The drugs circulate in the bloodstream throughout the body and disrupt the growth of cancer cells. Patients may have chemotherapy before and after the surgery, to diminish or control symptoms in advanced cancer, to slow an advanced cancer down. If the patient has a removable gastric cancer, the chemotherapy can be administered both before and after surgery. This method is called perioperative chemotherapy. A chemotherapy can be administered in the patients as injection, through a drip into the arm, through a pump as a very slow continuous infusion, or as tablets. It helps to reduce the size of the cancer and make easier to remove from the body. Although it reduces the chance of the cancer coming back, but it has side effects and not every patient is fit enough to take this therapy [20]. Chemotherapeutic regimens presently being used for the treatment of gastric cancer comprise anthracycline, fluoropyrimidine, taxane, and platinum-based agents [29]. With the advent of oral fluoropyrimidine, new cytotoxic agent and targeted therapy have led to the development in chemotherapy. A combination of fluoropyrimidine and platinum with or without anthracycline is prescribed as cytotoxic chemotherapy for the treatment of advanced gastric cancer [30].

9.2.9.3 Molecular-Targeted Therapies

During the past few decades, substantial improvements in cancer biology have led to the identification of chief factors responsible for tumorigenesis through a new way. A number of molecular-targeted agents have shown vital antitumor activity in different sorts of tumors, for example, hematologic malignancies, breast cancer, renal cell carcinoma, colorectal cancer, and gastrointestinal stromal tumors. Different sorts of pathways like cell growth, the cell cycle, apoptosis, angiogenesis, and invasion offer molecular targets for gastric cancer treatment. Target therapeutic strategies include epidermal growth factor receptor (EGFR) inhibitors, angiogenesis inhibitors, cell-cycle inhibitors, and matrix metalloproteinase (MMP) inhibitors [25]. When the general chemotherapy does not work well, targeted therapy is used to eradicate gastric cancer. The side effects of targeted therapy are lower than those of general chemotherapy. In case of some gastric cancers, the surface of the cell walls accumulates the HER2 protein responsible for the cancer. Trastuzumab is used to treat gastric cancers that block HER2 protein. EGFR is another type of protein that is accumulated on the cell and creates gastric cancer. Panitumumab is a drug that targets and destroys EGFR protein developed on the cells [3]. It is a multifunctional

receptor transmembrane glycoprotein and one of the members of the tyrosine kinase group of growth factor receptors. Epidermal growth factor (EGF) is the definite ligand of the EGFR and it stimulates the receptor by combining and phosphorylating the tyrosine kinase receptor. Receptor activation results in a number of intracellular transduction pathways and accelerates tumor cell division, migration, and angiogenesis. So, EGFR signal transduction may be targeted and gridlocked to inhibit tumor propagation, invasion, and remote metastasis in the molecular-targeted treatment of stomach cancer. The chief anti-EGFR therapeutic agents are anti-EGFR monoclonal antibodies and EGFR tyrosine kinase inhibitors (EGFR-TKI) [25].

The growth of tumor has a clear vascular dependence and tumors grow new blood vessels to get nutrients from the host that increase the capability of the tumor to metastasize to remote sites. In case of most of the solid tumors, angiogenesis, metastasis, and vascular formation are firmly associated with the vascular epithelial growth factor (VEGF) pathway. To know about this way is very important for the development of drug targeting VEGF, for instance, neutralizing antibodies targeting VEGF or its receptor (VEGFR) and targeted TKIs against VEGFR. Bevacizumab is a recombinant humanized monoclonal antibody that exhibits its action by blocking VEGF and amalgamates with VEGF to inhibit the activation of VEGFR, accelerating the inhibition of tumor angiogenesis. Sunitinib is one kind of kinase inhibitors and it inhibits the VEGFR, Raf, platelet-derived growth factor-beta receptor, fibroblast growth factor receptor, and c-KIT mechanism pathways. Sorafenib is one type of protein inhibitor of Raf and other receptor tyrosine kinase inhibitors in advanced stomach cancer and it could block the growth and angiogenesis of gastric carcinoma xenografts. Abnormal cell-cycle control is intimately connected to the cellular carcinogenesis and the expression and control of cyclin-dependent kinase (CDKs) play a vital role in the cdk cycle advancement. Flavopiridol and its derivatives are small molecule blockers of CDKs and flavopiridol is recently being used to advance its efficacy. It may improve the inhibitory activity of docetaxel on tumor growth [25].

Matrix metalloproteinases (MMPs) exhibit roles in a number of physiological and pathological processes, for example, inflammation, tissue fibrosis, angiogenesis, and tumor invasion and metastasis. They can dissolve the vascular basement membrane and extracellular cells that result in the detaching of endothelial cells from the vascular wall, angiogenesis, tumor growth, invasion, and metastasis. So, blocking MMPs can result in the inhibition of angiogenesis. The incidence, progression, and prognosis of gastric cancer, like most of the solid tumors, rely on crosstalk between multiple complex targets and regulatory signaling ways. Moreover, tumor cells at various stages of diversity present absolute heterogenicity. Therefore, the targeted treatment of a single pathway in sometimes is not enough to forestall the tumor progression [25].

9.2.9.4 Radiotherapy

Radiotherapy can be applied for the treatment of gastric cancer in conjunction with chemotherapy and/or surgery [11]. In recent times, radiation therapy has received great attention for the treatment of gastric cancer. It is used as a palliative remedy and adjuvant to neoadjuvant therapy for stomach cancer. Because of the anatomical and pathological morphology of gastric-specific applications, conventional radiotherapy that uses two-dimensional radiotherapy (2DRT) is strongly prohibited. Furthermore, the tolerance of the regular gastric mucosa and connected liver, small intestines, and other organs of the body, for example, kidney, is low [25]. In radiotherapy, the highly energetic rays or particles are used to destroy cancer cells in a specific part of the body. After surgery of gastric cancer, radiotherapy can be used to destroy a very small remnant amount of cancer that cannot be observed and removed during the surgery. Radiotherapy along with certain chemotherapy drugs may delay or prevent the recurrence or return after surgery and may assist patients to live longer. It can also be used to reduce the symptoms of advanced gastric cancer, for example, pain, bleeding, and dietary complications. The side effects of radiotherapy may include mild skin disorders at the site of radiotherapy, diarrhea, fatigue, nausea and vomiting, and low blood cell count [20].

9.2.9.5 Immunotherapy

Immunotherapy is the new technique of cancer treatment that applies immune tumor vaccines or antitumor antibodies to stimulate the body's own immune system to fight against the cancer. Immune system could be used for the identification of malignant tumors and inhibition of tumor development [25]. Immunotherapy is the process that uses medicines and assist the patient's body's immune system to combat against the cancer. Pembrolizumab was the first approved (year 2017) immunotherapy drug to treat gastric cancer. It is an immune checkpoint inhibitor and targets a protein obtained on some stomach cancer cells called PD-L1 [3].

The advent of new chemotherapy, targeted therapy drugs, and development in tumor molecular biology study will explore the new possibilities for the comprehensive treatment of gastric cancer. Immune cell adaptive therapy, tumor vaccines, monoclonal antibodies, and combined immunoassay point blockers can have wide treatment-related future possibilities. Consequently, novel research and developments will improve the treatment of advanced stomach cancer [25].

9.3 Conclusion

Gastric cancer is one of the multifactorial cancers and a number of factors are responsible for the gastric cancers, for example, diet, *Helicobacter pylori* infection, smoking, industrial chemical exposure, overweight, or obesity. Maintaining healthy weight, being physically active, eating healthy and fresh diet, and limiting alcohol consumption may prevent gastric cancer in general cases. It is suggested that health policy makers must take proper steps to prevent and reduce the occurrence of gastric cancer through promoting community education and awareness.

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Chapter 10
An Intergenic Variant rs4779584 Between SCG5 and GREM1 Contributes to the Increased Risk of Colorectal Cancer: A Meta-Analysis

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Abstract Colorectal cancer (CRC) is very common malignancy all over the world. Adoption of Western diet (red meat and high fat foods) in many countries has increased the incidence of colorectal cancer. There are genetic factors as well as environmental factors contributed to the etiology of CRC. Current meta-analysis is envisioned to investigate the association between rs4779584 variant and risk of CRC. PubMed, Google Scholar, and Embase were used for the collection of publication to retrieve data. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the association between rs4779584 variant and risk of CRC. To determine heterogeneity, Cochrane Q test and I^2 statistic were employed. Subgroup analysis and sensitivity analysis were performed to assess between-study heterogeneity. Publication bias was determined through Funnel plots and Egger's test. Total 14 publications with 26 different studies comprising 25,469 CRC cases and 32,745 controls were finally considered for meta-analysis. Overall, a positive association of rs4779584 polymorphism with CRC risk was found in all genetic models (allelic model: OR = 1.13; 95% CI 1.08–1.18; p = <0.001; I^2: 53%; dominant model: OR = 1.14; 95% CI 1.08–1.21; $p < 0.001$; I^2: 41%; and recessive model: OR = 1.19; 95% CI 1.09–1.30; $p < 0.001$; I^2: 44%). The level of heterogeneity was significant for all ethnic groups. No significant publication bias was found in this meta-analysis. Based on this meta-analysis, it can be confirmed that the rs4779584 polymorphism and CRC risk shares a positive correlation in patients where T allele was a susceptible factor.

Keywords Colorectal cancer · Colorectal carcinoma · rs4779584

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Abbreviation

BMP	Bone morphogenetic protein
CI	Confidence interval
CRC	Colorectal cancer
FEM	Fixed-effects model
FMN 1	Formin 1
GREM1	Gremlin 1
GWAS	Genome-wide association studies
IBD	Inflammatory bowel disease
OR	Odds ratio
REM	Random-effects model
SCG5	Secretogranin V
SNP	Single nucleotide polymorphism
TGF-β	Transforming growth factor β

10.1 Introduction

Colorectal cancer (CRC) is the fourth most common malignancy leading to more than >8% cancer deaths every year [1]. Individuals with Crohn's disease, inflammatory bowel disease (IBD), and ulcerative colitis are much prone to CRC. In many counties, adoption of the Western diet consisting mainly of red meat and high-fat foods has increased the incidence of CRC [2]. The risk of CRC among the first degree-relatives of cases is two-to-three times more common than the general population as CRC tends to accumulate in families. From the literature, it is evident that genetic factors as well as environmental factors played important role in the etiology of CRC [3, 4]. As only 5% of cases with CRC showed presence of high-penetrance germline mutations, genetic factors that contribute to CRC risk are still not known. Further, GWAS identified some low-penetrance SNPs associated with increased CRC risk [4, 5]. However, a recent GWAS identified a hotspot for CRC susceptibility on chromosome 15q13.3 region [6]. Chromosome 15q13.3 contains SCG5, GREM1, and FMN1 genes.

A high-penetrant SNP (rs4779584) that is associated with CRC risk was identified near GREM1 and SCG5 genes [7]. GREM1 encodes for a signaling molecule gremlin 1, which acts in the TGF-β pathway. TGF-β signaling modulates tumor invasion as well as metastasis. Any changes in TGF-beta pathway components triggered the risk of CRC [8]. Further, restoration of TGF-β pathway in CRC cells abrogates proliferation and tumorigenicity [9]. SCG5 encodes a neuroendocrine signaling molecule, secretogranin V that influence cellular proliferation [10]. Several research groups have analyzed the associations between this rs4779584 and the risk of CRC. A thorough review of the available literature showed that the studies related to rs4779584 and CRC risk are inconclusive due to inadequate sample size and other

factors. This motivated us to perform a meta-analysis for a precise characterization of the association between the rs4779584 polymorphism and colorectal cancer [11, 12].

10.2 Materials and Methods

10.2.1 Selection of Data

This meta-analysis was conducted to synthesize evidence of association between colorectal cancer and rs4779584 SNP. To retrieve relevant association between studies, a systematic search was conducted in PubMed, Embase, and Google Scholar databases. Keywords such as "Colorectal cancer", "Colorectal carcinoma," and "rs4779584" were used to retrieve articles published only in English language. For the inclusion of relevant studies in the meta analyses, the following eligibility criteria were adopted: (1) case–control studies assessing the association between rs4779584 and CRC risk, (2) CRC studies with genotypic data included for rs4779584, (3) cases of CRC confirmed histologically or pathologically, and (4) sufficient data to calculate the odds ratio (OR) with its 95% confidence interval (CI) and p value. Studies were excluded if studies had (1) overlapping data, (2) case-only studies, and (3) case–control studies with no rs4779584 genotype data. From all eligible studies, lead author's name, publication year, country of origin, ethnicity, and rs4779584 genotypes from CRC and control groups were extracted.

10.2.2 Statistical Analyses

To determine the association between colorectal cancer and rs4779584 polymorphism, individual study as well as pooled ORs with 95% CIs were calculated in allelic, dominant, and recessive genetic models. To find out the occurrence of heterogeneity between studies, the Q test and I^2 statistics were applied. Based on the magnitude of heterogenicity, random-effects model (REM) or fixed-effects model (FEM) was used in assessing the pooled OR. A sensitivity analyses were employed to test for robustness of the meta-analysis. To do the sensitivity analyses, we excluded one study at a time and determined the pooled OR for the rest of the studies. Funnel plot was used to assess publication bias for comparisons and was reconfirmed by Egger's test. To assess the effect of geographic specific population on the meta-analysis, an ethnicity-based subgroup analysis was performed. The MetaGenyo web tool was used to perform analyses of the data used in the meta-analysis [13].

10.3 Results

10.3.1 Study Characteristics

Search strategy adopted for the meta-analysis is depicted in Fig. 10.1. Two individual authors too retrieved 60 papers through extensive search. By thorough screening, we have identified 40 such papers analyzing the association between colorectal cancer and rs4779584 polymorphism. By adopting stringent inclusion and exclusion criteria, we finally selected 14 papers that contain 26 studies [4, 7, 14–25]. We had to exclude 26 papers that did not have usable data for the meta-analysis. Out of these 26 studies, 5 studies included Asian patients, while 21 studies included Caucasian population. The study by Serrano-Fernandez et al. included Estonia, Latvia, Lithuania, and Polish patients and hence it was considered as four independent studies [14]. The study by Tomlinson et al. included data from 10 sample series (UK2, Scotland2, UK1, VQNBS, EPICOLON, Helsinki, UK4, Scotland1, CCFR, and Australia); hence, it was considered as 10 independent studies [17]. Genotype frequencies of rs4779584 polymorphism of each study were presented in Table 10.1. The genotype frequencies of all studies followed Hardy–Weinberg equilibrium. I^2 values based on Q statistics showed significant between-study heterogenicity and hence we used REM to determine pooled ORs.

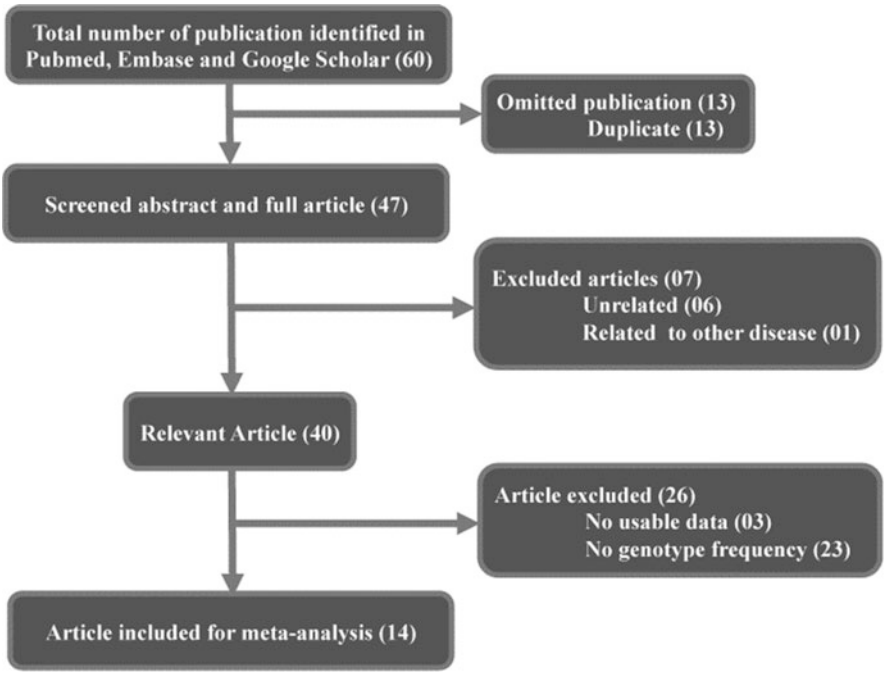

Fig. 10.1 Flowchart of literature selection for the meta-analysis

Table 10.1 The distribution of rs4779584 SNP genotypes in CRC and control subjects

Reference	Country	Ethnicity	CRC			Control			HW *p*-value
			CC	CT	TT	CC	CT	TT	
Jaeger et al. 2008 [7]	UK	Caucasian	426	248	44	637	292	31	0.726
Xiong et al. 2010 [16]	China	Caucasian	128	627	1353	109	682	1333	0.076
Von Holst et al. 2010 [18]	Sweden	Caucasian	1050	572	94	1104	551	89	0.063
Hawken et al. 2010 [25]	Canada	Caucasian	710	388	35	753	332	40	0.650
Tomlinson et al. 2011_1 [17]	UK2	Asian	1762	934	155	1858	857	102	0.796
Tomlinson et al. 2011_2 [17]	Scotland2	Caucasian	1276	603	84	1332	608	76	0.524
Tomlinson et al. 2011_3 [17]	UK1	Caucasian	533	316	52	611	288	30	0.577
Tomlinson et al. 2011_4 [17]	VQNBS	Caucasian	1155	564	81	1601	797	102	0.822
Tomlinson et al. 2011_5 [17]	EPICOLON	Caucasian	878	434	61	934	396	51	0.266
Tomlinson et al. 2011_6 [17]	Helsinki	Caucasian	378	362	88	418	352	69	0.671
Tomlinson et al. 2011_7 [17]	UK4	Caucasian	361	174	33	426	241	27	0.324
Tomlinson et al. 2011_8 [17]	Scotland1	Caucasian	591	331	55	676	286	39	0.209
Tomlinson et al. 2011_9 [17]	CCFR	Caucasian	716	412	58	647	319	32	0.333
Tomlinson et al. 2011_10 [17]	Australia	Caucasian	269	149	22	285	136	17	0.878
Ho et al. 2011 [19]	Japan	Asian	26	191	492	32	232	450	0.763
Talseth-Palmer et al. 2011 [23]	Australia, Poland	Caucasian	161	84	13	188	109	16	0.969

(continued)

Table 10.1 (continued)

Reference	Country	Ethnicity	CRC			Control			HW *p*-value
			CC	CT	TT	CC	CT	TT	
Carvajal-Carmona et al. 2013 [22]	UK	Caucasian	70	528	936	289	2317	4359	0.389
Talseth-Palmer et al. 2013 [24]	Australia, Poland, Dutch	Caucasian	230	110	16	448	232	33	0.673
Hong et al. 2015 [20]	Korea	Asian	4	50	139	7	44	129	0.199
Serrano-Fernandez et al. 2015_1 [14]	Estonia	Caucasian	99	58	9	97	59	10	0.797
Serrano-Fernandez et al. 2015_2 [14]	Latvia	Caucasian	49	29	3	52	22	7	0.054
Serrano-Fernandez et al. 2015_3 [14]	Lithuania	Caucasian	58	53	12	70	44	9	0.570
Serrano-Fernandez et al. 2015_4 [14]	Poland	Caucasian	446	301	48	467	272	56	0.062
Baert-Desurmont et al. 2016 [15]	France	Caucasian	563	402	64	223	115	12	0.545
Hosono et al. 2016 [21]	Japan	Asian	9	147	402	37	311	768	0.426
Abe et al. 2017 [4]	Japan	Asian	25	280	800	53	479	1131	0.793

10.3.2 Association of rs4779584 with CRC

Individual studies as well as pooled analysis of our study ($n = 26$) showed that the rs4779584 has significantly increased the risk of colorectal cancer in all the genetic models as depicted in forest plot (Fig. 10.2). All the values relating the association between colorectal cancer and rs4779584 from allele contrast (OR = 1.13; 95% CI 1.08–1.18; $p < 0.001$; I^2: 53%), dominant model (OR = 1.14; 95% CI 1.08–1.21; $p < 0.001$; I^2: 41%) and recessive model (OR = 1.19; 95% CI 1.09–1.30; $p < 0.001$; I^2: 44%) are presented in Fig. 10.2. Subgroup analysis by ethnicity was performed and it revealed that rs4779584 significantly increased the risk of CRC in both Asian

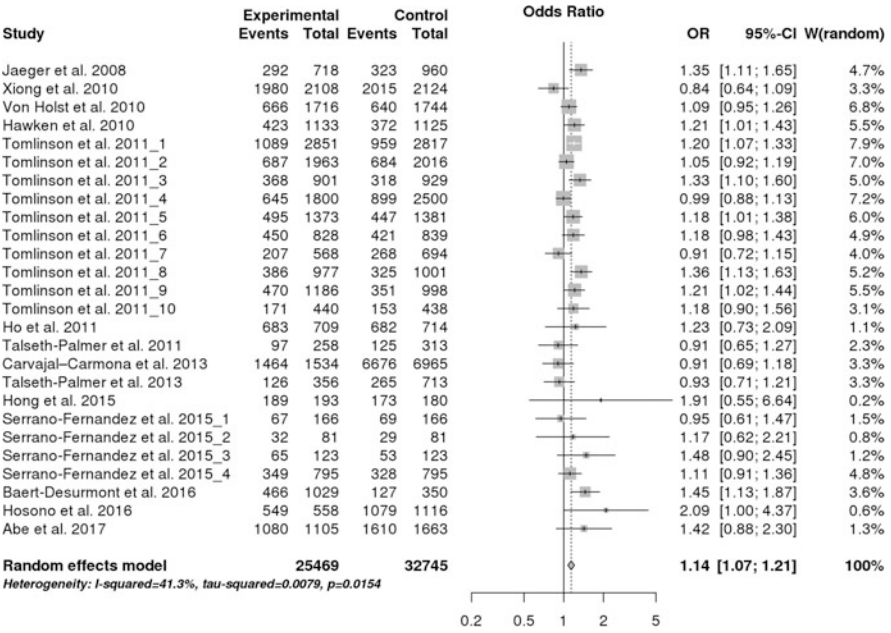

Fig. 10.2 Forest plot from the meta-analysis of CRC and rs4779584 SNP using dominant genetic model

Table 10.2 Association of rs4779584 SNP and colorectal cancer in different genetic comparison models

	CRC vs. control		
		By ethnicity	
rs4779584	Overall	Asian	Caucasian
Number of studies	26	5	21
Allele contrast (T vs. C)			
Heterogeneity I^2 % (p value)	53 (<0.001)	0 (0.972)	56 (<0.001)
Association OR (95% CI)	1.13 (1.08–1.18)	1.21 (1.13–1.29)	1.11 (1.05–1.17)
Publication bias (Egger's p-value)	0.332	0.661	0.216
Recessive model (TT vs. CT + CC)			
Heterogeneity I^2 % (p value)	44 (0.009)	0 (0.435)	41 (0.027)
Association OR (95% CI)	1.19 (1.09–1.30)	1.27 (1.15–1.41)	1.17 (1.05–1.30)
Publication bias (Egger's p-value)	0.086	0.789	0.042
Dominant model (CT + TT vs. CC)			
Heterogeneity I^2 % (p value)	41 (0.015)	0 (0.548)	46 (0.011)
Association OR (95% CI)	1.14 (1.08–1.21)	1.23 (1.11–1.36)	1.12 (1.05–1.20)
Publication bias (Egger's p-value)	0.529	0.074	0.976

and Caucasian population without having a substantial difference between the two populations (Table 10.2).

10.3.3 Sensitivity Analysis and Publication Bias

We performed sensitivity analysis by repeating the pooled analysis by excluding one study each time. Sensitivity analysis revealed that the results remain essentially unchanged (Fig. 10.3). Begs funnel plot revealed no significant asymmetry in the shape (Fig. 10.4). This indicates that there is no publication bias. This was also confirmed by Egger's test (Dominant model, $p = 0.529$).

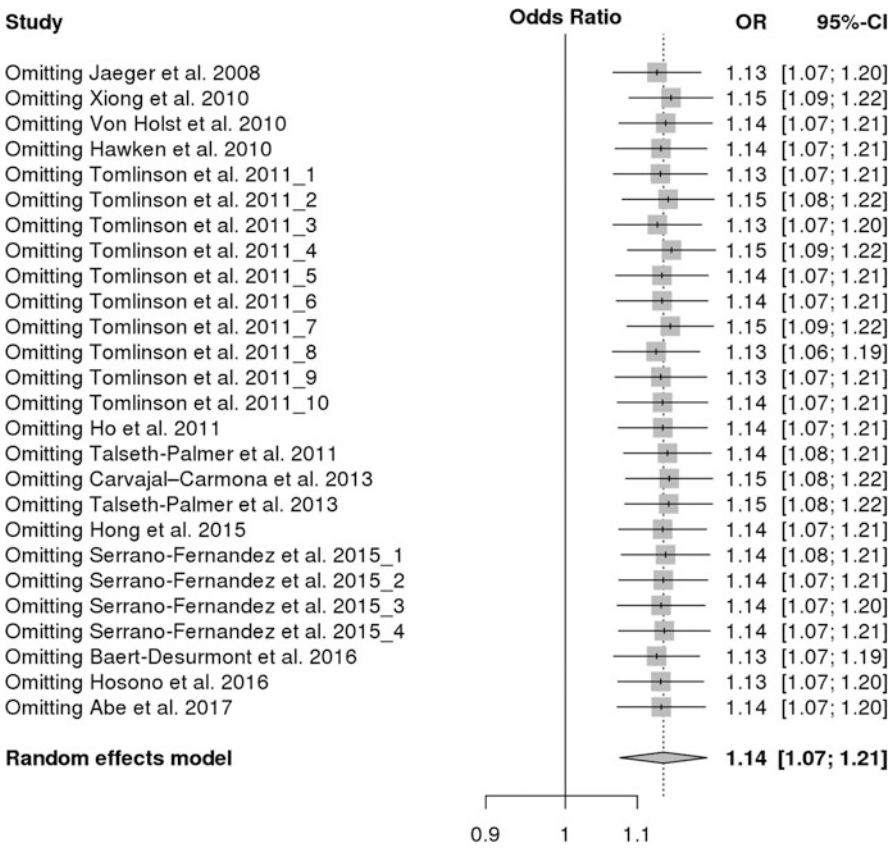

Fig. 10.3 Sensitivity analysis of this meta-analysis

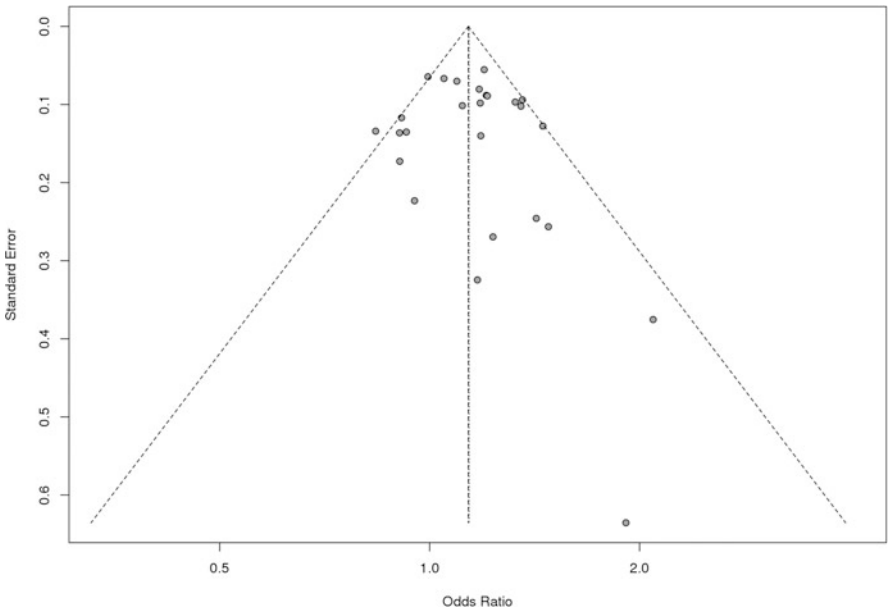

Fig. 10.4 Assessment of publication bias in meta-analysis using a funnel plot

10.4 Discussion

This meta-analysis is conducted based on 26 independent studies that investigate association between rs4779584 polymorphism and risk of CRC. All the three genetic models as well as subgroup analysis between Asian and Caucasian ethnic backgrounds showed significant association between rs4779584 polymorphism and risk of CRC. Significant between-study heterogeneity was observed. No publication bias is one of the highlighted observations of our meta-analysis. Diverse genotyping methods, small sample sizes, and a mixed population of different geographic regions lead to the heterogenicity.

Although linkage studies have identified some genes that are responsible for 2–6% of CRCs, all the susceptible genes are still unknown. Further, association studies have identified some low-penetrance alleles that are responsible for genetic risk for CRC [12, 26]. A number of studies have now confirmed the association between rs4779584 polymorphism and susceptibility to colorectal cancer. Previous studies showed that the SNP rs4779584 located in between GREM1 and SCG5 increased the risk of CRC [7]. In human, GREM1 gene inhibits BMP2, BMP4, and BMP7 proteins through promoter hyper-methylation and plays a major role cellular differentiation [27, 28]. Several lines of evidence reported that the GREM1 is overexpressed in colon tumors compared to surrounding normal tissues [29–31]. The SCG5 is involved in neuroendocrine signaling and alter cellular proliferation [32]. Association of rs4779584 with risk of CRC is controversial. Some studies

showed that the rs4779584 increased the risk of CRC [4, 7, 19–21], while others showed protection against CRC [16, 22, 24]. The current meta-analysis demonstrated that the rs4779584 increased the CRC risk. In consistent with our results, two previous meta-analyses showed evidence of association between rs4779584 and CRC risk [11, 12]. In summary, this meta-analysis revealed that rs4779584 is a major risk for developing CRC. Further research is still needed to investigate the clinical and biological implications of these associations.

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Chapter 11
Phytochemicals Plus Nanomaterial's on Colorectal Cancer

Prameswari Kasa, Gayathri Chalikonda, and Ganji Seeta Rama Raju

Abstract Colorectal cancer (CRC) is the most prevalent cancer around the world, and advancements in therapy continue to reduce illness and death. CRC examination and therapy with various nanotechnology-based methods have led to promising results, under scientific panel to establish novel therapeutic strategies. Today, the use of nanoparticles as a drug delivery has become the most auspicious form of cancer therapy. Many studies have revealed a keen benefit of phytochemicals and nanoparticles combined therapy for various cancers. In this chapter, we ensure a deeper discussion regarding the latest advancement of board nanoparticle drug-based phytochemical fabrication against colorectal cancer detection and treatment.

Keywords Phytochemicals · Nanotechnology · Colorectal cancer · Detection · Targets

Abbreviations

Ag	Silver
Al_2O_3	Aluminum oxide
Au	Gold
Bcl-2	B-cell lymphoma 2
CaCO3	$CaCO_3$
CAPE	Caffeic acid phenethyl ester

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CD-31	Cluster of differentiation 31
COX-2	cyclooxygenase-2
CRC	Colorectal cancer
CuO	Copper oxide
CURCS	Curcumin loaded chitosan
DHA	Docosahexaenoic acid
DOX	Doxorubicin
EGCG	Epigallocatechin gallate
EGFR	Epidermal growth factor receptor
FAP	Familial adenomatous polyposis
Fe_2O_3	Iron oxide
HNPCC	Hereditary non-polyposis colon cancer
HPβCD	Hydroxypropyl β-cyclodextrin
IC50	Inhibitory concentration
IL-8	Interleukin-8
IPR	Increased permeability and retaining
LCL	Long circulating liposome
LNA	Linolenic acid
MCF-7	Michigan Cancer Foundation-7
MPS	Mononuclear phagocytic system
NCI	National Cancer Institute
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NIH	National Institutes of Health
NIR	Near-infrared radiation
nm	Nanometer
NPs	Nanoparticles
PEG	Polyoxyethylene glycol
PLGA	Poly (lactic-co-glycolic acid)
RES	Reticuloendothelial system
RNAs	Ribonucleic acids
STAT3	Signal transducer and activator of transcription 3
TiO_2	Titanium dioxide
VEGF	Vascular endothelial growth factor
WHO	World health organization
ZnO	Zinc Oxide
μm	Micrometer

11.1 Introduction

Cancer, which is classified among the main causes of death, grows unrestricted and often spreads to distant organ systems. There are more than 100 types of cancers; each classified the cell type affected. Per WHO data, epidemiologists expect a rise of 8 million in new cancer cases in the upcoming decades [1, 2]. South and Central

America, Asian, and African countries report 70% of malignance expiries and 60% of yearly new cases of total cancers worldwide [3]. While many different therapeutic applications are available, chemotherapy with cytotoxic medicinal products is the most frequently employed therapy to monitor various types of cancers [4]. These treatments are connected with serious side effects and multidrug resistance [5–7]. With reference to these unwanted side effects, the NCI, USA, promotes the study of the potential antitumor activities of plant compounds [8, 9].

Throughout history, plants are the best source for the natural compounds used as medicinal products. Although these chemical products were improved by pharmacist, the increasing demand for herbal products is a result of the adverse effects produced by chemical products. Today, herbal medicines are improving and approximately 25% of the pharma products and its derivatives are available and procured from natural sources [10, 11]. Herbal products with various molecular backgrounds introduce a core for finding novelty in several drugs. At present, the trend in finding the herbal product-based drugs is the key point in making synthetically flexible lead molecules that mime their counterparts chemically [12]. Natural compounds reveal remarkable traits like extraordinary chemical divergence, and biochemical characteristics with macromolecular peculiarity, and lesser toxicity. Therefore, this makes research and the discovery of new drugs favorable [13]. Further computerized studies have allowed consideration of molecular interactions in drug development for the next-generation drug inventors like target-based and delivery-based drug discovery.

Though several advantages in pharmacy companies are unsure to spend more in herbal product derived drug systems and rather study the availability of chemical compound library to identify the new drugs. Herbal compounds can treat various major diseases, particularly cancer, inflammatory, diabetes, microbial diseases, and cardiovascular. In addition, herbal drugs possess extraordinary advantages like less toxicity, low side effects, low costs, and excellent therapeutic ability. Nevertheless, issues related to the biocompatibility and low toxicity of biological compounds bolster the case against herbal medicine. As a result, many herbal products are not cleared the clinical trials phase [14] as their large particle size required for delivery of drug which poses major difficulties, including in vivo volatility, inferior bioavailability, week solubility, inadequate absorption, difficulties in target particular delivery, tonic efficiency and liable damaging properties of drugs. Hence, using novel drug delivery schemes for against drugs to a particular body parts might be an alternative that can solve these crucial issues [15]. Nanotechnology has helped to improve the modern medicine's drug preparations, targeting stratagies, and monitoring of drug release and delivery.

11.2 Nanoparticles and Their Characteristics

Nanotechnology is a novel field in medicine predicted to bridge the barrier between biology and physics by implementing nanostructures and nanocarriers at different divisions of science particularly in medicine and nanomaterial-based drug deliveries [16]. Nanoparticle (NP) size ranges from 1 to 100 nm and contains materials configured at the molecular level or the atomic level; they are commonly small sized nanospheres [17]. NPs enhance the solubility and solidity of a medication, allowing for precise release, site directed delivery, and reduced malignancy [18]. Thereby, these particles might transfer more easily in the men/women body in comparison to bigger particles. Nanoscale-sized materials expose unique characteristics like structural, biological, electrical, mechanical, and chemical. Nanomedicines can be used as delivery agents for drugs released in a controlled manner to target sites [15, 19]. Nanotechnology is an arising field that can carry out the use of awareness and techniques of nanomedicine and also in biological medicine for the prevention of disease and rehabilitation. Nano-dimensional materials containing nanosensors and nanorobots are useful for delivery, diagnosing purposes, and activates materials in cells [20]. Additionally, nanomaterial's reportedly aid in avoiding drugs from being damaged in gastrointestinal region and assistance the delivery of poorly water-soluble drugs to different target locations. NPs show increased oral bioaccumulation as they reveal typical intake mechanisms of assimilative endocytosis.

Nanomaterial's stay inside the bloodstream for a longer period and provide the production of combined drugs according to the prescribed dose. In this way, they reduce the number of plasma variations with less adverse effects [21]. Because of their size, these particles permeate in to the cancerous tissues and enable easy absorption of the drugs, which allows an effective drug distribution and target at the specific site. The uptake of nanomaterials by the cells is considerably greater than 10 μm [13]. Thereby, they interact directly and treat the cancer cells with enhanced effectiveness and have fewer side effects. Therefore, in this study we focused on phytochemicals and their effect with nanomaterial's on gastrointestinal cancers in particularly on colorectal cancer (CRC).

11.3 Background of Colorectal Cancer

CRC is the third most common malignancy around the world, following breast and lung malignancy; also, it is the second most popular cause for malignance death [1]. CRC is responsible for 8.5% of recently identified malignance cases, and 8.5% of malignance deaths in 2020. The 5-year chances of survival ratio of a CRC patient following diagnosed is 65% [1]. Standard medical treatments for cancer are chemotherapy, radiotherapy, and surgery. Chemoradiotherapy is associated with significant toxicity, which impacts patient's quality of life. The notable risk factors for CRC are

genetic factors and age. CRC risk increases after the age of 40 years, as over 90% of colon cases are identified in patients above 50 years age groups. Personal history of colon cancer or organ polyps is responsible for 20%. Further, inborn genetic mutations like FAP and HNPCC are responsible for around 5–10% of CRC. The cancer is most commonly found in the colon, although it may be located in the rectum as well. Various polyp sites in CRC require several clinical and biologic presentations, prognostics, and response to therapy [22]. Adenoids polyps have the potential to transform into malignant tissues, and are therefore responsible for 96% of colon cancer [22]. With genetic mutations and changes, the polyp remains to attack adjacent tissues and extend into the intestine wall. The tumor is then vascularized and extends to detached metastatic sites over the blood stream and lymph [22]. As a result, screening and earlier identification of pre-malignance polyps are essential to avoid the growth of polyp to malignancy and decreases the frequency CRC. The ACS suggests that people with medium chance of getting CRC go through screening at the age of 50. Patients with higher chance of getting CRC may start diagnosis even earlier. CRC preliminary examination includes pictorial screening and stool tests, where results are followed with a colonoscopy [22].

11.4 Sources of Phytochemicals and Applications in Cancer

The intended system with plant compounds impact on human malignances are numerous and complex. The different phases of malignances possibly restrained and several in vitro or else *animal model* systems utilized to design these preventive properties in in vitro studies. Hence, describing the potential chemical constituents there from plant compounds and growth of effective in vitro or animal survey preceding clinical studies is essential. Phytochemicals, as a result of their dietary source are expected safety and much better allowed with comparatively lower toxicity.

From the past several decades, many of the plant extracts and their lively constituents were reports possess feasible uses as anti-malignancy agents. Polyphenols, namely phenolic acids, alkaloids, flavonoids, and terpenes, have the biological ability of medicinal plants [23–25]. Triterpenoids were reported to strain cytotoxic effects [26]. Likewise, flavonoids were stated to exhibit anticancer properties [25]. In addition, various alkaloids were reported to have anticancer activity [27]. Scientists have proved the feasible mode of action of plants which are used for medical purposes and their vigorous compounds, should be apply this mode of action individual or in combined form with other components exist in the medicinal plants. One method of decreasing damage triggered by illness is antioxidation [28]. Liu stated the several potential efforts of phytochemicals in different cancers [16]. Further study elaborated the biological effectiveness, particularly flavonoids potential activity to fight against cancer [29]. Various phytochemicals exist in different plants may stimulate cytotoxicity against several types of cancer cells.

Few of the plant sources and their chemical structures with anticancer properties are illustrated in Table 11.1.

11.5 Phytochemicals Derived Edible Nanoparticles

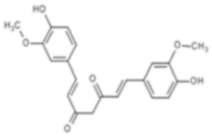
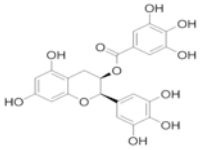
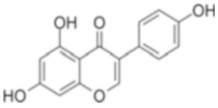
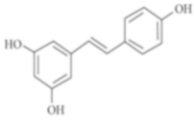
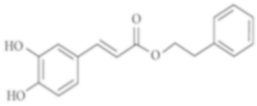
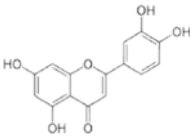
Earlier reports recommended that edible nanosized compounds from plant sources may function like exosomes to carryout anti-inflammatory characteristics, cause inter-specific transmission, and work against cancers [30, 31]. While exosomes were originated from human cells, plant derived nanomaterials exhibit an economic benefits to increase mass production [32]. Notably, bio-compatible and bio-safety are the huge obstacles between research laboratories and hospitals in nanomedicine. Therefore, plant derived NPs have a competitive advantage over traditional medicines; herbal medicine have high standards of lipids as well as fewer proteins and RNAs, which make them safer alternatives [33].

A major obstacle to malignance therapy is the development of resistance to chemo and radiation therapy. To address this, scientists are investigating further treatments with phytochemicals such as curcumin, genistein, resveratrol and lycopene. Use of these has shown reduction of side effects [34, 35]. However, some drawback to phytochemicals are weak absorption, reduced aqueous solubility and fast metabolism. Thus, the development of phytomedicines with NPs can enhance drug transport with lesser side effects.

11.5.1 Curcumin from Turmeric

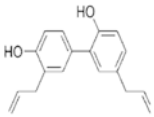
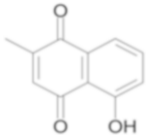
Curcumin has proven active chemo-prevention and anticancer properties [36]. It damages the growth of several cancers, including CRC, by suppressing signaling pathways involving EGFR, STAT3, and NF-κB which participates in tumor cell progression and spread [37]. The curcumin delivery and water solubility can be improved by nanoformulation. Nanoformulation is done with liposomes, lipids gold-nanogels, polymers, cyclodextrin, and micelles [38]. Nanoformulation on liposomes increases the incorporation of curcumin from endocytic action, while free curcumin scatters across the plasma membrane [39]. Several researchers have reviewed the potentiality of liposome preparations of curcumin to minimize CRC growth [40]. For example, curcumin embedded in liposomes displayed a more effective growth inhibition and tumor regression with a minor IC50 value against oxaliplatin in CRC. Curcumin-liposome treatment resulted in reduction of angiogenesis related factors, including VEGF, CD-31 and IL-8. As a result, this combination promoted apoptosis and reduced angiogenesis. Another investigation suggest that Lovo cells has revealed synergism between liposomal-coated curcumin and oxaliplatin [41]. Additionally, Sesarman et al. proved that, the combination of LCL-DOX-curcumin controls tumor growth of CRC cells by inhibiting NF-κB activation

Table 11.1 Phytochemicals with chemical structures and their sources

Plant compounds	Phytochemicals
 Turmeric	 Curcumin
 Green tea	 EGCG
 Soybean	 Genistein
 Grapes	 Resveratrol
 Tomatoes	 Lycopene
 Honey	 CAPE
 Celery	 Luteolin

(continued)

Table 11.1 (continued)

Plant compounds	Phytochemicals
 Magnolia	 Honokiol
 Pumblago	 Plumbagin

[39]. However, several cell lines countered differentially to the nanoformulations, signifying that additional clinical lessons are required to measure these NPs.

Chuah et al. [42] prepared a curcumin filled chitosan NPs (CURCS-NPs) with mucoadhesion to enhance curcumin aggregation in CRC tissues. Further, this formulation increased the G2/M cycle arrest and causes cell death in cells of CRC. The polymeric NPs merged with ligands that enhance curcumin transport to the tumor site. The nano-formulated PLGA-lecithin-PEG-curcumin was combined to aptamer, which effectively connects epithelial adhesion particles on the surface of cancer cell. This formulation increases the anti-proliferative activities of curcumin in CRC cells [43]. Curcumin and 5-FU loaded with chitosan NPs improves the medication's antitumor activities down-regulated COX-2 expression in colon cancer cells [44]. In addition, Xiao et al. [45] established functionally PLGA chitosan filled with curcumin and camptothecin, down-regulated Bcl-2 expression and subsequently increased apoptosis. Further, the curcumin-filled polymeric NPs were displayed increased tumor regression in mice [46]. Xie et al. [47] evolved a native silk fibroin polymer as a substitution for synthetic polymers to conquer biocompatibility problems; in this research, the native polymer revealed enhanced inhibition of CRC cells when compared with 5-FU and free curcumin, with lesser toxic properties on mucosal epithelial healthy colon cells.

Nanogel nano-formulations are developed from properties of gelatin polymers and acrylamidoglycolic acid to create inter penetration polymeric nanogels, which can directly coat hydrophobic curcumin. The benefits of this combination are its pH susceptibility and ability to curcumin deliver at pH 7.4 instead of 1.2. Therefore, nanogels formulation with curcumin results in excellent growth inhibitory property on CRC cells [48]. These nanogels revealed high antitumor potential, higher water solubility, and increased curcumin bioavailability. These unique properties of nanogels consequently shelter curcumin degradation.

AuNPs helps for the production of NPs (having pH-sensitive, heat-responsive) that are sensitive to radiofrequency. CT26 xenograft mice were used to measure the positioning of AU-CRC-TRC-NPs; the formulated AuNPs embedded with curcumin in vivo demonstrated higher retaining capacity and improved drug release. For 6 h following administration, therapeutic levels of curcumin were maintained [49]. Therefore, this method could constitute a new therapeutic scheme for CRC treatment.

11.5.2 Genistein from Soybeans

Genistein is an isoflavone found in plants like soybeans, psoralea lupine, fava beans, *Flemingia vestita*, coffee, and kudzu. It functions as an antioxidative, antiproliferative, and deworming agent. Genistein has been found to have antiangiogenic properties, which thereby limits cell growth and cell division [50]. Nevertheless, the usage of genistein is difficult due to its natural restrictions: lower water solubility, lower bioavailability, higher thermo-instability and less pH. Pool et al. [51] proposed that by developing a hybrid nanomaterial to enclose the genistein compound into PEGylated silica NPs to enhance its water solubility anti-proliferative property. This formulation improved hydrogen peroxide (H_2O_2) production and up-regulated antioxidative enzyme. On the other hand, free genistein did not produce similar results. Moreover, genistein increases the pro-apoptotic anticancer effects, including the cytochrome-c mitochondrial pathway [52]. Further, indirect proofs imply that genistein exhibited its impact on mitochondria of malignant cells by destroying them and promoting caspase-9 in upper levels. Also, several cancers, including pancreatic, prostate, and breast, are associated with the inability to activate NF-κB [53]. The special physicochemical properties of NPs, including its size, charge, and surface chemistry, play an important role in the absorption of medicine by the epithelial cells [54–56]. Therefore, knowing the biological communications are much crucial for the development of nano drug delivery approaches. Bannunah et al. [57] resolved the cell absorption and drug translocation challenges with a $CaCO_2$ mono-layer typical of duodenal epithelial cells with therapeutic suppressors. Their studies revealed that genstein is a tyrosine kinase inhibitor, and it does not impact positively charged NPs, yet it blocks the caveolae (clathrin)-dependent endocytosis as well cell uptake and the interchange of negatively charged NPs. Another research report indicated that genistein also triggered the inhibition about (50%) on the intake of positively charged NPs in HeLa cells [58]. In addition, Del Gaudio et al. with a creative ideology developed NPs integrated with soybean dry and the nano spray-drying method [59]. They also prepared soy product with carboxymethyl cellulose to enhance the stability of soy extract, thereby increasing the water solubility and penetration using the membrane also electrostatically charged. Results revealed fourfold higher permeation, as compared to intact soy extract. This formulated soy isoflavone carboxymethyl cellulose NP powder could be handled as a constituent in food supplements and administered as oral

neutraceutical. Therefore, the genistein-NPs formulation allow for non-invasive therapeutic drug delivery probably passing them over the mucosal barriers [60].

11.5.3 Gingerol from Gingers

Zingiber officinale Roscoe (Ginger) is the perfect natural root to obtain edible NPs. According to Zhang et al., nanoparticles were isolated from the root of ginger with ample 6-gingerol and 6-shogaol execute best constancy, tissue discrimination, anti-inflammatory impact, and possibility of cancer therapy in mice [61]. The administration of NPs orally may travel via the circulatory system to other organs, instead of merely binding to the cells of intestinal tract. It should also be pointed out that reaping of esculent NPs in high efficiency and attribute is difficult.

11.6 Nanomaterial's in Treatment of Colorectal Cancer

The research field of nanotechnology retains the possibility to convert cancer diagnosed methods and curative technologies. Progress in materials research and bioengineering has led to new nanoscale identifying approaches and increase of safety and therapeutic efficiency in cancer patients. Nano-based technology involves the structures, valuation, pattern, equipment, output, and methods at the nanometer scale. The problems associated with current cancer treatments include positioning of the treatment to the tumor sites, drug immunity by tumors, and small drug dissemination times. Apart from this, cancer medication toxicity causes major complexities, in particular cardiac problems and reduced white-blood cell number. There are a number of methods for release of nanomaterial's in to tumor cells, such as liposome vesicles mediated drug administration, eco-friendly, and biologically compatible copolymer nanocarriers delivery, and drug administration of dendrimers [24, 62].

11.7 Nanotechnology Based Drug Delivery Systems

Recently, there has been significant progress in the field of drug delivery systems to ensure therapeutic agents or herbal based compounds to its target site for the treatment of various factors [63]. Although there are a several drug delivery systems favorably used in recent times, there are still specific challenges that must be addressed and an modern technology need to be created for successful drug delivery to its target location. Therefore, today's nanotechnology-based drug delivering systems will promote the modern drug delivery systems.

NPs drug delivery have been heavily studied in recent years. Several solid tumors, including breast cancer, lung cancer, prostate cancer, and colon cancer, have special structural characteristics containing the hyper permeable vascularity and damaged lymphatic drainage. Thus, tumor tissues are penetrable to macromolecules and nanocarriers [64, 65]. The cell-specific targeting has two major mechanisms for nanocarriers: first one is active and the second one is passive. The first method depends on the inter-linkages among the nanocarriers and receptors on the target cell. The second method implies mechanisms enhance the vascular permeability as well as to maintain long-circulating nanocarriers at cancer sites in the flow to damaged lymphatic system [66]. The increased permeability and retaining (EPR) effect, NP approval by the mononuclear phagocytic system (MPS), and unique NP characteristics for cancer bids are all important factors in NP-based drug delivery systems. The EPR effect has a crucial role in identifying the effectiveness of the NP-based drug delivery system [67]. One common issue regarding NPs efficacy, is the MPS—alternatively the reticuloendothelial system (RES)—which is responsible for clearing macromolecules from circulation [68]. One of the main methods of avoiding rapid RES uptake is covering of the particles with detergents or covalent linkage of polyoxyethylene [69]. There are several methods for transferring standard therapeutics to solid tumors; size less than 200 nm, spherical shape, and smooth texture [70].

11.8 Particles Used in Drug Delivery System

Nanotechnology is the best-known field for developing new biological applications in medical stream. Only a fewer nanotechnology-based outputs that are at present employed in cancer applications. Yet, scientists have primarily concentrated on metallic nanocarriers due to their faster actions. Metallic nanocarriers have extraordinary physical and also chemical characteristics in accordance with the quantum size, resulting in broad spectrum of exciting biomedical applications. Gold, copper oxide, silver, iron oxide, aluminum oxide, and zinc oxide are often used. Gold and silver nanocarriers are the most significant, susceptible, authentic, and eminent ions using in green synthesis with different phytochemicals from various medicinal plants and their constituent elements (Fig. 11.1).

The common methods of producing NPs usually involve strong chemical reducing mediators, which are expensive, complicated, and create toxic byproducts [71]. Thus, in-expensive and eco-friendly alternative approaches can directly reduce the risk of polluting the environment. We aim to review several NPs available for anti-CRC therapies and consider their recent simulated techniques and treatment.

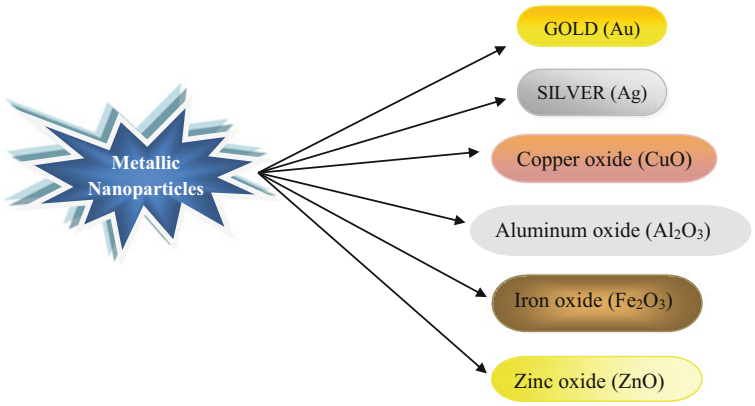


Fig. 11.1 Metallic oxide NPs

11.8.1 Gold NPs

Although gold (Au) has been used in disease treatment in ancient India and China, nanotechnology has introduced a new method for Au therapies. Gold nanoparticles (AuNPs) have lower cytotoxicity, higher surface area quantity and consistency, which indicates their use in chemo as well as immunotherapy in treatment of cancer [72]. AuNPs have also come into focus as an perfect imaging agent in CT and X-ray and appeared as a radio-sensitizer for cancer early identification, symptoms, and remedy [73, 74]. Additionally, various studies have suggested that AuNP has antitumor cell properties [75]. AuNPs have captured the focus of researchers for use as a drug carrier, and as a result facilitate the use of green chemistry. Previously, AuNPs were synthesized with the use of solvents, which often have a negative impact on surroundings and men health [76]. Currently, AuNPs synthesized with active derivates from extracts of plants are more often declared non-toxic to cells [77]. In addition, synthesis is simple and eco-friendly [78]. AuNPs also reveal special characteristics, namely Surface Plasmon Resonance (SPR) and the potential to bind to a thiol and amine group. The preclinical cytotoxic impacts of AuNPs were briefed in several investigations: AuNPs show anticancer features under oxidative stress [79]. A recent research stated that AuNPs produced from *A. leptopus* shows improved anticancer potential [80]. Other research revealed the cytotoxic efficiency of *Cassia tora* over CRC cells. The study evaluated the activity of *C. tora* at different doses (25, 50, and 75 μg/mL), and observed that higher dose demonstrated increased apoptosis [81]. Additionally, *Moringa oleifera* flowers aqueous extract synthesized using AuNPs displayed anticancer activity against lung cancer [82].

11.8.2 Silver NPs (AgNPs)

Similar to AuNPs, sliver nanoparticles (AgNPs) also show antibacterial, anticancer and antimicrobial effect [83]. In contrast to Au, Ag is more accessible for the treatment of cancer. Researchers are enthusiastic about green synthesis, as plant extracts both minimize and stabilize agents [84]. Green synthesis of AgNPs utilizing phytochemicals has several benefits, including cost efficiency, environmental friendliness, and biocompatibility [85]. AgNPs without phytochemicals were demonstrate excellent anticancer activities through green synthesis. Although, the phytochemicals role in AgNPs cannot not be ignored, some clues indicate the bioactive compound could bind to the byproduct AgNPs [86]. Although the anticancer characteristics of AgNPs have been demonstrated the composition of AgNPs controls the stability, toxicity, and the interaction [87].

11.8.3 Iron Oxide NPs (Fe_2O_3NPs)

Iron oxide NPs (Fe_2O_3NPs) stimulate antitumor activity direct and indirect via nontoxic frequency of radiation swinging magnetic fields and is immediately immersed by toxic incentive of ROS [88]. The microparticle of $\mathbf{Fe_2O_3}$ allows them to attach covalently to the cancer site [89]. Further, $\mathbf{Fe_2O_3}$ can convert radiant energy into ROS, which decreases damage to normal cells. Nanomaterials obtain energy from exterior sources, like NIR and magnetic fields, which can directly kill the cancer cells [90].

11.8.4 Other Metallic NPs

Other metals such as copper, aluminum, platinum, and zinc can also be incorporated into phytochemical-inert metal NPs [91]. Nevertheless, the nanotoxicity of metallic NPs must be noted. The application of metallic NPs needs careful in vivo testing, as Au and Ag toxicity is associated with tissue accumulation over prolonged use.

11.9 Disputes for Phytochemicals in Cancer Treatment and Novel Alternates

Phytochemicals demonstrate success in in-vitro and animal studies, occurred small to lack of progress within passage of phytochemicals named as front-line treatment. The constraints of in vitro trail models are stringent, as straight disclosure of cancer cell lines throughout in vitro test can cause a severe display of phytochemicals,

promoting major anticancer and anti-proliferative action, which is generally not accomplished with their physiological state. While these preclinical studies offer substantial understandings into the signaling pathways, they do not shed light on the impact of test agent over this organism. Though the evidence is encouraging, constraints of involving human research without significant laboratory data cause research to depend on such in vitro forms as the initial step. Hence, the urgency for preclinical forms can directly emulate systemic display to phytomedicines, with resulting metabolic and pharmacokinetic modifications. The main challenge is usage rate [92–94]. Since most of these phytomedicines are portion of regular human food, they are efficient metabolism and cleared toxins from the body. As a result, phytochemicals are not preserved in homeostasis, and the remedial consequences are usually short term [94]. Also, another problem regarding the use of phytochemicals in cancer treatment is its failure to provide target particularities. Since it is used with few restrictions, the multi-focused consequences of phytomedicines—the "pleiotropic" effect—emphasize the very core of these antimalignant agents [95, 96]. Cancer cells usually activate substitute pathways that lead to the inability to deliver targeted therapy. Beneath such conditions, a multi-focused anti-cancer agent is probably as compared more efficient by virtue of being potential to identify the initiation of substitute endurance paths. While the problem of utilization rate cannot be solved simply by rising the rate of management, there are specific proxy ways to evade this problem. These comprise of three strategies: (a) syntheses of fresh analog of phytomedicines to enhance the effectiveness and utilization rate, (b) new preparations to selective and better phytomedicine deliver to their designed targeting areas, and (c) development of new delivery vehicles that modify the kinetics of the therapeutic drug. Here we explain how the phytochemicals loaded with the nanocarriers are delivered o the target cancerous cell (Fig. 11.2).

11.10 Future Perspectives of Nanotechnology Advances of Phytochemicals

At present, clinical research aims to improve the efficiency of nanosized phytochemicals in biological systems over 20 NPs therapeutic agents accessible for several clinical applications. Abraxane (Albumin-bound paclitaxel) and DaunoXome (liposomal daunorubicin) are the two best examples of succeed fabrications of natural products preparations established on nanotechnological methods [97]. The progress of nanotechnology may offer a solution to constraints adjacent to lots of phytochemicals' chemical and pharmacokinetics parameters. With the applications of proper nano-range carriers, phytochemicals can be released in a slow and stable manner [97]. Sozer and Kokini [98] reported another example of active transport of nutrients, quick controlled methods of biochemical contaminants, protein bioseparation, and nano-packaging of healthy meal, DNA microarrays, and micro-fluidics. In addition, the series of NPs and phytochemicals show improvement in cosmetics field. As an

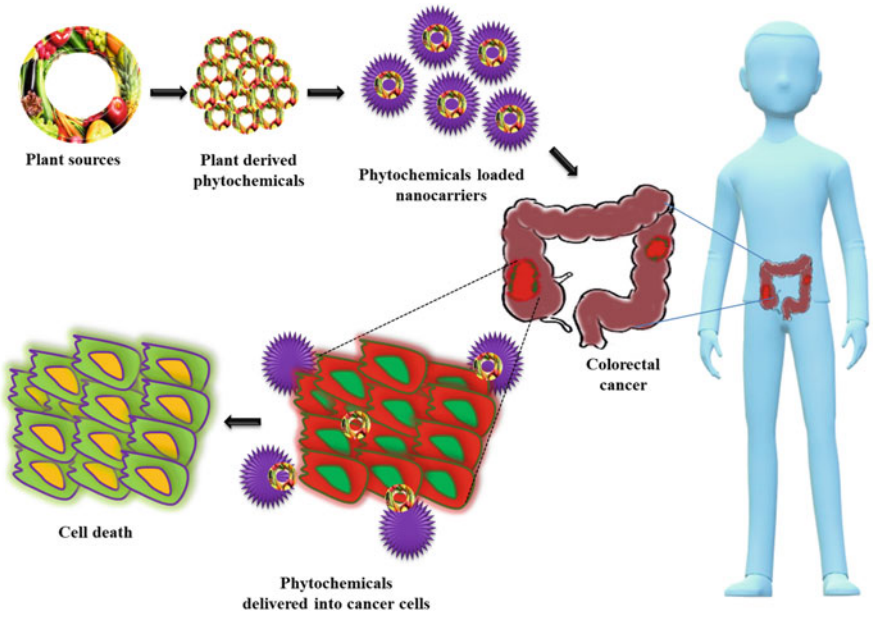


Fig. 11.2 Break the barrier of colorectal cancer cell wall with NP charged with phytochemicals

example, the integration of Zinc Oxide (ZnO) and titanium dioxide (TiO_2) nanocarriers help shield phytochemicals from sun damage. *Aloe vera* extracts with liposome based products of lesser than that of 200 nm diameter were approved to allow increased proliferation and result in improved collagenase in-vitro utilizing epidermal keratinocytes and fibroblast [99]. The correlation of phytochemicals and NP technology elevates the outlook on food industries, including simplified food-graded lipids, numerous emulsifications, and solid-lipid nanomaterials.

Nanoemulsion-established transfer systems enhance the biological effectiveness of various phytochemicals and their oral bioaccumulation. Similarly, polymer micelle is able to increase water dispersity of numerous crystalline phytochemicals containing β-carotene and curcumin through greater in vitro anticancerous activities. In addition, lot of measures was dedicated for the progress and pattern of various nutraceutical delivery systems with substantial advances [100]. Also, NPs produced with plant products can be utilized in discovering of biomarkers and elegance of analysis, so formation of novel medicine for neurological diseases somewhere modern method of administration of drug across the blood-brain barrier may be possible. Malignant treatment with phytochemicals from different plants and future benefits in the advanced neoplasm of gold NPs may expand the possibilities of improved structure and expansions of effective gold NPs and that can be better synthetic and employed in oncology research [101].

Alternatively, nanotechnology-based plants can promote the rehabilitation in nervous system, while femto-lasers (Innovative ultrafast laser solutions), nano-

robots, and N = nanotechnology-originated equipment can make development in neurosurgery zone [102]. Therefore, the factors that provide to the eminent nanomedicine-based phytodrug delivery are enhanced disintegrative and bioaccumulation, lower toxicant and adverse reactions of phytochemicals. Future investigation must focus on the progress of modern technology for nanotoxicology, origin of nano-biomonitoring, and identification of biological properties of NPs present in the atmosphere [103]. As a result, the integration of phytochemicals and NPs will likely have a large role in the future of biological medicine.

11.11 Conclusion

This study mainly concentrates on the anti-malignant properties of plants drugs and the NPs. Medicinal herbs are the primary source of highly active standard medicines for the treating of several types of diseases and illnesses. The effective compounds separated from medicinal plant products cannot function as anticancer drug, but they can make substitutes for the improvement of future cytotoxic agents. Research advances, novel technology supports the progress of the anti-cancerous activity of the drug. Nanomedicine is a successful field associated to NPs plus drugs, which have a greatest feasibility with nano-sized compounds. Some of NPs (Metallic) developed through plant extracts exhibit improved tumor peculiarity, encouraging activity, and lower toxicity to healthy cells, due to its great surface area, that allows effective drug delivery. Many research studies of medicinal plant products and metallic NPs were shown *invitro,* hence it is essential to carry out investigation on phytochemicals and metallic NPs in animal models as well. Further research must be done to explain the mode of action of potential substances and metallic NPs for the improvement of anti-malignant drugs.

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Chapter 12
Role of Selected Transcription Factors in Pancreatic and Colorectal Cancer Growth and Metastasis

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Abstract The gastrointestinal cancers particularly, pancreatic cancer (PC) and colorectal cancer (CRC), are the widely diagnosed cancers with high mortality rate worldwide. Metastasis is the primary cause for mortality in patients of CRC and PC. The metastatic stages of these cancers are mainly due to the dysregulated activity of transcription factors like signal transducer and activator of transcription 3 (STAT3), nuclear factor kappa B (NF-κB), and hypoxia inducible factor-1 alpha (HIF-1α). In this chapter, we have focused on the role of STAT3 and HIF-1α in the progression of CRC and PC. The prognosis of the patients is poorly associated with the overexpression of these transcription factors. They play a crucial role in developing resistance against therapeutic drugs and significantly resulting in the cancer recurrence. The hypoxic conditions developed by the activation of HIF-1α induces metastasis that later in the presence of a series of signaling cascades develops resistance. Similarly, STAT3 is also suggested as a biomarker for developing resistance against therapeutic drugs. They are also responsible for developing tumor microenvironment and promoting metastasis. In this chapter, we have focused on the role of STAT3 and HIF-1α that promote PC and CRC progression and metastasis.

Keywords Pancreatic cancer · colorectal cancer · STAT3 · HIF-1α · Metastasis · Resistance

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Abbreviations

AP-1	Activator protein-1
bFGF	Basic fibroblast growth factors
CDK	Cyclin dependent kinase
COX	Cyclo-oxygenase
CRC	Colorectal cancer
EGFR	Epidermal growth factor receptor
EMT	Epithelial–mesenchymal transition
HIF-1α	Hypoxia inducible factor-1 alpha
HSP90	Heat shock protein 90
MMP	Matrix metalloproteinases
MOAP-1	Modulator of apoptosis 1
MTA1	Metastasis associated gene 1
NF-κB	Nuclear factor kappa B
PC	Pancreatic cancer
PCNA	Proliferating cell nuclear antigen
PDAC	Pancreatic ductal adenocarcinoma
PTBP3	Polypyrimidine tract binding protein 3
STAT3	Signal transducer and activator of transcription 3
TGF	Transcription growth factor
VEGF	Vascular endothelial growth factor

12.1 Introduction

Among cancers, digestive cancers particularly colorectal cancer (CRC) and pancreatic cancer (PC), are the major diagnosed cancers with high incidence and fatality rate worldwide. CRC is the third most common cancer diagnosed in both male and female [1]. As estimated by the American Cancer Society, CRC new cases for the year 2020 would be 104,610 for colon and 43,340 cases for rectal cancer. PC otherwise called as pancreatic ductal adenocarcinoma (PDAC) with a 5 year of survival rate is estimated as the second most common cause for cancer-related mortality [1]. As estimated from American Cancer Society, 57,600 cases are newly diagnosed and 47,050 are fatality recorded till now. Despite of the advanced technologies for diagnosis and novel chemotherapeutic regimens for cancers, the prognosis always remains poor. This is because of adaptation of cancer cells to limited O_2 delivery inducing invasion and developing resistance against therapy. This triggers metastasis at an early stage of cancer irrespective of the novel adjuvant therapies and resections performed. There are a few mechanisms involved that play pivotal role in promoting invasion and metastasis [2]. The epithelial and mesenchymal transition (EMT) is a key mechanism where in the epithelial cells lose their cell-to-cell adhesion and obtain mesenchymal transition essential for invasion and

metastasis [3]. Additionally, they enhance resistance against apoptosis. E-cadherin and vimentin are certain transmembrane protein that plays crucial role in regulating EMT and metastasis [3]. The function of transcription factors lies in inducing resistance and controls various proteins like E-cadherin to maintain the polarity of epithelial cells [4]. The dysregulated transcription factors like signal transducer and activator of transcription 3 (STAT3), hypoxia inducible factor-1 alpha (HIF-1α), nuclear factor kappa B (NF-κB), and c-myc are the primary cause for the survival of cancer cells and resistance to be developed against therapy. The cancer genome studies also evidenced mutations in the transcription factors as the major cause for tumorigenesis [5]. Moreover, the oncogenic signaling pathways results in altering the downstream transcription factors and its encoded gene expression that drive cell proliferations [6]. This justifies the therapeutic strategies to target the transcription factors to treat the aberrantly acting cancer cells. In this chapter, we focused on HIF-1α and STAT3 transcription factors that play primary role for CRC and PC progression.

The expression of HIF-1α is correlated with poor prognosis in PDAC patient [7]. Its expression is encountered with advanced stage and incidence for lymph node and hepatic metastasis. It interacts with transcriptional regulators including p53, Notch, and myc regulating various downstream pathways. HIF-1α targets pyruvate dehydrogenase kinase-1, vascular endothelial growth factor (VEGF), and TWIST factor that compromises nutrition and hypoxic condition in tumor stroma [8]. VEGF and HIF-1α play a pivotal role in promoting angiogenesis and metastasis in PC [9]. Metastasis-associated gene1 (MTA1) is an oncogene and its aberrant expression is associated with overexpression of HIF-1α and VEGF that are correlated with invasion and migration of PC cells [10]. Moreover, under hypoxic conditions HIF-1α was also found to induce MTA1 expression [10]. Thus, MTA1 can be taken as a potential therapeutic target for PC therapies. HIF-1α expression is equally responsible for the cause of CRC. The immunohistochemistry studies revealed that HIF-1α expression is found positive in 66.7% of CRC patients mostly seen in stage III patients [11]. Additionally, its expression is correlated with the expression of VEGF and is associated with downregulated expression of tumor suppressor proteins PTEN and upregulated expression of PI3K/Akt pathways [12]. Polypyrimidine tract binding protein 3 (PTBP3) is a protein that functions oncogenic in various cancers. It enhances HIF-1α translation that further promotes tumor progression and metastasis in CRC [13, 14]. More recently, Hou et al. [15] found that PTBP3 regulates HIF-1α; thus, it could serve as a prognostic biomarker and a therapeutic target for CRC therapy.

STAT3 pathway activates various signaling pathways including EGFR, Src kinase (a gene found in Rous sarcoma virus that encodes tyrosine kinase), and interleukin-6 (IL-6) in various tumor cells like CRC and PC. It plays crucial role in regulating cell cycle and inhibiting apoptosis. Ma et al. [16] analyzed 45 colon cancer cases that showed expression of p-STAT3 in almost 57.8% cases. Moreover, the expression is associated with lymph node metastasis mostly detected in stage III and stage IV. Additionally, STAT3 mRNA expression is positively associated with expression of survivin, Bcl-xl and cyclin D1 [17]. Mir-572 negatively regulates

modulator of apoptosis 1 (MOAP-1) to induce CRC progression. Previous studies also suggested that STAT3 induced CRC progression through miR-572-MOAP-1 pathway [18]. miR-18a was also found to be upregulated via STAT3 along with NF-κB in CRC cell lines [19]. STAT3 also inhibits tumor suppressor microRNA. For instance, miR-34a is blocked via IL-6-STAT3 signaling pathway; thus, it enhances EMT and promotes invasion in CRC [20]. Rac1 in its active state reacts with STAT3 and promotes its phosphorylation. Thus, it induces EMT in CRC cells [3, 21]. Rac1 in its active form directly promotes phosphorylation of STAT3 that induce EMT in CRC cells. In an indirect way it promotes STAT3 phosphorylation via upregulating the expression of Bcl-2. This determines a strong correlation between the expression of Bcl-2 and STAT3 phosphorylation. Thus, there exists a crosstalk between Rac-1 activation, overexpression of Bcl-2 and STAT3 phosphorylation to promote cell survival [22]. STAT3 signaling pathway also promotes PDAC. Its activation is correlated with tumor progression and altering tumor microenvironment. The dysregulated STAT3 along with mutated KRAS significantly potentiated PC [23, 24]. The previous studies also showed the reactivation of STAT3 signaling cascade that mediates resistance against inhibitors of EGFR and Src kinase [5, 6]. Later, Finger and Giaccia also determined STAT3 activation that developed resistance against monotherapy is blocked through the inhibition of EGFR and Src kinase in vitro in PDAC cell lines [13]. Recent novel mechanisms that include combined inhibition of EGFR and Src to promote stromal alteration through STAT3 inhibition are well studied [25]. Thus, STAT3 can be taken as a target for therapeutic strategies. In this chapter, we have discussed the role of HIF-1α and STAT3 activation in cancer. We have highlighted novel research updates about these transcription factors in both CRC and PC.

12.2 Role of HIF-1α in Pancreatic Cancer Proliferation

HIF-1α plays an important role in pancreatic cancer (PC) cell proliferation. Even though, the detailed mechanism is unknown, there is a strong possibility of the involvement of an oxygen-independent metabolism cascade [26]. Assuming that cell proliferation involves significant protein, nucleic acid, and lipid synthesis, it is vital for the cell proliferation signals to rearrange metabolic activities in order to initiate the proliferation of inactive cells [27]. For instance, when partial pressure of oxygen reduces, the rate of glycolysis is elevated that drives energy generation. Similarly, increased rate of glycolysis is a key consequence of upregulated HIF-1 activity [28]. It has been previously reported that glioma cells are categorized through a positive feedback loop, which involves HIF-1 activation, pyruvic acid, as well as lactic acid [29]. This investigation revealed that upon silencing of HIF-1α, PCNA expression levels was reduced and the contributory effect of HIF-1α on cell proliferation disappeared. In vitro studies suggest that, during hypoxic conditions, the growth rate of pG1 cells was more rapid as compared to normoxia [30]. Previous research report determined that the silencing of HIF-1α downregulates the

expression of VEGF in pGenesil-1-HIF-1α cells (pG2) there by restrain metastasis. The pG2 cells are the cells that are transfected with plasmid encoding five siRNA against HIF-1α. These pG2 cells are susceptible under hypoxic conditions and grow very slowly under normal conditions and hypoxic conditions. This determines the unique role of HIF-1α both under normal oxygen status and hypoxia against PC [30]. The study conducted by Wie et al. [31] revealed that HIF-1 under hypoxic conditions promotes PC cell proliferation. In vivo studies revealed that pG2 cells that were silenced by HIF-1 could not form tumor under hypoxic environments because of the decreased resistance to hypoxia. Under normal conditions, tumors resulting from pG1 cells outnumbered those obtained from pG2 cells, suggesting a unique characteristic of HIF-1 on PC cell proliferation under hypoxia as well as normal conditions.

A large number of oncogenes are regulated by HIF-1α, which are involved in metabolism, oncogenic gene expression, metastasis, angiogenesis, and so on [32]. HIF-1α is also recognized as a vital target for cancer therapies due to its importance [33]. Transcription of hypoxia inducible genes is also activated by HIF-1, which control various cellular functions such as angiogenesis, invasion, and metabolism. For instance, HIF-1α upregulates VEGF expression and promotes angiogenesis in PC. Moreover, HIF-1α is a key transcription factor that activates EGFR signaling cascade eventually promoting gene expression of survivin. However, use of HIF-1α siRNA downregulates HIF-1α expression and significantly causes survivin inhibition in cancer cells. The altered HIF-1α siRNA markedly enhanced the efficacy of docetaxel via promoting apoptosis in docetaxel-treated cancer cells. HIF-1α overexpression stimulates survivin protein expression and decreases the apoptotic response [34]. Survivin is a protein that regulates many signaling pathways including PI3k/AKT pathway and it also controls cell migration, cell mitosis, and apoptosis [35]. Further, survivin also helps in proliferation and metastasis of cancer cells. HIF-1α regulates the metastatic nature of PC and serves as a potential therapeutic target. Various studies have revealed that hypoxia significantly induces the cell survival, cell division, and metastasis. For example, inhibition of HIF-1α expression may impair/reduce the cell proliferation and metastasis [35].

12.3 Role of HIF-1α in Colorectal Cancer (CRC) Proliferation

Under hypoxic conditions, HIF-1α plays an essential role in tumor survival via stimulation of survival angiogenic growth factors like VEGF. Induced HIF-1α and VEGF expression is associated with advanced tumor stages and an extremely poor prognosis in colorectal cancer (CRC) cases. Furthermore, previous studies suggest that HIF-1α controls angiogenesis via VEGF induction among primary and metastatic cancers [36]. However, proficient treatments that target HIF-1α remain uncertain. Abnormal activation of Wnt/β-catenin pathway is necessary for the

pathogenesis of CRC, and the molecular regulation of this signaling pathway has become an important therapeutic strategy [37]. In CRC cells, β-catenin degradation is decreased/damaged and the nuclear translocation is elevated, which makes the Wnt-signaling overactive and cells prone to tumorigenesis. The extracellular antagonist including secreted Frizzled-related protein (sFRP), Wnt inhibitory factor-1 (WIF), and dickkopf (DKK) for Wnt signaling pathway prevents molecular interaction of ligand and receptor. However, better understanding of these inhibitors is still essential to determine specificity of the antagonist and its binding affinity. Together, for the antagonist of Wnt and/or β-catenin the inhibitor efficiency offered with promising preclinical outcomes [38–40]. Plasmid p-HIF-1α RNAi can specifically and efficiently inhibit HIF-1α activity, cell proliferation, as well as control the expression levels of important constituents in the Wnt/β-catenin pathway, such as β-catenin and VEGF. Consequently, p-HIF-1α RNAi is also novel and exceptionally promising therapeutic inhibitor of HIF-1α.

12.4 PC and CRC Metastasis

Cancer is interrelated with the capability of malignant cells to spread outside its primary tissue where it originates onto secondary tumors, also known as metastasis. A prerequisite for cancer metastasis is the invasive migration, which is also considered as an onset of cancer [41]. PC is extremely invasive in nature and extends to others areas of the body such as the portal veins, regional veins as well the peritoneum [42]. It also metastasizes to distant organs such as the lungs and the liver and to regional lymph nodes. Likewise, CRC is also an aggressive disease that metastasized to liver, lungs, bones and to other parts of the body in its advanced stage.

The primary cause of PC and CRC associated mortality is metastasis [43, 44]. Regional and local invasion could obstruct tumor resection and are also considered as the key cause of malignancy since they could also lead to severe pain derived from perineural invasion, jaundice, and duodenal obstruction [45]. PC and CRC metastasized to the regional lymph nodes. This is correlated with reduced overall survival of the patient and is defined as the negative prognostic factor [46–48]. In order for a cancer cell to invade its neighboring tissue, malignant cells must cross a specialized region of the extracellular matrix (ECM) known as the epithelial basement membrane, which separates the mesenchyme and the epithelium. In other words, an intact basement membrane is one of the prominent features, which is used to distinguish cancerous cells from the premalignant tumors [49]. A recent investigation suggests that during PC and CRC progression, invasion could begin earlier than previously understood. According to this in vivo study, premalignant PC cell lines (PanIN-3) that were delaminated from the epithelia were able to cross the basement membrane, enter the circulation, and were able to invade the liver of a mouse model [50]. Whether or not these dispersed cells form metastasis is still uncertain; however, earlier pre-tumorous cell dispersion in breast cancer models has

been known to metastasize [51]. The probability of initial dissemination often indicates that primary PC tumors and metastasis could form and progress simultaneously [50] instead of sequentially [52]. Upon crossing the epithelial basement membrane, the invasive property is vital at various steps of the metastatic signaling pathway in order to migrate through the surrounding stroma, disrupt the endothelial basement membranes, as well as reach the circulatory systems [53]. Upon reaching the capillary bed of different organ, invasive features of the tumor cells influence the extravasation followed by dissemination. Dispersed cells might remain inactive in the distant organ, as micro-metastasis or as solitary cells before they begin to proliferate and form metastasize [53]. It is clinically important to maintain the inactive state of the dispersed cells to restraint them from metastasizing in the distant organ and potentially limiting the effects of PC as a chronic ailment instead of a life-threatening malignancy.

The activity of HIF-1α is upregulated in various cancers including CRC. Elevated HIF-1α expression is closely associated with tumor aggressiveness and increased metastatic abilities of PC and CRC [54, 55]. At molecular levels, HIF-1α exerts its biological properties by stimulating target genes like TCF3, Snail, and Twist that are all related with epithelial–mesenchymal transition (EMT) and metastasis [56, 57]. EMT is one of the most vital mechanisms that underlines PC and CRC metastasis and tumor invasion leading to the production of proteases that facilitate ECM degradation, loss cell adhesion, transformation of cytoskeletal elements as well as synthesis of novel ECM components. Many carcinogenic signaling pathways are mediated through Src, Wnt/β-catenin, Ras, and Notch-induced EMT [58]. The researchers also determined from the matrigel invasion assay that overexpression of HIF-1α induces invasion. Further, siRNA against HIF-1α and the counteracting antibodies against urokinase-type plasminogen activator receptor (uPAR) block the overexpression of HIF-1α and stimulatory effect of hypoxia [59, 60]. During hypoxia, HIF-1α is protected from the proteasomal and ubiquitination degradation [61]. HIF-1α also stimulates transcription of protein sequences that encode VEGF, glycolytic enzymes, and glucose transporters. Hypoxic HIF-1α controls the activity of various genes that are involved in PC and CRC progression by its ability to bind to the HRE promoter regions of COX2, VEGF, and MMP genes [62, 63]. Angiogenic inducers such as bFGF (basic fibroblast growth factors), VEGF, IL-8, and TGF (transforming growth factor) also activate tumor angiogenesis in PC and CRC [64, 65]. HIF-1α is a well-known modulator of angiogenesis [66] and a vital factor in PC and CRC progression [67]. Interaction of β-catenin and HIF-1α improves the transcriptional activation of HIF-1α activity; in turn, it promotes PC and CRC cell survival under hypoxia [68, 69]. Silenced HIF-1α downregulates the expression levels of β-catenin [70, 71]. The expression levels of Nur77 and β-catenin are elevated in PC and CRC cell lines as compared to normal cells [72, 73]. All together, these observations reveal that HIF1α assists β-catenin in inducing Nur77 expression, Nur77 positively controls β-catenin through the PI3K/AKT signaling pathway, and Nur77-modulated β-catenin improves transcriptional activation of HIF-1α that also enhances the aggressiveness of PCs and CRCs.

12.5 STAT-3 Role in PC and CRC Cell Proliferation

Tumor development is mainly dependent on cell proliferation and angiogenesis, formation of new blood vessels from existing capillaries. VEGF is one of the most vital molecules for proliferation in PC and CRC [74, 75]. The analysis of VEGF promoter revealed that binding of proteins like Sp1, AP-1, and HIF-1α at their respective binding sites present on the growth factors would regulate the expression of VEGF if exposed to any inhibitor, and thus determines the involvement of many signaling pathways in VEGF regulation [76–78]. STAT-3 is a member of the JAK-STAT signaling pathway [79]. STAT-3 is known to bind on the VEGF promoter and plays a role in regulating tumor growth [80]. STAT-3 is stimulated by phosphorylation and commonly observed in various tumors including PC and CRC [79, 81]. STAT-3 activation has been observed in transformed cell lines with oncogenes such as tyrosine-protein kinase ABL1 (encoded by ABL1 gene) and v-Src [82]. Many malignancies including PC and CRC have elevated STAT-3 expression as compared to normal cell lines. Moreover, subsequently active STAT-3 expression increased cell transformation and tumor formation in in vivo investigations [83], suggesting that STAT-3 could function as a potential oncogene and play a major role in pancreatic and colorectal tumor progression.

STAT-3 inhibition in PC cell lines facilitates apoptosis and inhibits chemoresistance [84]. Subsequent activation of STAT-3 during PanIN progression also facilitates proliferation of the pancreatic preneoplasmic lesions [85]. c-Myc, cyclinD1, and CDK4 are important in driving tumors from G0/G1 to S phase, thereby supporting cell cycle progression [86]. Likewise, isolated PC cell lines from STAT-3 deficient mice revealed decreased proliferation in response to hyper-IL-6, FCS, and IL-6 [24]. Intracellular modulation of STAT-3 activity is regulated by the expression of Socs3, which is the endogenous inhibitor of STAT-3 [24]. In vivo investigations revealed increased levels of Socs3 expression, which suggests that STAT-3 activation and inhibition are both balanced during PanIN progression [24]. Deleting the alleles of Socs3 caused phosphorylation of STAT-3, which also accelerates PanIN progression and PC development [24]. Socs3 deficient mice showed decreased tumor survival, suggesting that the STAT-3/Socs3 signaling cascade plays a central role in PC proliferation. Similar observations were also found in CRC [87].

12.6 Role of STAT-3 in PC and CRC Metastasis

STAT-3 plays an important role in PC and CRC growth and metastasis [88, 89]. STAT-3 expression is associated with metastatic properties including migration, invasion, and angiogenesis [88, 90]. STAT-3 regulates P70S6K at transcript level and induces migration. STAT-3 also increases invasion by elevating the expression of uPAR, MMP-2, and MMP-9 [88]. STAT-3 induces the new blood

vessel formation by increasing the expression of VEGF. STAT-3 is also involved in EMT by increasing the expression of Vimentin and decreasing the expression of E-cadherin [91, 92]. These results strongly suggest that STAT-3 plays a vital role for metastasis in PC and CRC. Further, these data all indicate that STAT-3 inhibition could serve as a therapeutic strategy in the treatment of PC and CRC.

12.7 STAT3 Is Necessary for Induction of HIF-1α by Oncogenic/Growth Signals

Reports mainly show that increased HIF-1α production by growth signals results from enhanced protein synthesis. Experiments were conducted to determine whether this was also the case with IL-6. MCF-7 cells were treated with cycloheximide for varied time intervals following treatment with IL-6. If the IL-6 effect enhanced the protein synthesis rate, it would have been responded by the cycloheximide to the cells. HIF-1α levels would reach the baseline rapidly. If the increase in HIF-1α levels was due to the termination of degradation, cycloheximide addition would not have any effect as the levels of protein would stay elevated over time. Within 1 h, levels of HIF-1α were the same as in cells that were not treated. These results show that the outcome of IL-6 on HIF-1α is due to protein synthesis.

12.8 What Do We Know About Resistance to Chemotherapy and HIF-1α, and STAT-3?

Several signaling pathways that contribute to chemotherapy resistance have been identified in preclinical models and clinical trials in PC or CRC. HIF-1α is an essential mediator of cellular reaction to hypoxia, and regulates gene expression for resistance to oxidative stress, glucose metabolism, and tumor angiogenesis [93, 94]. Activation of HIF-1α is related with poor diagnosis in PC and CRC and with radio and chemo-resistance [95–98]. The activation of STAT-3 has been correlated with uncontrolled proliferation, angiogenesis, and apoptosis regulation. Several studies indicate that STAT-3 would be an effective mediator of chemo-resistance [99, 100]. In PC, the inactivation of STAT-3 sensitized the cells to radio-chemotherapy and increased their rate of apoptosis [95]. This evidence suggests that these pathways contribute to resistance to chemotherapy in PC and CRC. In addition, we also demonstrated in CRC and PC models that the mechanism of this chemo-sensitization was mediated through inhibition of HIF-1α and HSP90 [101, 102]. As a combinational approach, curcumin when combined with 5-FU sensitizes the resistant CRC cells to 5-FU, thereby inhibiting cell proliferation and survival of CRC cells [103]. Similarly, Yu et al. [104] demonstrated that curcumin is a very potent chemo-sensitizer of CRC cells.

The above-published literature supports our hypothesis regarding the mode of action of HIF-1α and STAT-3 transcriptional inhibitors in PC or CRC cell lines and justifies for the testing of these novel agents in PC and CRC. In addition, the above literature provides a rationale for the selection of the molecular pathways being evaluated.

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Chapter 13
Pancreatic Ductal Adenocarcinoma and Type 2 Diabetes Mellitus: Distant Relatives or the Close Ones?

Kumari Subham, Sonali Mohanty, Sonali Jena, Monalisha Ojha, and Suman Jha

Abstract Tumors are the charismatic pirated cells, which get distracted from the well-maintained metabolic route. In oncology, metabolic alterations in tumor cells, to adopt the challenging physiological condition, are emerging attraction for investigation. Pancreatic ductal adenocarcinoma (PDAC) is the aggressive malignancy that develops in a nutrient-deficient, hypoxic and acid environment, by altering their biosynthetic machinery. Despite significant advances in the understanding of PDAC genetics, biology, and clinical behavior, PDAC is one of the dominating agents of cancer-related death in all over the world. The two major risk factors contributing to the incidence of PDAC are obesity and Type 2 diabetes mellitus (T2DM). These three diseases are intricately related contributing to the cause and effect of one another. This chapter discusses the underlying mechanism of obesity and T2DM that leads to PDAC, mediated by various factors like redox stress, inflammation, insulin resistance, and β-cell mass degeneration. At the end, this chapter focuses on the therapeutic approach for PDAC, taking into account the two efficient branches of medicine which have recently gained much of attention, one being phytochemicals and the other, nanomedicines. Many epidemiological studies have already proved the anticancer activity of both the approaches, and ongoing researches are in favor too.

Keywords Pancreatic ductal adenocarcinoma · Type 2 diabetes mellitus · Obesity · Tumor microenvironment · Phytochemicals · Phytonanomedicines

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Abbreviations

ADM	Acinar ductal metaplasia
AgNP	Silver nanoparticle
AuNP	Gold nanoparticle
CAF	Cancer associated fibroblasts
CONP	Cerium oxide nanoparticle
CPE	Carboxypeptidase E
ECM	Extracellular matrix
ERS	Endoplasmic reticulum stress
FLIP	FLICE inhibitory protein
HBP	Hexosamine biosynthesis pathway
HFD	High fat diet
HIF	Hypoxia inducible factor
HSPGs	Heparan sulfate proteoglycans
IAPP	Islet amyloid polypeptide
IGF	Insulin like growth factor
IL	Interleukin
MDSC	Myeloid-derived suppressor cells
MMP	Matrix metalloprotease
NanoCurc	Nanoparticle encapsulated curcumin
NP	Nanoparticle
PanIN	Pancreatic intra-epithelial neoplasia
PC1/3	Prohormone convertase 1/3
PC2	Prohormone convertase 2
PDAC	Pancreatic ductal adenocarcinoma
PPP	Pentose phosphate pathway
PSCs	Pancreatic stellate cells
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T2DM	Type 2 diabetes mellitus
TANs	Tumor associated neutrophils
TCA	Tricarboxylic acid
TME	Tumor microenvironment
UPR	Unfolded protein response
ZnONP	Zinc oxide nanoparticle

13.1 Introduction

In human pathology, pancreas is directly related to two different, somehow interlinked diseases that is, pancreatic cancer and type 2 diabetes mellitus (T2DM). Nowadays pancreatic cancer is the principal cause of death in USA,

according to American Cancer Society, Cancer fact and figures (2019). By 2030, it is expected that it will become second most main cause of cancer-related death and surpass the other dominating cancer in terms of number of deaths [1]. As there are various risk factors involved in pancreatic cancer, at genetic as well as molecular level, the rate of average 5-year survival is less than 5% [2]. The literature suggests that although there are significant advances in understanding of pancreatic ductal adenocarcinoma (PDAC) biology, little has been improved in terms of stretching the survivorship graph of PDAC patients [3]. The American Cancer Society estimates that the occurrence of pancreatic cancer is 56,670 in 2019, with a concomitant mortality of 45,750 in both male and female. The 5-year survival has been lengthened to a mere 8% [1].

Pancreatic ductal adenocarcinoma is the typical destructive malignant cells that flourish in nutrient-deficient, hypoxic environments [4]. Success in treating solid tumor is limited by serious side effects of chemotherapeutic agents and the chaotic and complex tumor environments that cause drug resistance [4]. Recent literature suggests that T2DM and obesity are major risk and prognostic factors for pancreatic cancer. Obese people are at a greater risk for development of T2DM [5]. According to a health survey for England conducted during 2009–11, doctors compared waist circumference to occurrence of diabetes. They provided evidence that women with wider waist circumference (greater than 88 cm) are at three times higher risk for development of T2DM [5]. T2DM is a systemic disease characterized by chronic hyperglycemia due to insulin resistance. One of the hallmark features of islet cells in T2DM is the presence of amyloid lesions. Islet amyloid polypeptide (IAPP or amylin) and insulin are the major secretory products of β-cells in pancreatic islets of Langerhans [6]. IAPP has the ability to aggregate in pancreatic islets forming amyloid deposits in humans with T2DM [6]. The classical antioxidants of β-cells, which are unable to cope up with the enhanced redox stress caused by aggregate in extracellular matrix, result in apoptotic cell death. Thus, the accumulation of toxic, misfolded and soluble oligomeric proteins leads to apoptosis, eventually resulting in β-cell mass degeneration [7]. In T2DM, defects in the misfolded protein removal machinery (i.e., protein remodeling factors, aggresome formation, ubiquitin proteasome system, and autophagy) lead to accumulation of aggregated and misfolded proteins [8]. All these physiological events that occur in T2DM (oxidative stress, inflammation, insulin secretion and resistance, and β-cell death) enable the acinar and ductal cells of pancreas to proliferate and surpass signals of apoptosis leading to tumor progression and angiogenesis causing pancreatic cancer [9, 10].

The cure of PDAC is difficult to achieve due to its poor response to most chemotherapeutic agents [11]. Therefore, there is an urgent need to find out an alternative to chemotherapy. The phytochemicals present in medicinal plants are a blessing in disguise, but the lack of technological advancement has kept its use restricted for a longer time. In the last few decades, various phytochemicals have been proved to be good candidates for the development of novel anticancer agents [12–15]. A variety of phytochemicals from medicinal plants have shown anti-metastatic, antiproliferative, pro-apoptotic, and anti-angiogenic effects in in vitro experiments and animal trials [16–18]. Some phytochemicals have been found to

potentiate the impact of synthetic drugs which ultimately proved helpful in treating PDAC [19]. Unfortunately, clinical utilization of various phytochemicals has been hindered due to their poor systemic bioavailability [20]. To overcome the drawback and to enhance the efficacy in cancer treatment, nanoparticles can be explored. Nanoparticles, due to their very small size and high surface area to volume ratio, can diffuse easily to different inaccessible body parts, even the blood–brain barrier. Additionally, nanoparticles surface can be easily modified by adsorbing the therapeutic drugs for delivery, which invade the tumors with higher specificity [21, 22]. Applications of nanotechnology in cancer have been introduced in clinical laboratory analysis, imaging, and therapeutics [23]. Among all types of nanoparticles that are synthesized, metal and metal oxide nanoparticles are of greater importance [24]. Gold, cerium oxide, silver, and zinc oxide nanoparticles were reported to be active against PDAC [21, 25–27]. Biofabricated nanoparticles are also gaining attention in the present context. Nowadays phytochemicals and nanoparticles are used together for additive effects in treating different types of cancer.

13.2 Type 2 Diabetes Mellitus and IAPP

T2DM is a systemic disease characterized by chronic hyperglycemia due to insulin resistance or delayed insulin secretion. It is generally found in adults where hyperglycemia can be controlled by diet or by oral hypoglycemic drugs. One of the hallmark features of islet cells in T2DM is the presence of amyloid lesions. Although non-diabetic individuals also have amyloid lesions, the severity and frequency increases with progression of diabetes and aging [28]. Peripheral insulin resistance is recompensed by increasing insulin production during development of T2DM. The increased expression of insulin gene also results in co-expression of another proteinaceous hormone, that is, IAPP, which is also known for its highest amyloidogenic peptide among the proteome of pancreatic cells in vitro. Interestingly, both the hormones' expression, processing, and storage share common stimulant, enzymes, and vesicles. With the persistent need of insulin in prediabetic stage, either β-cell mass degeneration or β-cell exhaustion or both results in reduced production of functional insulin, and hence T2DM prevails [9]. IAPP and insulin are the major secretory products of β-cells of pancreatic islets of Langerhans [6]. Both the hormones are expressed as preproprotein against same stimulant. IAPP is cleaved at double basic amino acid residues similar to proinsulin, resulting in a mature IAPP of 37 amino acids [29, 30]. Both proIAPP and proinsulin are processed by prohormone convertase 2 (PC2), prohormone convertase 1/3 (PC1/3), and by carboxypeptidase E (CPE) [31, 32]. IAPP has relatively higher propensity to aggregate in pancreatic islets forming amyloid deposits, which is also present in abundant fraction in amyloid plaques taken from patients with T2DM [33]. It has been found that insulin acts as a strong inhibitor for IAPP fibril formation [34]. Insulin to IAPP ratios of 1:5 and 1:100 have been reported to have a strong inhibitory

effect [35]. The positively charged N-terminal region of IAPP is more flexible and known to interact with negatively charged lipid fraction. The interaction results in insertion of N-terminus into the membrane, making it more favorable for aggregation at the membrane interface [36, 37]. The aggregation at membrane interface either mechanically punctures the membrane or forms pore in the membrane. In both the cases, it leads to unregulated Ca^{2+} influx affecting the cell viability [36, 37].

13.2.1 Effects of IAPP on β-Cell Mass Degeneration

The β-cells deficient with the classic antioxidants are unable to protect itself from the surrounding redox stress. With the development of T2DM, the antioxidant machinery is compromised with the deficiency of catalase, superoxide dismutase, and glutathione peroxidase [38, 39]. Redox stress (ROS, RNS, and reactive thiol species) and increased insulin production post-transcriptionally modify IAPP leading to endoplasmic reticulum stress (ERS), induction of unfolded protein response (UPR), and protein misfolding. When the cells' quality control system becomes saturated, IAPP intermediates are modified generating stable oligomers with an antiparallel crossed β-pleated sheet structure that accumulates as space-occupying lesions within the islets. Accumulation of toxic, misfolded and soluble oligomeric proteins leads to apoptosis, eventually causing β-cell death [7]. In the later phases of T2DM, β-cell mass (both number and volume of cells) is reduced as a result of apoptosis by IAPP oligomers especially in rapidly replicating β-cells [7]. In T2DM, defects in the misfolded protein removal machinery (aggresome formation, autophagy, and ubiquitin proteasome system) lead to accumulation of aggregated and misfolded proteins [8].

13.2.2 Type 2 Diabetes Mellitus and Inflammation

T2DM is characterized by insulin resistance and β-cell dysfunction. Even before the onset of impaired glucose tolerance, β-cell function starts to decline [40]. Including glucose, many other factors like autoimmunity, leptin, cytokines, dyslipidemia, and certain sulfonylureas contribute to malfunction of β-cells [41]. The key regulator for insulin secretion is glucose. Therefore, it seems logical that it controls the long-term adaptation of insulin production by regulating β-cell turnover [41]. However, prolonged exposure to increased glucose concentration reduces the proliferative capacity of β cells [41]. Factors like IAPP [42, 43], palmitate [44], and endocannabinoid [45] stimulate islet macrophages to secrete Interleukin-1β (IL-1β) in vivo [44]. One of the major contributors to β-cell decline in T2DM is islet inflammation. The biopsy sample from T2DM patients shows the presence of IAPP aggregates in macrophages present in pancreatic tissues [46]. In vitro studies show that internalization of IAPP aggregates lead to secretion of multiple

inflammatory cytokines including IL-1β. Phagocytized IAPP oligomers lead to IL-1β secretion by activating NLRP3 inflammasome [42]. These are the common mechanism of tissue damage by protein aggregation [47]. Studies done both in vitro and in vivo showed that hyperglycemic shock elicits IL-1β production followed by Fas upregulation [48]. The Fas-regulated proliferation occurs in presence of caspase-8 inhibitor FLICE inhibitory protein (FLIP). However, decrease in FLIP due to excessive glucose production will switch the adaptive pathway to detrimental signals which will eventually lead to degenerative form of diabetes, that is, T2DM [49].

13.2.3 Consequences of Mechanisms Underlying Type 2 Diabetes Mellitus

Some of the main physiological events occurring during T2DM in the body are β-cell destruction, insulin resistance, inflammation, and release of adipokines and cytokines. To maintain the glucose homeostasis, β-cells of T2DM patients secrete more insulin, which eventually increases the intrapancreatic insulin levels. The insulin is diffused to the acinar and ductal cells of pancreas, which are adjacent to the islet cells through the intra pancreatic portal circulation. The high levels of insulin exert proxicrine effects on insulin receptors present on the acinar cells thereby promoting its survival and proliferation [10].

According to several epidemiological studies, T2DM has been known to elevate the risk of a number of human cancers, like pancreatic, colorectal, bladder, breast, colon, endometrial, and liver cancer. About 68% of patients with several human cancers were diagnosed with concurrent diabetes in a survey of 100 patients confirming the association of diabetes to human cancers [50]. Diabetes is more likely related to PDAC than any other type of cancer. The patients are more likely to develop PDAC within few years of diagnosis of diabetes rather than the patients who are diagnosed with diabetes from a relatively longer duration [10]. The hyperglycemic condition observed in T2DM patients due to excessive hepatic gluconeogenesis and peripheral glucose uptake, all cumulated by an impaired insulin signaling [51]. High blood sugar helps in activation of transforming growth factor β-1 pathway, leading to reduced E-cadherin expression in ductal epithelial cells. This results in more mesenchymal and pro-metastatic morphology [52].

Insulin, being a growth-promoting hormone, increases glucose usage and proliferation of cells leading to tumor development and progression [9]. Initial peripheral resistance is either compensated by overproduction of endogenous insulin or exogenously introduced for treatment of T2DM [51]. It also downregulates the formation of IGF-binding proteins, which originally bind to IGF (Insulin like growth factor) and decrease its availability [53, 54]. IGF1 acts as a potent growth stimulus for the cells containing insulin receptors and IGF1 receptors (IGF1R). Both IGF1 and IGF1R are overexpressed in the PDAC cells leading to their proliferation, invasion, and angiogenesis. Also, it reduces apoptosis in the PDAC cells [55–57]. IGF1R-

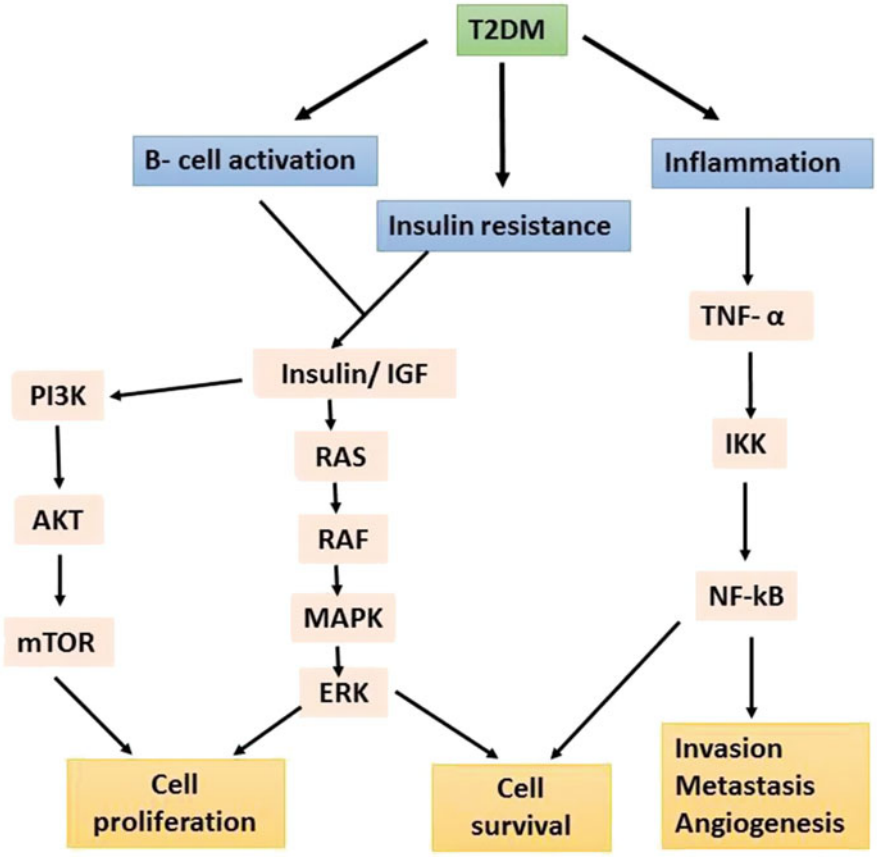

Fig. 13.1 Plausible signaling mechanisms of T2DM leading to progression of PDAC. (Adapted from [9])

mediated signal transduction activates important intracellular signaling pathways, such as phosphoinositide-3 kinase/Akt/mammalian target of rapamycin (mTOR), and Ras/Raf/MAPK pathways which further aid in cell survival and proliferation [58]. Insulin provides a supportive niche in synergism with IGF-1 via activation of ERK1/2 pathway [59]. The upregulation of ERK1/2 pathway is associated with development of insulin resistance acting as a connecting link between PDAC and T2DM [60].

T2DM manifests the risk of pancreatic cancer by increasing inflammatory responses and oxidative stress. It has been suggested that oxidative stress acts as a pioneering event leading to development of insulin resistance [61, 62]. Glucose intake can be held responsible for the increase of oxidative stress activating transcriptional factors such as NFκB leading to cell survivability and eliciting an immune response. Another mechanism leading to PDAC is mediated by adipose

tissues which secrete adipokines and proinflammatory cytokines, such as resistin, IL-6, and tumor necrosis factor-a (TNF-a) [63]. These adipokines and cytokines play major part in triggering innate immunity, inflammation, and arresting apoptosis. The enhanced cytokines level promotes tumor progression, angiogenesis, and metastasis of pancreatic cells [63, 64] (Fig. 13.1).

13.2.4 Diabetes: Cause or Effect of Pancreatic Cancer?

By the above-mentioned mechanisms, it is now clear that pancreatic cancer and diabetes are closely related. Recent evidences suggest that in 74–88% of patients with PDAC having diabetes, the latter was diagnosed in less than 24 months prior to the diagnosis of pancreatic cancer [65]. Also, other epidemiological evidences suggest that early onset of diabetes increases the chances of acquiring PDAC by 1.5–2.0-folds [9]. These facts demonstrate that diabetes and pancreatic cancer show "dual causality." T2DM is an important risk factor for the pathogenesis of pancreatic cancer, and alternatively, pancreatic cancer is presumed to be a potential cause of diabetes as a pre-disease symptom in a large number of cases. The mechanisms of these intricate relationships are still not completely clear [10]. Another set of interesting findings states that the risk of occurrence of PDAC is higher in patients with short-term exposure to diabetes than those with long-term exposure. A plausible hypothesis to explain the cause of decreased risk of PDAC in long-term affected diabetic patients can be linked to the lifestyle changes occurred after diabetes diagnosis. Also, some anti-diabetic medications like metformin are proved to partially block the inflammatory and pro-oncogenic stimulus rendering a protective effect against PDAC in diabetic patients [66, 67].

13.2.5 Obesity: A Missing Link

Obesity and T2DM are the recognized risk factors for development of pancreatic cancer [51]. Obesity triggers adipose tissues to release increased amounts of glycerol, hormones, fatty acids, proinflammatory cytokines, and other factors that lead to T2DM due to increased insulin resistance [68]. In addition, the chances for development of pancreatic precancerous lesions are higher in obese individuals [69]. In early adulthood, excessive fat deposition in adipose tissues increases the risk of PDAC [70]. High fat diet (HFD) rich in omega-6 lipids and processed meat lead to increased invasiveness with an inflamed microenvironment which is directly correlated with pancreatic intraepithelial lesions (PanINs) [71, 72]. The abrogation of TNFR1 signaling significantly blocks the central role of TNF-α in obesity-mediated enhancement of PanINs lesions. Improved glucose tolerance is attributed to changes in energy metabolism by amplification of metabolic stress, pancreatic exocrine insufficiency, and expression of genes involved in mitochondrial fatty acid

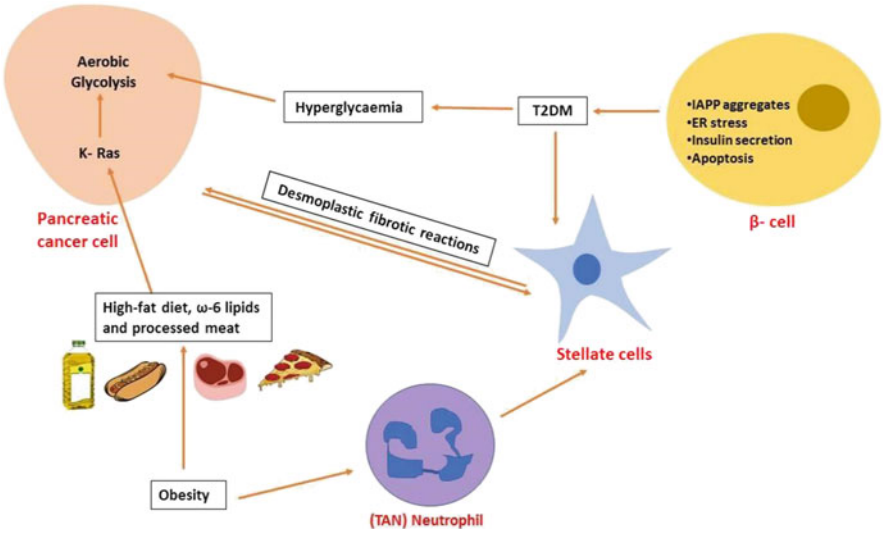

Fig. 13.2 Obsogenic diets rich in fat, ω-6 lipids, and processed meat sustain a K-Ras mediated aerobic glycolysis supporting cellular proliferation [81]. Obesity regulates activation of TANs that activate stellate cells responsible for desmoplastic fibrotic reactions through hyperglycemia, supporting the metastatic dissemination and growth of primary tumor as seen in PDAC [77]. (Adapted from [51])

β-oxidation [73]. Obesity induced due to HFD and hyperglycemia causes low-grade inflammatory conditions and by activation of IKKβ-NF-κB pathway, insulin resistance is developed [74–76]. Hyperglycemia increases post-translational O-GlcNAcylation which leads to nucleotide alterations and genomic mutability, supporting KRAS (proto-oncogene) mutations [77]. An HFD leads to oncogenic K-ras (product of KRAS) activation that accelerates pancreatic intraepithelial neoplasm development via Ras/MAPK pathway [73]. It also drives a tilt toward aerobic glycolysis by upregulating KRAS-G12D, leading to intense invasive PanINs and ultimately to PDAC [78]. Obesity acts as a proinflammatory agent with ability to activate tumor associated neutrophils (TANs). This in turn induces pancreatic stellate cells (PSCs), which causes desmoplastic fibrotic reactions as seen in PDAC [79]. It has been suggested that infiltration of immune cells and excessive production of inflammatory cytokines during chronic pancreatitis play a significant role in increment of acinar progenitor cells of a certain pool that are susceptible to transformation by K-ras activation. Also, it may facilitate trans-differentiation of mature acinar cells showing inflammation to be essential for development of panINs and PDAC [80] (Fig. 13.2).

13.3 Pancreatic Ductal Adenocarcinoma

Among all the types of cancer, pancreatic cancer is the third leading cause of cancer related death across the world. Pancreatic cancer invites competition in the cancer society due to various factors including aggressive molecular response driven by the loss in function of multiple tumor suppressor genes, gain in function of various oncogenes, incompetent immune response with low immunogenicity and intricated tumor microenvironment (TME) [3]. These are some major factors, which help pancreatic cancer to form malignancy. Until now, the sustaining treatment of pancreatic cancer is adjuvant therapy and surgical section, as PDAC is highly resistant to chemotherapy and radiation therapy. The combination of cytotoxic agents and adjuvant altering the metastatic settings is the mainstay treatment.

13.3.1 Microenvironment of PDAC

In the past, various strategies were used to cure PDAC, but these approaches did not yield any major improvement in clinical outcomes due to its complicated signaling pathways.

The peculiar characteristic features of PDAC microenvironment makes it particularly challenging to treat [82]. The challenging microenvironment of PDAC is profoundly immunosuppressive due to presence of various immunosuppressive regulatory cells [83]. The dense TME consists mainly of pancreatic stellate cells which produce a fairly large amount of stromal elements like fibronectin, laminin, and collagens by the process of desmoplasia [84]. The major drawback in the management of PDAC is emergence of multidrug resistant malignant clones, due to the presence of aggravated desmoplastic response that severely compromises tumor blood perfusion and drug delivery [85]. The tumor microenvironment is composed of a complex dynamic neoplasm that contains the desmoplastic tumor and helps it to efficiently grow in a challenging environment [86]. Tumor microenvironment of cancer consists of tumor stroma, including fibroblasts, immune cells, and cells containing blood vessels. In addition, to support the growth of cancer cells, TME includes some proteins secreted by stromal cells such as extracellular matrix (ECM) proteins and angiogenesis proteins [87]. The cooperation of cancer cells with stromal cells is considered as an important factor for cancer progression. Since the tumor–stroma relationship is very complex, further work is being carried out to elucidate the biology of PDAC. The chaotic microenvironment of pancreatic cancer consists of cancer associated fibroblasts (CAF, also known as pancreas stellate cells), acellular stroma, immune cells, and soluble factors including cytokines, chemokines, growth factors, and pro-angiogenic factors [88]. Melstrom et al. stated, “The eventual development of PDAC is thought to stem from acinar ductal metaplasia (ADM), and subsequent morphological changes of pancreatic intra-epithelial neoplasia (PanIN 1–3) that are in part regulated by oncogenic inflammation” [89]. The unique

characteristics of TME have direct effects on the molecular biology of cancer cells. Current evidences suggest that the proinflammatory cytokines, orchestrated by myeloid-derived suppressor cells (MDSC), which are frequently present in the stroma of pancreatic cancer provoke the activity of stellate cells. The desmoplasia condition induced by stellate cell results in angiogenesis, which is a hypo-vascular microenvironment that builds the molecular impression of cancer cells [86]. For example, hypoxia microenvironment transforms cancer cell and alters the gene expression profile, which facilitates adaptation to the continuously changing micro-environment [90]. Another study reported that the upregulation of multidrug resistance gene is associated with hypoxia-inducible factor-1α (HIF-1α). Along with multidrug resistance, the hypoxia and hypoxia inducible factor is also involved in metabolic reprogramming of tumor cells by overexpressing the genes involved in metabolic process [91].

13.3.2 Impact on Metabolism

The most notable feature of cancer cell is unbridled growth, invasion, and metastasis. To accomplish this uncontrolled growth, cancer cell undergoes metabolic reprogramming and modulate their biosynthetic machinery [92]. The TME differs from normal cell's internal environment in various ways. In tumor cells, glycolysis is augmented to fulfill the high energy demands of cancerous cell [93]. As cancer cells are growing under oxygen starving condition; these cells are known to be hypoxic cells. The key factor involved in hypoxia condition is HIF which is responsible for various factors involved in major signaling pathways of tumor cell, and also responsible for the resistance of cancer cell from apoptosis [94]. The idea that tumors have a reprogrammed metabolism is associated with enhanced glycolysis, which is supported by molecular and functional data. Microarray data collected from various studies shows that upregulation of the genetic machinery, which is involved in glucose intake, uptake, and glycolysis, is reported in several cancers. The hypoxia condition of TME is associated with severity of pancreatic cancer due to metastasis, larger risk of local spread, lack of success of the treatment, and mortality for the patient. Hypoxia condition of TME also acts as a shield for tumor cell mitochondria to prevent programmed cell death (PCD). In response to reduction in partial pressure of oxygen in tumor microenvironment, tumor cells switch its metabolic fluxes and replenish the Krebs cycle by enhancing anaerobic glycolysis (Fig. 13.3).

The enhanced demand for energy and macromolecular biosynthesis is compensated by increased nutrient acquisition that is coupled with increased flux through downstream metabolic pathways. Thus, it is not surprising that *KRAS* mutation, loss in function of tumor suppressor genes (e.g., *TP53*, *RB*, and *PTEN*), and gain in function of other canonical oncogenes (e.g., *AKT*, *MYC*, and *PI3K*) can directly reprogram cellular metabolism, thereby accelerating the cell growth [94, 96, 97]. These genes involved in hastened growth, by acting at genetic level, can also directly remodel the cellular metabolism of PDAC. A frequent subject associated

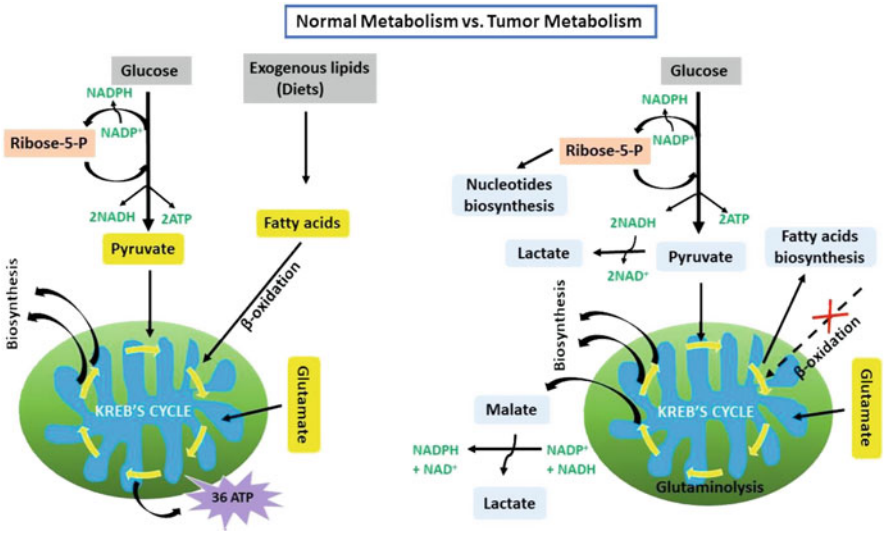

Fig. 13.3 Metabolism of normal cells vs tumor cells. The highlighted (yellow) terms are the major anaplerotic precursor of both normal cell and tumor cells. Glutamine supports cell's energy demand by replenishing TCA cycle. (Adapted from [95])

with this cancer metabolism is the upregulation of glucose metabolism, providing a major nutrient source for ATP synthesis as well as acting as building block for anabolic process [98].

Aside from glucose, glutamine—which is the most abundant and versatile amino acid in the cytoplasm—is essential for rapidly dividing cancer cell to fulfill their energy demand and for macromolecular biosynthesis [93]. The non-essential AA glutamine acts as a precursor and amine donor for biosynthesis of other AAs, nucleotides, hexosamine, and so on. Glutamine also acts as carbon skeleton donor for replacement of tricarboxylic acid (TCA) cycle intermediates (anaplerosis). This amino acid becomes conditionally essential for growth of cancer cells as some cells in culture show addiction to glutamine [99, 100]. The development of some inhibitors of enzyme, as well as glutamine metabolism pathway is aroused due to the active participation of glutamine in fueling tumor cell metabolism. The rapidly developing tumor cells generate high levels of ROS, which is also mitigated by the KRAS by activating the transcription factor NRF2 [101].

In various types of cancer, coordination between glucose and lactate metabolism has been reported. As the cancer cells are growing under hypoxic environment, glucose tries to skip the mitochondrial pathways and convert into lactate by lactate dehydrogenase (LDH). This phenomenon is referred as the Warburg effect [102]. The pseudo hypoxic cells present in the cancer environment devour lactate, which is eliminated by the hypoxic cells [99, 103, 104]. The intake and uptake of lactate are carried out by some transporters, which are overexpressed in tumor cells. For intake purpose, the pseudo hypoxic cells express lactate importer MCT1, which

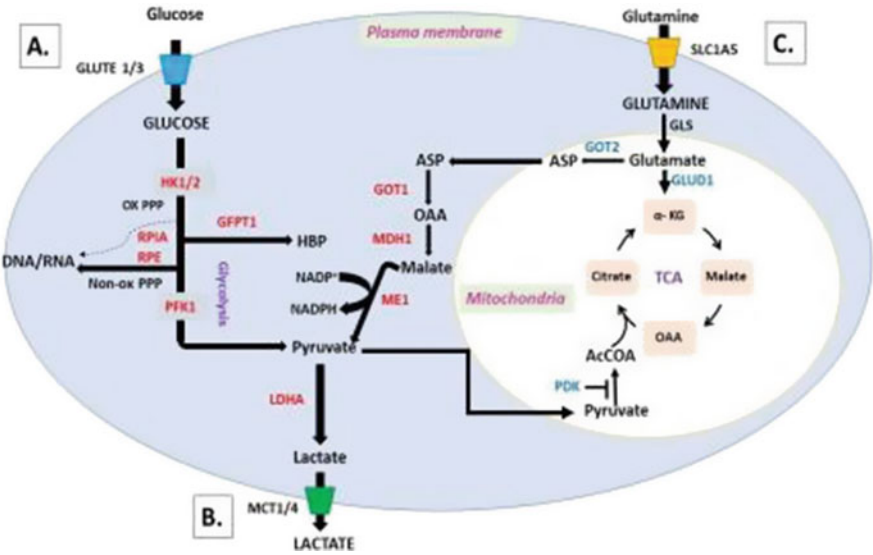

Fig. 13.4 (**a**) KRAS involved in upregulation of expression level of glycolytic enzymes and multiple glucose transporter in PDAC. In addition to this, glycolytic intermediates are involved in non-oxidative pathways like pentose phosphate pathway and hexamine biosynthetic pathway, which gives rise to precursor for generation of glycolipid, glycosaminoglycans, glycoproteins, and proteoglycans. (**b**) To prevent intracellular accumulation of lactate and also to maintain the acidic environment, lactate transporters such as MCT1 and MCT4 are overexpressed in PDAC. (**c**) KRAS is also involved in glutamine metabolism. Through aspartate-malate shunt, glutamate is involved in HMP pathways to produce NADPH. It also maintains the redox balance of PDAC environment. The blue portion indicates the enzymes whose expression levels are regulated by mutant KRAS. (Adapted from [93])

directly import and metabolize lactate. Lactate is excreted out from the hypoxic tumor cell through the export system MCT4 [103, 105].

To promote the cancer growth, tumor microenvironment provides fatty acid, which undergoes β oxidation to the biosynthetic machinery by providing energy. In the hypoxic environment of PDAC, the partial pressure of oxygen is found to be 2–5 mmHg, which is ten times lesser than the normal cell. Some studies reported that, in the TME of PDAC, levels of certain proangiogenic factors like VEGF, COX-2, and NRP-1 are elevated [106]. Apart from all these factors, VEGF-independent angiogenesis is promoted by KRAS oncogenic signaling and hypoxic condition [87] (Fig. 13.4).

13.3.3 Impact on Extracellular Matrix

The structural support of the tissues and the continuous cell-to-cell communication to maintain the tissue homeostasis are accomplished by ECM. The ECM surrounds the cell, which is composed of many macromolecules like structural proteins to maintain the framework of cells. These structural proteins are collagen, laminin, fibronectin, and heparan sulfate proteoglycans (HSPGs), and so on. [107]. The tissue homeostasis is maintained to prevent neoplastic transformation of normal tissue by maintaining the tight junction proteins and cell adhesion proteins. However, in cancer cells these limitations are altered by reframing the ECM unit. Several families of matrix degrading enzymes, such as cysteine, serine proteases, and matrix metalloprotease (MMP) classes, as well as heparinase like endoglycosidases, mediate ECM reframing in an orchestrated manner [108]. Some literature suggests that "In desmoplastic tumors, in particular, mechanical interactions among rapidly proliferating cancer cells, cancer associated fibroblasts (CAFs), ECM fibers (collagen and hyaluronan), and the surrounding normal tissue lead to accumulation of intratumoral solid stresses, causing vessel compression and hypo-perfusion" [109–111]. The invasive and migratory potential of PDAC is increased due to the changes occurring in the ECM protein level.

However, various strategies are used to cure PDAC, but these are less effective against the complex microenvironment of PDAC. Recent studies state that "The 5-year survival rate for PDAC patients is less than 5%, which is an indicator of the failure of current therapies." Therefore, a more promising therapy that is more reliable with fewer risk factors as phytochemicals with the aid of nanomedicine should be explored.

13.4 Therapeutic Approach

From time immemorial till date, cancer treatment is the most worked on subject by scientists and researchers. Synthetic drugs have become the part and parcel of most cancer patients' life and are being employed to treat various types of cancer, including pancreatic cancer. Heavy usage of synthetic drugs have several side effects. Therefore, it was essential to find out a favorable means of medication for treating cancer more efficiently and with lesser side effects. Recently phytochemicals, compounds obtained from plant sources, have become a major focus as an anticancer agent. Over the past few decades, several epidemiological studies have proved that there exists a positive correlation between intake of phytochemicals present in plant products and reduced incidence of cancer [112]. Phytochemicals can act as therapeutic agents as well as components of regular diet for chemoprevention. Unfortunately, clinical utilization of phytochemicals has been hindered to some extent due to their poor systemic bioavailability [20]. The extrinsic pathological features of cancer cell that characterize PDAC, such as compressed blood vessels

and the desmoplastic stroma, are also found to limit the therapeutic efficacy of the treatments [113]. To overcome these obstacles and enhance the therapeutic approach, cancer nanotechnology emerged. It eventually led to development of new research aids, ways to diagnose, site-specific drug delivery systems, and efficient cancer treatment [114]. An emerging branch of medicine, that is, "Nanomedicine," has gathered much attention in treating various ailments along with cancer. Precisely, nanomedicine is the clinical application of nanotechnology, which seeks to deliver a valuable set of formulations, research tools, and medical equipment in the near future [115, 116].

13.4.1 Phytochemicals

Since time immemorial, medicinal plants have been widely used as a remedy for curing and preventing several diseases throughout the world. Knowledge about various medicinal plants makes us aware of their potential to cure headache, cold, cough, fever, poisonous stings, and other ailments [117]. Phytochemicals are active agents obtained from these medicinal plants, which aid in curing diseases. Experimental evidences have shown that medicinal plants and herbal preparations have anticancer effects [118, 119]. Various phytochemicals have shown anti-metastatic, antiproliferative, pro-apoptosis and anti-angiogenic effects in in vitro experiments and animal trials [16–18]. Some phytochemicals have been found to potentiate the impact of synthetic drugs, which ultimately proved to be helpful in treating cancer [19]. As efficient metabolic machinery of cancer cell is the key factor in aiding its unbridled growth, it can act as an effective target for chemoprevention. To this end, phytochemicals can be used to interfere cancer cell metabolism by different mechanisms like interrupting glucose uptake by downregulating the expression of its transporter, GLUT1, impairment of glutamine uptake, and so on [120]. Listed below are some major phytochemicals and their molecular mechanism that has earned positive results in terms of treating PDAC with lesser side effects (Table 13.1).

13.4.2 Phytonanomedecine

Research and development in the field of nanotechnology are growing across the world [147]. The nanoparticles are structures usually ranging from 1 to 100 nanometers (nm). The knowledge that functionalities can be added to nanomaterials by interfacing them with biomolecules has opened up a new window for biomedical research. Nanoparticles, due to their very small size and high surface area to volume ratio, can diffuse easily to different inaccessible body parts, even the blood–brain barrier. Additionally, nanoparticle surface can be easily modified by adsorbing the therapeutic drugs for delivery, which in turn penetrate the tumors with a higher specificity [22, 148]. In last few decades, nanotechnology has been used in imaging,

Table 13.1 Phytochemicals and their molecular mechanisms in PDAC chemoprevention

	Plant name	Phyto-chemicals	Molecular targets	Reference
1.	*Curcuma longa*	Curcumin	Inhibits NFkB, MMP2, and ERK1/2, WT1, STAT3, Notch-1, SP1, COX-II, ATM/Chk1	[12, 121–126]
2.	Cruciferous vegetables	Isothiocyanates	Inhibits AKT, STAT3, HDAC, NFkB	[13, 127, 128]
3.	*Camellia sinensis* var. *Sinensis*	Catechins	Inhibits HSP90, HSP75, HSP27, and inhibits the phosphorylation of AKT and p53	[129]
4.	Pines, berries, red grapes, and peanuts	Resveratrol	Reduces phosphorylation of FOXO, FOXO3a, AKT, PI3K, and ERK	[130–132]
			Inhibits STAT3	
			Targets Hedgehog pathway inducing apoptosis	
5.	*Zingiber officinale*	Gingerol	Inhibition of Cyclin A and (Cdk) expression	[14]
6.	*Scutellaria baicalensis*	Baicalein	Targets NEDD9 to decrease Akt and ERK activities	[133]
7.	*Glycine max*	Genistein	Decreases the number of mammospheres and CD44+ cells	[134]
8.	*Brassica oleracea*	Sulforaphane	Induces apoptosis, activating caspase 3	[135]
			Downregulates β-catenin	
9.	*Brassica oleracea*, *Allium sepa*, apple, cranberry, mango	Quercetin	Inhibits Wnt signaling	[136]
10.	*Reseda luteola*	Luteolin	Inhibits GSK-3β and NF-κB	[137]
11.	*Solanum lycopersicum*	Lycopene	Inhibits cerulein-induced upregulation of intracellular ROS, activation of NF-κB, and expression levels of IL-6 in pancreatic acinar cells	[15]
12.	*Allium sativum*	Extract	Increase the number and activity of natural-killer cells	[138]
13.	Tea, broccoli, grape fruit	Kaempferol	Inhibition of AKT, ERK1/2, and EGFR related Src pathways	[139]
14.	*Crocus sativus* L.	Crocetin	Cell cycle arrest and DNA fragmentation leading to PCD	[140]
15.	Citrus fruits	Obacunone	Initiation of caspase-9 and caspase-3, increases p53, Bax, and decreases Bcl2	[141]
			Inhibits NFkB and Cox-2 leading to cytochrome-c mediated intrinsic apoptosis pathway and inflammatory activity	

(continued)

Table 13.1 (continued)

	Plant name	Phyto-chemicals	Molecular targets	Reference
16.	Mushroom	Vitamin D	Increases production of prohormone 25-hydroxyvitamin D3 regulating cellular proliferation and differentiation	[142]
17.	Plant oil	Vitamin E (tocotrienols)	Suppressing PI3-kinase/AKT and ERK/MAP kinases via downregulation of Her2/ErbB2 expression leading to apoptosis	[143]
18.	*Momordica charantia*	Juiced fruit extract (freeze-dried)	Caspases activation, alteration of Bcl-2 family members expression and cytochrome, decreases inhibitor of apoptosis, and increases p21, CHOP, and phosphorylated mitogen-activated protein kinases levels leading to apoptosis	[144]
19.	Apples, strawberries, cucumber, and onions	Fisetin	Suppression of DR3 mediated NF-κB activation inducing apoptosis	[145]
20.	Fruits and vegetables	Apigenin	Induce G2/M phase cell cycle arrest, inhibits HIF-1α, GLUT-1, VEGF mRNA, and protein expression	[3, 146]

clinical diagnosis, and therapeutics. Nanoparticle-mediated site-specific delivery of drugs in cancer therapeutics can significantly minimize the drugs dosage with low toxicities and enhanced bioavailability [23]. The "**nanomedicine"** strategies have been shown to target autophagy, Hedgehog-signaling, and specific RAS-mutant phenotypes, among other pathological processes of PDAC. These unique therapies have shown to increase efficacy and reduce off-target toxicities [149]. Among all types of nanoparticles that are synthesized, metal and metal oxide nanoparticles are of greater importance [24]. Below is the list of some well-studied metal oxide nanoparticles in the context of PDAC therapeutics:

1. *Gold nanoparticles (AuNPs)*: The reason behind the use of AuNPs in cancer nanotechnology is its unique physiochemical properties such as (a) the ease with which it is synthesized by employing several simple, economical and reliable methods; (b) its broad shape and size range (2–500 nm) obtained by altering the reaction parameters; (c) high surface reactivity due to negative surface potential that aids in better interaction with biomolecules [150]; (d) novel electronic and optical characteristics making it an ideal material to be used as biosensors; (e) biocompatibility and non-toxicity [151–153]. In PDAC, EGFR (tyrosine kinase) expression levels are upregulated. So, blocking receptor tyrosine kinases (RTKs) can be used as a target to treat PDAC [154]. Patra et al. (2008) have fabricated the drug delivery system using AuNPs as carriage, gemcitabine as the anti-cancer drug, and cetuximab (anti-EGFR antibody) as targeting agent. They

have successfully shown that the above-mentioned delivery system has resulted in notable suppression of pancreatic tumor cell (PANC-1, AsPC-1, and MIA Paca2) proliferation in vitro and orthotopic pancreatic tumor growth in vivo [21].

2. *Cerium oxide nanoparticles (CONPs)*: ROS can operate both the initial progression and advancement of cancer cells as well as can downregulate antioxidant enzymes that normally combat free radicals [155]. CONPs are known to display a number of antioxidant behaviors like superoxide dismutase (SOD) activity [156], catalase mimetic activity [157], nitric oxide radical scavenging [158], hydroxyl radical scavenging [159], and direct oxidant behavior [160]. A study on mice model bearing pancreatic tumor has revealed that ROS in cancer cells are modulated by CONPs in a dual manner, one by direct toxic effect and the other by therapeutic properties that extend to radio-sensitization of cells [26]. CONPs are also found to address various radical associated problems driving and resulting from diabetes [161].
3. *Silver nanoparticles (AgNPs)*: Several research studies have shown that AgNPs induce oxidative stress that exert acute toxic effects on various cultured cells. However, the process by which AgNPs damaged cells remained veiled for many years. For the very first time Zang et al. (2012) have demonstrated that AgNPs-induced apoptosis is mediated by the ER stress-signaling pathway [162]. One of the recent studies has shown that size- and concentration-dependent synthesis of AgNPs is successful in reducing the viability and progression of PDAC cells ultimately leading to its death. Also, the ultrastructural analysis is suggestive about the cellular uptake of AgNPs that has led to apoptosis, autophagy, necroptosis, and mitotic catastrophe [27].

Discussing further, phytochemicals and nanoparticles have been used separately as a therapeutic agent in curing and preventing PDAC for a long time. But recently, researchers have started to amalgamate both together for better results. Zhao et al. (2019) have successfully biofabricated the ZnONPs using polyphenol with average particle size of 33 nm and confirmed their concentration-dependent cytotoxicity against PDAC cell lines [25]. Another study that deals with the systemic administration of polymeric nanoparticle encapsulated curcumin (NanoCurc) in preclinical models of PDAC has shown that NanoCurc increases the systemic bioavailability of free curcumin, which leads to halt in primary tumor growth [163]. But the above-mentioned studies are just an initiative step and it requires further investigation in the field of green nanotechnology, which will for sure lead to better outcomes in the near future.

13.5 Conclusion

Obesity and T2DM are the major key players governing the development of PDAC. There is a complex relationship between these two factors as they are often correlated but independently exercise their effects on PDAC. The change in inflammatory cytokines and insulin resistance as an effect of obesity and T2DM leads to altered

tumor microenvironment causing onset of PDAC. Several factors, like HFD, palmitate, endocannabinoids, and IAPP aggregates, further aid to cytotoxicity, increasing oxidative stress and elucidating inflammation eventually leading to progression of PDAC. The risk of PDAC in obese diabetic patients can become clinical as well as economic burden in future due to the growing population of these diseased individuals. Although the stated studies establish an expectant platform for future cancer therapy, the complex microenvironment of tumor creates hassle over the therapeutic approaches of PDAC. Furthermore, studies should be carried out to reveal the complex tumor milieu and the tumor-stromal interaction in cancer progression. There are ample of evidences proving that phytochemicals aided with nanoparticles show positive effect against the major hallmarks of cancer. Particularly, metal and metal oxide nanoparticles prove their efficacy in various fields such as efficient drug delivery, prolonged drug availability, reduced drug dosage, and eliciting suitable responses against the signaling pathways that help PDAC to grow. Nevertheless, more work has to be done in order to obtain sustainable and unfailing therapy to improve the status of diabetes and PDAC.

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Chapter 14
Tumor Biomarkers and Diagnosis of Pancreatic Adenocarcinoma

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Abstract Pancreatic adenocarcinoma is one of the highly malignant cancers of gastrointestinal tract. Patients during the initial stages usually do not present characteristic signs and symptoms. Even if malignancy is suspected, differentiation between the malignant and benign conditions might be challenging. Recently, an increasing interest has been focused on the utility of immunological tumor markers. The use of tumor biomarkers aims at catching/identifying the cancer at a relatively earlier stage, surveillance, response to the treatment, endpoints, and any adverse effects. Based on biochemical characteristics, tumor markers can be classified into multiple groups including genetic, glycoproteins, ribonucleic acids (RNAs), proteins, oncofetal antigenic markers, metabolite-based markers, and so on. In this chapter, we sought to discuss some important biomarkers that can be useful in the diagnostic evaluation of pancreatic adenocarcinoma.

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Abbreviations

AUC	Area under the curve
CA	Carbohydrate antigen
CSF	Cerebrospinal fluid
CTC	Circulating tumor cells
ctDNA	Circulating tumor deoxyribonucleic acid
EUS	Endoscopic ultrasound
FDA	Food and Drug Administration
FNA	Fine needle aspiration
IGF	Insulin-like growth factor
MIC-1	Macrophage inhibitory cytokine-1
PAC	Pancreatic adenocarcinoma
RNA	Ribonucleic acid
TGF-β	Transforming growth factor-β

14.1 Introduction

Tumor biomarkers are small molecules (usually proteins and glycoproteins) that are released either by the tumor cells or other tissues because of the tumor or other disease conditions. The main aim of a screening test is to catch the cancer at an early treatable stage prior to progressing to advanced non-curable stage [1, 2]. The efficient way to increase life expectancy and/or better quality-of-life for cancer patients is to catch the disease at an early stage using biomarkers.

The biomarkers constitute an essential part of the evaluation and management process in numerous cancers; therefore, they are included in several clinical practice guidelines for appropriate diagnosis and better management of persons with cancer. As a result of its importance, biomarkers are the focus of modern research as attempts are made to discover new ones with superior sensitivity, specificity, and cost-effectiveness. The use of tumor biomarkers includes, but is not limited to, in catching/identifying the cancer at a relatively earlier stage, in surveillance, in response to the treatment, at endpoints, in adverse effects, and of course in making an essential informed decision based on the results of biomarkers in minimizing the clinical course.

Based on biochemical characteristics, tumor markers can be classified into multiple groups including genetic, glycoproteins, ribonucleic acids (RNAs), proteins, oncofetal antigenic markers, metabolite-based markers, and so on. Significant advancements in the laboratory techniques (technologies) and expansion in the relevant scholarly research literature failed to significantly increase the use of these

biomarkers in the clinical settings. To date, only a few of the biomarkers are approved through regulatory agencies with limited use clinically. There are many regulations on the approval process of these biomarkers and, on average, approximately one biomarker is approved yearly by the United States Food and Drug Administration (FDA) agency. Despite broad research works being done in the field of biomarkers, only a few have made to the common practice use.

Tumor markers are non-invasive, more advantageous, and readily available aid during the diagnostic process. These can be utilized either alone or in combination with other biomarkers or diagnostic investigations to increase its efficacy, since they have low sensitivity and specificity when used alone, and due to this reason, they are highly criticized. That is why when combined with the standard diagnostic techniques and standard-of-care, biomarkers become more effective than being used alone [3, 4]. Therefore, in this chapter, we sought to summarize some of the key tumor biomarkers that are potentially helpful in the diagnosis of pancreatic adenocarcinoma (PAC; see also Table 14.1).

14.2 Biomarkers of Pancreatic Adenocarcinoma

14.2.1 Carbohydrate Antigen 19-9 (CA19-9)

Carbohydrate antigen 19-9 (CA19-9), also called Sialyl Lewis A, is one of the important and frequently used markers of pancreatic adenocarcinoma [23]. Structurally, it is an O-linked glycoprotein present on the adeno-cancerous cell of pancreas [24, 25]. It is used in the diagnostic evaluation, management, and monitoring of the clinical path in pancreatic cancer. CA19-9 has the sensitivity of 82% and specificity of 90% for the PAC screening and diagnosis. One should keep in mind that CA19-9 level is also high in some other clinical conditions like hepatic and pancreatic cysts, jaundice, and pancreatitis along with certain neoplasia, like colorectal and breast cancers. Approximately 10–20% of white population lacks CA19-9 due to the absence of the enzyme fucosyltransferase. Even CA19-9 is not decisive in the screening and diagnosis of PAC routinely, and despite this, it has been used widely in the clinical practice, and is well studied in the patients suffering from PAC. Newer studies have proved that CA19-9 is revealed more on the cell surfaces 2 years ahead of the development of PAC [26]. These newly conducted studies also acknowledged the diagnostic certainty of CA19-9 and is increased when sequenced with other biomarkers [27] such as insulin-like growth factor (IGF) and albumin, and in combination, substantially improves the sensitivity and specificity to comprehend chronic pancreatitis from PAC.

Table 14.1 Biomarkers' expression with sensitivity and specificity as reported in the peer-reviewed literature for pancreatic adenocarcinoma

Name of the biomarker	Level of biomarker expression	Specimen type	Diagnostic ability in cancer diagnosis		References	Remarks
			Biomarker sensitivity (%)	Biomarker specificity (%)		
Carbohydrate antigens (CAs)						
CA19-9	Increased	Serum	81	81	Su et al. [5]	Most extensively used and FDA approved
CA50	Increased	Serum	71.1	93.5	Wu et al. [6]	
CA72–4	Increased	Serum	63.4	75.2	Jiang et al. [7]	
CA125	Increased	Serum	66.8	83.3	Ćwik et al. [8]	
CA242	Increased	Serum	67.8	83	Zhang et al. [9]	
CEA	Increased	Serum	39.5	81.3	Zhang et al. [9]	
MIC-1	Increased	Serum	79	86	Chen et al. [10]	Can be used to differentiate pancreatic cancer from chronic pancreatitis
PAM4	Increased	Serum	76	85	Gold et al. [11]	Helps differentiate pancreatic cancer from benign pancreatic disease
S100A6	Increased	EUS-FNAC	88.2	90	Zihao et al. [12]	
OPN and TIMP-1	Increased	Serum	87	91	Poruk et al. [13]	Associated with tumor invasiveness and metastases
Osteoprotegrin and ICAM-1	Increased	Serum	88	90	Brand et al. [14]	Increased sensitivity and specificity when combined with CA19-9

microRNAs (miRNAs)						
miR-21	Increased	Stool samples	90	66.7	Yang et al. [15]	Early detection of precursor lesions with malignant potential
miR-155	Increased	Stool samples	76.7	73.3	Yang et al. [15]	Early detection of precursor lesions with malignant potential
miR-196a	Increased	Plasma	n/a	n/a	Wang et al. [16].	Early detection of precursor lesions with malignant potential
miR-210	Increased	Plasma	n/a	n/a	Ho et al. [17]	
miR-216	Decreased	Pancreatic juice	n/a	n/a	Szafranska et al. [18]	
miR-143 and miR-30e	Increased	Urine	83.3	96.2	Debernardi et al. [19]	
miR-205, miR-210, miR-492, and miR-1427	Increased	Pancreatic juice	88	87	Wang et al. [16]	
miR-217	Decreased	Pancreatic juice	n/a	n/a	Szafranska et al. [18]	
REG-4	Increased	Serum	94.9	64	Takehara et al. [20]	Combination of REG-4 with CA19-9 has 100% diagnostic sensitivity, which helps in early detection of pancreatic cancer
siC3b	Increased	Serum	AUC 0.81	n/a	Märten et al. [21]	Helps in detecting tumor recurrence
M2-PK	Increased	Serum	75 (71–79)	80 (64–95)	Harsha et al. [22]	Increased diagnostic sensitivity when combined with CA19-9
DUPAN-2	Increased	Serum	80 (48–80)	80 (75–80)	Brand et al. [14]	Useful in Lewis-null blood type patients
SPAN-I	Increased	Serum	82 (81–89)	83 (67–85)	Harsha et al. [22]	Useful in Lewis-null blood type patients

Abbreviations: *AUC* area under the curve, *EUS-FNAC* endoscopic ultrasound guided-fine needle aspiration cytology, *FDA* Food and Drug Administration, *MIC-1* macrophage inhibitory cytokine-1, *n/a* not available, *RNA* ribonucleic acid, *CEA* carcinoembryonic antigen, *DUPAN-2* pancreatic cancer-associated antigen, *ICAM* intercellular adhesion molecule 1, *M2-PK* pyruvate kinase M2, *OPN* osteopontin, *PAM 4* monoclonal antibody (Clivatuzumab), *REG4* regenerating islet-derived protein 4, *SPAN 1* S-Pancreas Antigen-1, *TIMP 1* tissue inhibitor matrix metalloproteinase 1

14.2.2 MicroRNA

MicroRNA (miRNA) is a short non-coding RNA that consists of 18–24 nucleotides. Its main functions are to maintain balance in the transitional process of messenger RNA transcripts [28, 29]. There is enough evidence to support the carcinogenicity of miRNA as a tumor suppressor of oncogene and this makes miRNA as an essential marker in the diagnostic evaluation, management, and prognosis of PAC [30]. The miRNA level is generally elevated in several malignancies, which also includes pancreatic cancer; hence, it can be used in the diagnosis of PAC. The following subtypes of miRNAs are increased in PAC, such as miRNA-21, miRNA-155, miRNA-146a, miRNA-196a [31], miRNA196b, miRNA-200a/b/c, and miRNA-217. The presence and stability of miRNAs in the stool, cystic fluid, and plasma—and their easy separation—make them ideal biomarkers for the PAC diagnosis [32, 33].

Research studies have verified the diagnostic usefulness of miRNA, but its use in clinical practice is highly contentious due to its fluctuating results, which could be due to the variability in the subjects' race, control sources, type of miRNAs, and the miRNA samples used. A study conducted by Wang et al. [16] in Caucasian population demonstrated a sensitivity and specificity of 64% and 89%, respectively [16]; however, Liu et al.'s [34] observations were variable in the study population consisting of Asians and found a sensitivity and specificity of 71% and 69%, respectively [35]. On the same ground, the diagnostic certainty was shown to be lower for single miRNA profiling as demonstrated by several studies including one by Liu et al. [34]. This study demonstrated expression of miRNA-155 in plasma samples as a biomarker for PAC; however, the lower sensitivity and specificity of 63% and 84%, respectively, showed their limited ability to comprehend PAC from chronic pancreatitis [34]. On the other hand, Ganepola et al. [36] noted that combining some of the miRNAs such as miR-22-3p, miR-885-5p, and miR-642b-3p can enhance the diagnostic accuracy by increasing both the sensitivity and specificity to 91% for early detection of PAC [36]. Up till now, a lot of studies have shown the diagnostic capability of microRNA [37–39] including a systematic review and meta-analysis performed by Sun et al. [35] but due to the diverse nature and unpredictability in the diagnostic certainty, further studies are required to confirm the diagnostic value of microRNA in the clinical setting of PAC.

14.2.3 Macrophage Inhibitory Cytokine-1 Versus CA19-9

The macrophage inhibitory cytokine-1 (MIC-1) belongs to family of cytokines known as transforming growth factor-β (TGF-β) and was formerly called as macrophage activation-associated gene [40]. MIC-1 is highly expressed in a number of clinical conditions including inflammatory, tissue injury, and carcinomas such as colorectal and prostatic carcinomas [41–45]. It was found by Koopmann et al. [46]

that pancreatic cancer patients were found to have high levels of MIC-1 as compared to the healthy controls and study subjects with non-malignant pancreatic neoplasms. The sensitivity for MIC-1, which is 71% as related to only 59% for CA19-9 in PAC patients, is high enough in differentiating from the healthy controls and non-malignant neoplasms, but despite having a comparatively lower specificity [46], MIC-1 was found to be superior over CA19-9 in resectable PAC patients [45, 46]. Nonetheless, the diagnostic certainty of MIC-1 for PAC is still not confirmed as the data obtained from the studies with relatively smaller sample sizes are not satisfactory enough. MIC-1 as a single tumor marker has been shown to have comparable diagnostic certainty to CA19-9 for PAC diagnosis as shown by a meta-analysis by Yang et al. [47]. Obviously, more rigorous research studies are desirable to better investigate the prognostic value of MIC-1 in PAC.

14.2.4 Pancreatic Cancer Derived Serum Exosomes

Research studies have shown the important role(s) of exosomes in building the tumor microenvironment [48, 49]. Exosomes are small vesicles with a diameter ranging from 30 to 100 nm and are secreted extracellularly by the healthy or malignant cells. It is unknown how the exosomes are made; its function and contents are not yet explained by molecular processes [50]. Exosomes play important role (s) in the progression and distant as well near/proxy metastases of the cancer via interactions with the tumor microenvironment in various ways [51]. Body fluids such as milk, saliva, tears, blood, lymphatic fluid, cerebrospinal fluid (CSF), and urine are found to have exosomes, which can easily be identified [52, 53]. Hence, exosomes for a particular cancer can be an important potential source of diagnosis and target for therapeutic interventions. For this purpose, further translational research is needed to find out more about the importance of these exosomes at cellular/molecular levels (Fig. 14.1).

14.2.5 Glycoproteomics

The role of glycosylation in the pathogenesis of cancer has been widely studied, and more recently, we have gained better understanding about its biological roles and regulatory functions. Glycans have roles in nearly all human diseases, ranging from infections, inflammation, and diabetes to neurodegeneration [54–57]. After the synthesis of proteins, glycosylation is one of the most vital steps that occurs post-translational modification. Glycoproteomic concept has been used for the recognition of multiple N-linked glycoprotein modifications associated with a wide array of disorders. Aberrant changes in the glycosylation arrangement have been identified on these glycoproteins in malignancies. Glycan modifications are rapid and dramatic as a result of diseases, which renders it a reliable qualitative tumor marker in terms of

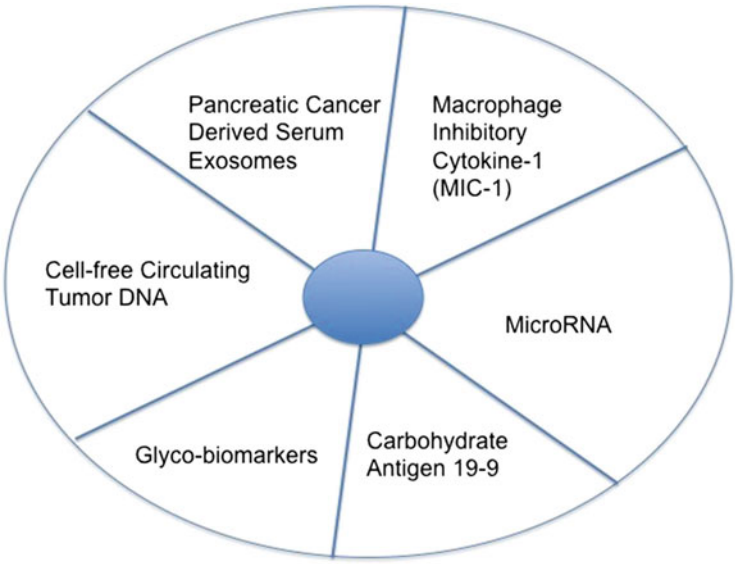

Fig. 14.1 Schematic illustration of various chemical biomarkes studied for the diagnostic utility of pancreatic adenocarcinoma

the predictive value for the disease [58]. Some glycoproteins with the altered glycosylation in tumor cells can be released into the serum, which can be used as clear-cut tumor markers [59, 60]. As a result, the distinct glycoforms of the changed glycoproteins, when identified, could be utilized as cancer biomarkers with relatively higher specificity in comparison to the quantitative levels of the glycoproteins. Because of this, glycoproteomic technologies are of paramount importance to detect the modified glycoforms on glycoproteins, which are produced as a result of specific tumor, and utilize these glycoforms as a potential cancer biomarker.

These so-called "glyco-biomarkers" can effectively be used for the early detection of PAC. The identification of a particular glycoform in combination with the protein levels could increase the diagnostic capability of the glycoproteins as biomarkers. We should keep in mind that aberrant glycosylation is disease specific but not tissue specific, which means that alterations in the glycoprotein such as fucosylation or sialylation could typically be common to epithelial cancers. Moreover, utilizing the multi-marker sets consisting of the particular glycoprotein(s) in combination with CA19-9 and other standard diagnostic utilities (such as imaging) could enhance the sensitivity and specificity of a single biomarker leading to better treatment/management decisions for patients with pancreatic cancer.

14.2.6 Other Blood-Based Markers

In addition to glycoproteomic methods, a lot of scholarly research works have been done in search of biomarker(s), which could detect pancreatic cancer at a comparatively early stage [61–64]. Harsha et al. [22] analyzed a wide range of new protein (biomarkers) candidates (207 over-expressed proteins), but many of the candidates were unable to differentiate between the benign and malignant conditions [65–67].

Some non-protein molecules have also been under research investigation. Pancreatic malignancy alterations in genes can also be identified by cell-independent circulating tumor deoxyribonucleic acid also called ctDNA or free-floating neoplastic cells [68–72]. Pancreatic neoplasia has been allocated based upon usually mutated genes like *KRAS*, *TP53*, *SMAD4*, and *CDKN2A* [73, 74], which may possibly be clinically linked. Recognition of these alterations in genes is based on ctDNA or circulating tumor cells (CTCs) [71, 72]. Bettegowda et al. [68] found that ctDNA has been identifiable in 40% of stages I–III patients with pancreatic cancer that increases to 90% in stage IV cancer. On the other side, Kinugasa et al. [69] have shown KRAS mutation in ctDNA in 63% of pancreatic cancer patients of all stages, 20% of chronic pancreatitis patients, and 5% of the healthy control individuals. Hence, they have the capability to be used as a biomarker for early identification of the disease, and of course they will have certain limitations such as a low sensitivity, methodology used for the detection, so we cannot say yet with certainty that these markers can be used to replace tissue biopsies [70, 75, 76].

14.2.7 Combination Biomarkers

Combining various biomarkers may enable to achieve high sensitivity and specificity for the very early diagnosis of pancreatic cancer [27, 77–80]. Sefrioui et al. [81] reported in a new research study that after combining the CA19-9, CTC, and KRAS gene alteration, the circulating DNA in patients who went through endoscopic ultrasound guided-fine needle aspiration (EUS-FNA) were relatively better prognostic markers [81]. The CA19-9 combination with CTC and circulating DNA analyses enhanced the sensitivity and specificity considerably (78% and 91%, respectively) as compared to the EUS-FNA alone (about 73% and 88%, respectively). Moreover, diagnostic achievement increased significantly when the CA19-9 was used in combination with other markers compared to CA19-9 alone in the patients of cholestasis. In addition, a study [77] assessed the plasma concentration of miR-16, miR-196a, and CA19-9 in pancreatic cancer cases, the chronic pancreatitis cases, and the healthy controls groups, and showed that adding these markers improved the diagnostic certainty as compared to CA19-9 or miRNA alone (area under the curve [AUC] for CA19-9 alone = 0.903, AUC for miRNA panel = 0.895, and AUC for combination panel = 0.979)] [77]. The importance of the obtained data is rather unsure and hence for more widespread clinical proofs, multi-center trials with larger-

size patients' groups need to be done to further strengthen these initial findings. Moreover, the methods employed for the detection of these biomarkers should be simplified to be able to combine into the regulatory-approved clinical laboratories [82].

14.3 Conclusion

With the advent of newer targeted therapies and chemotherapeutic regimens, pancreatic biomarkers can prove to be of significant importance in the overall management of pancreatic adenocarcinoma. However, in the current situation, more studies and translational research works are desirable to better evaluate the prognostic value of these biomarkers for improved diagnosis and management of patients with pancreatic adenocarcinoma.

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Chapter 15
The Role of HIF-1α in Hepatocellular Carcinoma

Saimila Momin and Ganji Purnachandra Nagaraju

Abstract Hepatocellular carcinoma (HCC) is one of the top leading causes of death around the world, with more than 800,000 individuals diagnosed on a yearly basis. There are many factors that contribute to the development of HCC and a strong risk factor includes chronic liver diseases or diseases that cause damage to the liver. Hypoxia-inducible factor 1 (HIF-1) alpha or HIF-1α subunit has shown to play a role in many types of cancers, including HCC. Understanding the role of this subunit in HCC will take us a step closer in finding strong therapies and treatment options for patients diagnosed with HCC and possibly with other cancers. The hypoxia-inducible factor is a transcription factor that consists of two subunits, HIF-1α and HIF-1 beta (HIF-1β). In normal conditions, when oxygen is readily available, the HIF-1α subunit degrades and the beta subunit translocates to the nucleus to exert its transcriptional function (Fig. 15.1). However, during time of hypoxia, when oxygen levels have significantly decreased, HIF-1α stabilizes and also translocates in the nucleus with the beta subunit. Expression of this heterodimer plays a major role in cellular response during hypoxia. However, over-expression of this heterodimer can lead to the promotion of tumorigenesis in many parts of the body, including the HCC (Fig. 15.2). Understanding the link between HIF-1α and HCC can allow researchers to potentially construct therapeutic options that focus on manipulating the HIF-1α pathway to decelerate the rate of cancer or even reverse its effects. In this chapter, we explore the pathophysiology of liver cancer and the role that HIF-1α plays and more importantly, focus on current investigations researching therapies associated with HIF-1α.

Keywords Hepatocellular carcinoma · Normoxia · Hypoxia · Hypoxia-inducible factor

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Abbreviations

CBP	CREB-binding protein
EMT	Epithelial mesenchymal transition
HCC	Hepatocellular carcinoma
HIF-1α	Hypoxia-inducible factor 1 alpha
HIF-1β	Hypoxia-inducible factor 1 beta
pVHL	von Hippel-Lindau tumor suppressor protein
VEGF	Vascular endothelial growth factor

15.1 Introduction

The liver is one of the major and largest internal organs of the human body that serves as a vital component of the human body. The liver functions to break down toxic wastes in the blood along with alcohol and drugs. It also produces and distributes bile, a digestive juice, to the intestines that aids in fat absorption and digestion. Additionally, the liver metabolizes drugs and nutrients that require chemical modifications before these substances can be used to function in the body. The liver is an important organ in the human body, which needs to remain healthy for the organism to survive; hence, carcinoma or tumors can impede its functions and lead to detrimental effects throughout the body.

Hepatocellular carcinoma (HCC) is one of the leading causes for global mortality. It is estimated that in the year 2020 in the United States of America, there will be 42,810 new cases and approximately 30,160 or approximately 71% of these cases will lead to fatality [1]. In many countries such as sub-Saharan Africa or Southeast Asia, HCC is one of the most common types of cancer with more than 800,00 people diagnosed annually [1]. Individuals who are prone to viral infections especially hepatitis B and C and other liver diseases such as cirrhosis and nonalcoholic steatohepatitis and metabolic disorders such as diabetes mellitus and hemochromatosis have increased chances of developing HCC [2, 3]. Additionally, other factors that contribute to HCC include but are not limited to tobacco use, alcohol consumption, diet, and obesity [3]. Signs and symptoms of HCC include but are not limited to unexplained weight loss, loss of appetite, feeling full, enlarged liver and/or spleen, abdominal pain, abdominal welling, jaundice, hypoglycemia, and high cholesterol levels [4]. There are also different types of hepatocellular carcinoma. These include intrahepatic cholangiocarcinoma, angiosarcoma, hemangiosarcoma, and hepatoblastoma. Additionally, different types and sizes of tumors can also appear. However, regardless of the type of carcinoma or tumor, the prognosis and treatment options remain the same across. Currently there are very limited treatment options for HCC; treatments depend on how aggressive the cancer is and the cancer's stage level. Treatments include liver transplant in individuals where the cancer has not spread beyond the liver, chemical ablation, embolization therapy, hepatectomy,

chemo and radiation therapy, and immunotherapy [3, 4]. However, there are no molecular targeted therapies for HCC, especially one targeting hypoxia-inducible factor 1 alpha (HIF-1α), which plays a major role in various types of cancers [5]. Determining a way to manipulate the HIF-1α and its pathway can lead to a promising future for patients with HCC [5]. In this chapter, the HIF-1α pathway is explained and its functional relevance to HCC along with current investigations that include or directly target the HIF-1α pathway.

15.2 HIF-1α

HIF1A gene encodes the hypoxia-inducible factor 1, which is a heterodimeric transcription factor that consists of an oxygen-sensitive alpha (α)-subunit and a helix–loop–helix structure. HIF-1α responds to the levels of oxygen available in the body [6, 7]. Its primary responsibility is to deliver oxygen throughout the body via angiogenesis [7]. HIF-1α reacts in two ways, depending on the oxygen levels. When oxygen is available, or under normal conditions, HIF-1α undergoes post-translational modifications; in this case it becomes hydroxylated at certain proline residue sites. Here it will then interact with the von Hippel-Lindau tumor suppressor protein (pVHL), inducing post-translation modification via ubiquitination and deterioration of proteasome (Fig. 15.1) [8]. Ultimately, this process will yield an inactivation or degradation of the α-subunit. Additionally, the beta (β)-subunit will

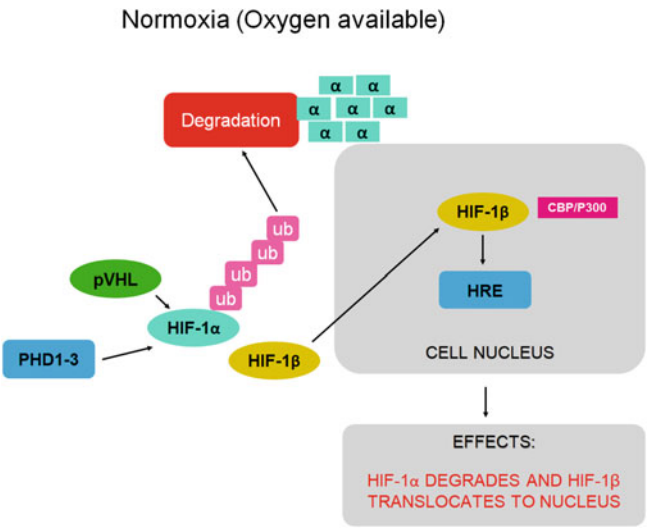

Fig. 15.1 During normal conditions, when oxygen is readily available, hypoxia-inducible factor 1 alpha (HIF-1α) subunit interacts with the von Hippel-Lindau tumor suppressor protein (pVHL) and induces post-translation modifications via ubiquitination and deterioration of proteasome, which leads to degradation of the α-subunit

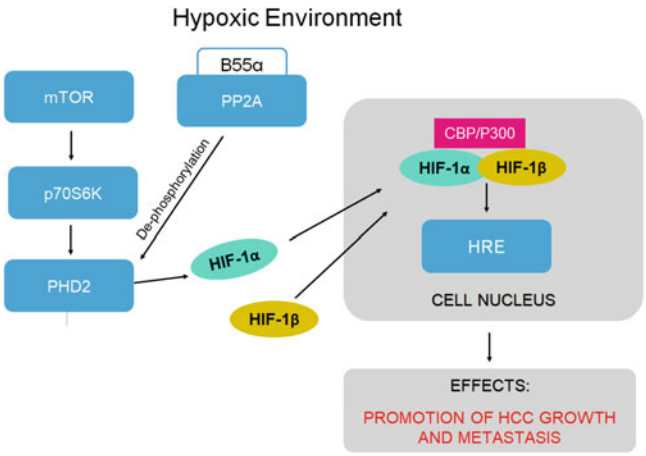

Fig. 15.2 During hypoxic conditions, the mammalian target of rapamycin (mTOR) and p70S6K are activated. Prolyl hydroxylase domain protein 2 (PHD2) undergoes dephosphorylation via PP2A-B55α. This leads to the activation of hypoxia-inducible factor 1 alpha (HIF-1α) and prevents its degradation, allowing it to translocate to the nucleus

translocate into the nucleus and interact with CREB-binding protein (CBP) and p300 transcriptional co-activators (Fig. 15.1) [3]. On the other hand, during times of inadequate oxygen levels, the HIF-1α is stabilized instead of degraded [9].

During hypoxic conditions, which is characterized as a condition in which the body is deprived of adequate oxygen supply at the tissue level, either throughout the body or in a specific region of the body, the proline residue sites, where HIF-1α undergoes hydroxylation, become inhibited [3]. Therefore, HIF-1α is unable to interact with pVHL and the subsequent processes, including ubiquitination and proteasomal degradation, are inhibited. Additionally, the HIF-1α subunit is not degraded during hypoxic conditions and instead the α-subunit is translocated to the nucleus, where both the α and β subunits undergo dimerization (Fig. 15.2) [3, 10].

This dimerization leads to both subunits binding to the deoxyribonucleic acid (DNA) and activation of specific genes. Over-expression of the dimer, especially in patients with chronic liver conditions, can lead to the promotion of HCC [5] (Fig. 15.2).

15.3 HIF-1α and Its Role in HCC

HIF-1α is a transcription factor that not only plays a role in HCC but also has been linked to many other cancers. Therefore, understanding the connection and its role in HCC can aid us in the future in creating therapies and treatment options for those who are diagnosed [11].

As mentioned above, during hypoxic conditions, the stabilized alpha and beta subunits heterodimerize and bind with the DNA to aid in compensating for the depleted oxygen levels in the body. HIFs in general regulate genes involved in cell proliferation, such as growth factors including insulin-like growth factor 2 (IGF-2) and transforming growth factor alpha (TGFα), angiogenesis, energy metabolism, and regulating immune cells, especially since HIF leads to inflammation [3, 11].

Additionally, individuals diagnosed with chronic liver diseases, such as nonalcoholic fatty liver disease and alcoholic liver disease, are more prone to a state of hypoxia [3]. Furthermore, the disease state leads to liver damage and deterioration, a perfect environment to host and nurture cancer cells and tumors. Over-expression of this heterodimer can lead to inappropriate levels of liver inflammation and above all inappropriate cellular proliferation, which can ultimately accelerate the rate of cancer [12].

Furthermore, in patients with HCC, other factors and pathways can be activated to expedite metastasis. During hypoxia, the Wnt/β-catenin pathway and PI3K/AKT pathway can be stimulated and can induce the epithelial mesenchymal transition (EMT), which leads to cellular motility [3, 13]. Additionally, the hypoxia can lead to decreased levels of IFT88/TG737, which increases cellular invasion. Also, factors such as CXCL6 and SERPINB3 can increase metastatic capability of HCC cells [3]. Another important target of HIF-1α is the vascular endothelial growth factor (VEGF), which leads to angiogenesis and tumor proliferation [11]. Overall, all these factors and pathways can accelerate HCC and possibly lead to the spreading of cancer to other body organs. However, there have been investigations conducted to see how HIF-1α can be manipulated to prevent tumorigenesis.

15.4 Current Investigations

In one study, the researchers investigated how angiogenesis could be halted using angiostatin gene therapy in EL-4 tumors in mice; angiostatin is a natural inhibitor of angiogenesis. With just angiostatin, angiogenesis stopped for a total of 6 days; however, afterwards the tumors increased the generation of HIF-1α and the vascular endothelial growth factor, which led to both angiogenesis and tumorigenesis [14]. However, the researchers then used antisense HIF-1α, which decreased HIF-1α levels and VEGF levels, along with other factors that were a result of HIF-1α expression [14]. The use of antisense HIF-1α completely destroyed small tumors (diameter of 0.1 cm); however, with larger tumors (diameter >0.4 cm) it was only able to temporarily suspend tumor growth [14]. However, when both angiostatin and antisense HIF-1α were injected into the mice, even the large tumors had completely disappeared within 2 weeks [14]. The data strongly suggest that HIF-1α plays a major role in cancer by up-regulating tumor growth and angiogenesis and also demonstrates a way to decrease HIF-1α expression [14]. This study shows promising ways to carefully manipulate HIF-1α to prevent the growth of tumors and eradicate them. However, this study definitely needs to be replicated in HCC and

eventually in human test studies. The implications of this study are quite significant and definitely pave the pathway for novel HCC treatment options.

HIF-1α not only accelerates and promotes tumorigenesis in HCC but also has the ability to generate resistance to anticancer drugs. In another study, it was investigated if antisense HIF-1α, combined with doxorubicin, an anticancer drug, can increase the efficacy of doxorubicin, in mice models [15]. The study revealed that the combination of antisense HIF-1α and doxorubicin definitely aided in multiple areas, including decreasing tumor growth, angiogenesis, cellular proliferation, and apoptosis [15]. Specifically, the antisense HIF-1α decreased HIF-1α and VEGF expression levels and doxorubicin decreased VEGF expression [15]. The combination of both antisense HIF-1α and doxorubicin led to a strong synergistic effect that helped to significantly decrease VEGF levels, which strongly suggests that antisense HIF-1α has the ability to improve the efficacy of doxorubicin [15]. The data show us that antisense HIF-1α not only has the ability to independently alter expression levels of HIF-1α and VEGF, but also has the ability to increase the efficacy of anticancer drugs. However, this study needs to be applied in HCC cases and with other anticancer drugs to see if the increased efficacy effects can be mimicked in other drugs.

15.5 Conclusion

As seen above, there are investigations that are being conducted to determine the correlation between HIF-1α and HCC and to also determine how the factor and its pathways can be manipulated positively in order to create new treatments or enhance old treatment options. However, it is important to note that HIF-1α is not the only nor the dominant factor that controls HCC. HCC is dominated by multiple molecular factors, cellular mechanisms, and even environmental factors; HIF-1α is simply one of the larger pieces of this convoluted puzzle.

There are many questions to be answered and studies that need to be conducted in order to understand the HIF-1α factor and its pathway. For example, in what ways can we prevent the activation of the Wnt/β-catenin pathway and PI3K/AKT pathway in order to block the epithelial mesenchymal transition. Additionally, how can we maintain the levels of IFT88/TG737 during hypoxia. And lastly, how can we decrease inflammation during hypoxia. Specifically, for the questions above, it is important to, first, understand how each of these mechanisms can be altered without disturbing other pathways and mechanisms and, second, to engineer drugs or treatment options that actually alter the above factors and pathways. The journey to finding long-lasting drugs and treatment options is definitely not complete. Nonetheless, determining its mechanism and role in HCC will definitely bring us one step closer to understanding how we can diagnose HCC earlier through prognostic markers, prevent the spread of HCC to other areas, and, one day, even find a full-proof treatment that will permanently end HCC.

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