RIPK pathway, as a Potential Target, for Cancer Treatment - A Review

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy
BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “RIPK pathway, as a potential target, for Cancer Treatment” submitted by Nusrat Jahan (19146057) of Summer, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on February 2023.

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Ethics Statement

This study does not involve any kind of animal and human trial.

Abstract

Cancer is one of the most feared diseases whose prevalence has been drastically increasing in recent years. Among different types of treatment options, the development of kinase-targeted cancer therapies is currently the most studied target for potential anti-cancer therapies. After G-protein-coupled receptors, protein kinases significantly grab the attraction as a target of developing anti-cancer drugs. Based on current research, this review article outlines receptor-interacting protein kinases specially RIP1 and RIP3 as attractive targets over other kinases in the treatment of numerous types of cancer, especially for their tumorigenesis functions in cancer metastasis.

Keywords: Cancer; RIPK; Pathway; Inhibitors; Therapy; Anti-cancer drugs.

Dedication

Dedicated to the innocent lives that cancer has taken

Acknowledgment

First of all, I want to thank God, the Almighty Allah, for granting me good health and the patience, dedication, and knowledge I needed to finish the thesis.

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List of Acronyms

HIV	human immunodeficiency virus
EGFR	epidermal growth factor receptor
PI3K	phosphatidylinositol 3-kinase
ERK	extracellular signal-regulated kinase
AKT	protein kinase B
Grb2	growth factor receptor-bound protein 2
SOS	son of sevenless
PIP3	phosphatidylinositol-3,4,5-triphosphate
MAPK	mitogen-activated protein kinase
ERK	extracellular signal-regulated kinase
IGF1	insulin-like growth factor 1
SKP2	S-phase kinase-associated protein ubiquitin ligase
TGFβ	Transforming growth factor-β
PTK6	Protein tyrosine kinase 6
IKKε	I-kappa-B kinase epsilon
TBK1	TANK-binding kinase 1
GTP	guanosine triphosphate
FAK	Integrin-focal adhesion kinase
NF-κB	Nuclear factor kappa B
ROS	reactive oxygen species
ID	intermediate domain

COR	C-terminus of Roc
RHIM	respective homotypic interaction motif
RIP	receptor-interacting serine/threonine kinase
DD	death domain
SgK288	Sugen kinase 288
LRRs	leucine-rich repeats
Roc	Ros of complex proteins
DRs	death receptors
TNFR1	Tumor necrosis factor receptor 1
TRAIL	TNF-related apoptosis-tempting ligand
AML	acute myeloid leukemia
CARD	caspase-recruiting domain
Raf	rapidly accelerated fibrosarcoma
TKIs	tyrosine kinase inhibitors
NSCLC	non-small cell lung cancer
SCC	squamous cell carcinoma
RIPK	Receptor interacting protein kinase
MEK	Mitogen-activated protein kinase
ECM	extracellular matrix

RIPK Pathway, as a Potential Target, for Cancer Treatment

Chapter 1

Introduction

1.1 Cancer

Cancer is the 6th leading cause of death globally in the 20th century and it has become one of the most dreadful diseases of the 21st century(*The Top 10 Causes of Death*, n.d.). Cancer is a combination of various diseases. According to National Cancer Institute Cancer can be simply defined as unmanageable and spreadable cell development in any body part. The birth of new cells occurs only due to the death or damage of the previous older cells. By breaking this universal cell cycle rule, sometimes different unusual types of cell growth and their uncontrollable multiplication occurs through cell division, and those are exposed as tissue lumps or tumors (cancerous or benign). Through the invasion of neighboring tissues (metastasis), cancerous or malignant tumors are transportable on their own. n. In recent decades, the rising trend of cancer has created anxiety among doctors, pharmacists, and people. By 2040, experts expect that 29.5 million new cases of cancer will be filed per year along with 16.4 million death. The disease affects the mental health of the patients and their families. A decrease in productivity in cancer patients and premature mortality cause a great hindrance to the national economy(*The Economic Burden of Cancer | The Cancer Atlas*, n.d.).

1.2 Types of Cancer

Cell-specific:

- Carcinoma: They are developed by epithelial cells, covering the body's inner and outer surface. Adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma are some subtypes covering breast, colon, prostate, bladder, ureters, and kidney cancers.
- Sarcoma: cancers that build in bones and soft tissues such as Osteosarcoma
- Leukemia: Cancers that build in the bone marrow and cause abnormal growth of white blood cells are called leukemias.
- Lymphoma: They build in lymphocytes which are basically T or B cells, part of the immune system of the body, and cause abnormal growth of lymphocytes in lymph vessels and lymph nodes of different organs.
- Multiple Myeloma: Cancers of plasma cells. They cause the development of abnormal plasma cells in the bone marrow.
- Melanoma: Cancers in melanocytes that produce melanin responsible for the pigmentation of the skin(*What Is Cancer? - NCI*, n.d.) .

Organ-specific:

- Based on the affected organ, cancer can be of different types. For example; breast cancer, kidney cancer, liver cancer, prostate cancer, intraocular, retinoblastoma, colon cancer, anal cancer, rectal cancer, pancreatic cancer, appendix cancer, gastric cancer, gallbladder cancer, and so on(*Cancers by Body Location/System - NCI*, n.d.)

1.3 Etiology

The impact of different types of cancer is increasing gradually. Different types of cancer have different causes affecting different body parts.

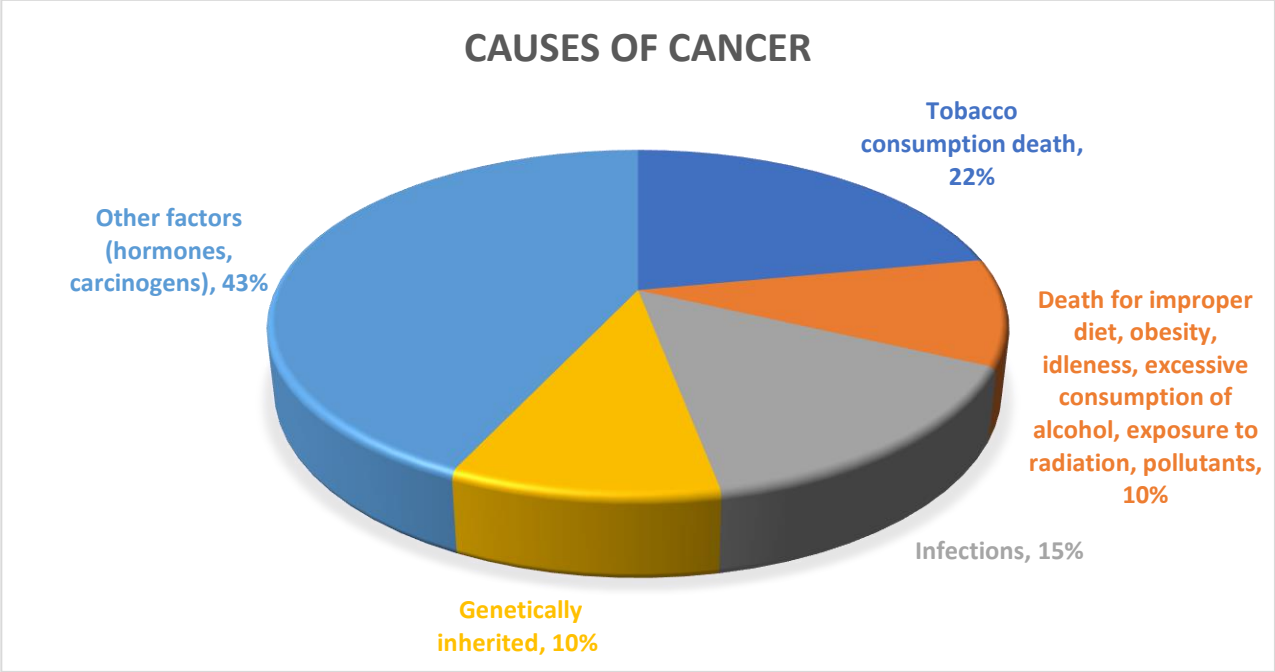

Figure 1: Causes of cancer (Saini A, Kumar M, 2020)

From the diagram, it is clear that 22% of worldwide cancer patients die as a consequence of tobacco consumption, and 10% of them die from an indiscipline lifestyle, exposure to radiation, and environmental pollution. 15% of cancer occurs worldwide because of different types of infections like Epstein - Barr virus, human papillomavirus infection, hepatitis b, hepatitis c, helicobacter pylori, and lastly human immunodeficiency virus (HIV) while 10% of cancer is proven as genetic defects which is inherited. Hormones and different types of carcinogens (physical, chemical, biological) are also accountable for different types of cancers.

1.4 Risk Factors

Various risk factors like obesity, smoking. consumption of alcohol, physical inactivity, smoking, air pollution, indoor smoking etc. act behind different types of cancers which are outlined below (Table1):

Table 1: Risk factors of different types of cancers (Danaei et al., 2005).

▪ **Risk factors**	▪ **Cancers**
▪ Obesity	▪ Corpus uteri cancer, colorectal cancer, kidney cancer, menopausal breast cancer, and gallbladder cancer.
▪ Insufficient intake of fruit and vegetables	▪ Colorectal cancer, lung cancer, stomach cancer, esophageal cancer.
▪ Physical inactivity	▪ Breast cancer, prostate cancer, colorectal cancer,
▪ Smoking	▪ Lung cancer, mouth cancer, oropharynx cancer, leukemia, esophageal cancer, stomach cancer, pancreatic cancer, liver cancer, cervix uterine cancer, and bladder cancer.
▪ Alcohol	▪ Liver cancer, breast cancer, mouth cancer, oropharynx cancer, esophageal cancer.
▪ Unsafe sexual and reproductive health	▪ Cervix uteri cancer
▪ Air pollution	▪ Lung cancer
▪ Indoor smoking	▪ Lung cancer
▪ Contaminated injection	▪ Liver cancer

1.5 Signaling Pathways in Cancer Therapy

The disease, cancer, is associated with several different pathways. Some of the common pathways are the epidermal growth factor receptor (EGFR) signaling pathway, vitamin D signaling pathway, PI3K (phosphatidylinositol 3-kinase)/AKT (protein kinase B) signaling pathway, and the extracellular signal-regulated kinase (ERK) signaling pathway.

1.5.1 EGFR signaling pathway

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor belonging to the ErbB family of cell membrane receptors. In addition, EGFR is also known as HER1/ErbB-1. Different types of receptors belong to this ErbB family e.g. HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). EGFR is composed of 3 different regions including an extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic tyrosine kinase-holding domain(Krasinskas, 2011). EGFR activates two important pathways such as the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase- (PI3K-) protein kinase B (AKT) pathway which ultimately activate many transcription factors that play a significant role in cellular responses like cell proliferation, migration, differentiation, and cell apoptosis(Citri & Yarden, 2006).

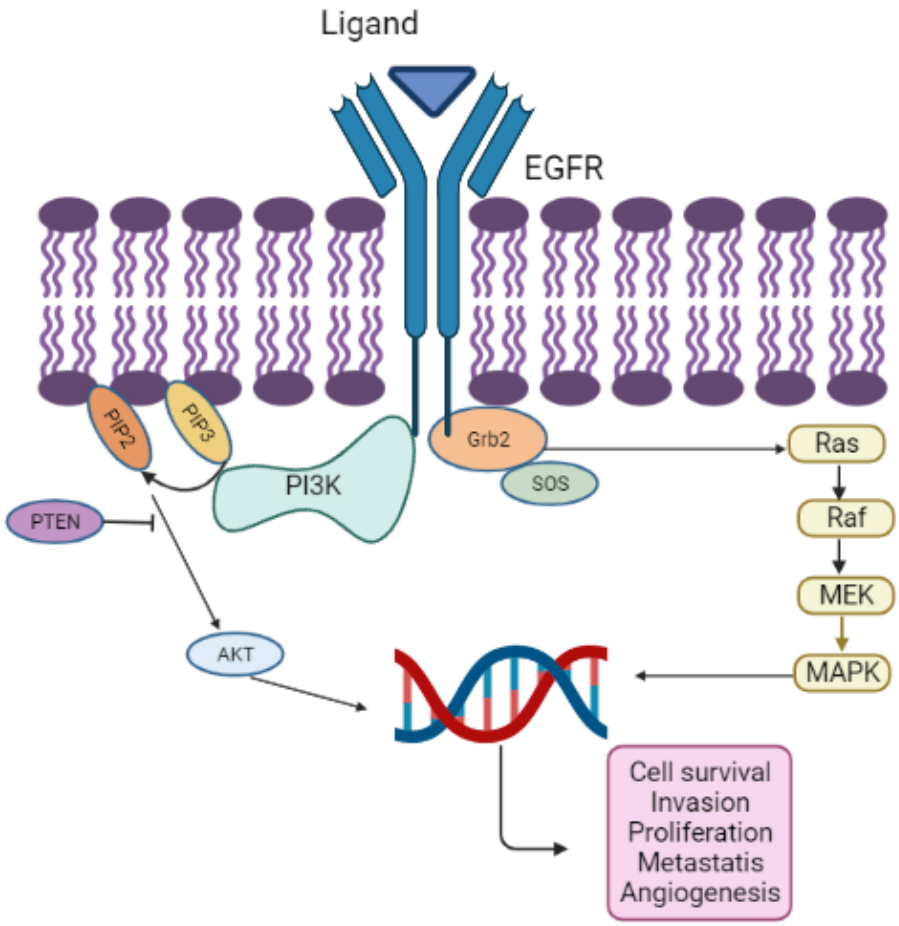

Figure 2: epidermal growth factor receptor (EGFR) signaling pathway (adapted from(Mitsudomi & Yatabe, 2010))

Dimerization occurs after Ligand binding and thus it activates the EGFR. Autophosphorylation of tyrosine residues promotes downstream signaling. In the EGFR signaling pathway, one of the patterns is Ras-Raf-MEK-MAPK, an adaptor protein complex composed of growth factor receptor-bound protein 2 (Grb2) and son of sevenless (SOS) activates the Ras GTPase. After activation, Ras recruits and then activates the serine protein Raf (i.e., B-Raf), and subsequent phosphorylation and activation of MEK, and then MAPK happens. As a consequence, transcription factors activate in the cell nucleus. The Ras-Raf-MAPK signaling pathway is responsible for controlling cell growth, differentiation, and survival(Mitsudomi & Yatabe, 2010; Spano et al., 2005). The other pattern of the EGFR signaling pathway is the PI3K-AKT

pathway. After phosphorylation of EFGR tyrosine residues, PI3K is translocated and binds to tyrosine phosphate in the cell membrane which eventually produces phosphatidylinositol-3,4,5-triphosphate (PIP3). PI3K then encourages AKT activation. Activated p-AKT activates numerous targets presenting within the cytoplasm of the cell(Krasinskas, 2011).

1.5.2 Vitamin D signaling pathway

Many Epidemiological studies found that Vitamin D deficiency increases the risk of numerous cancers like colon, breast, and prostate cancer.

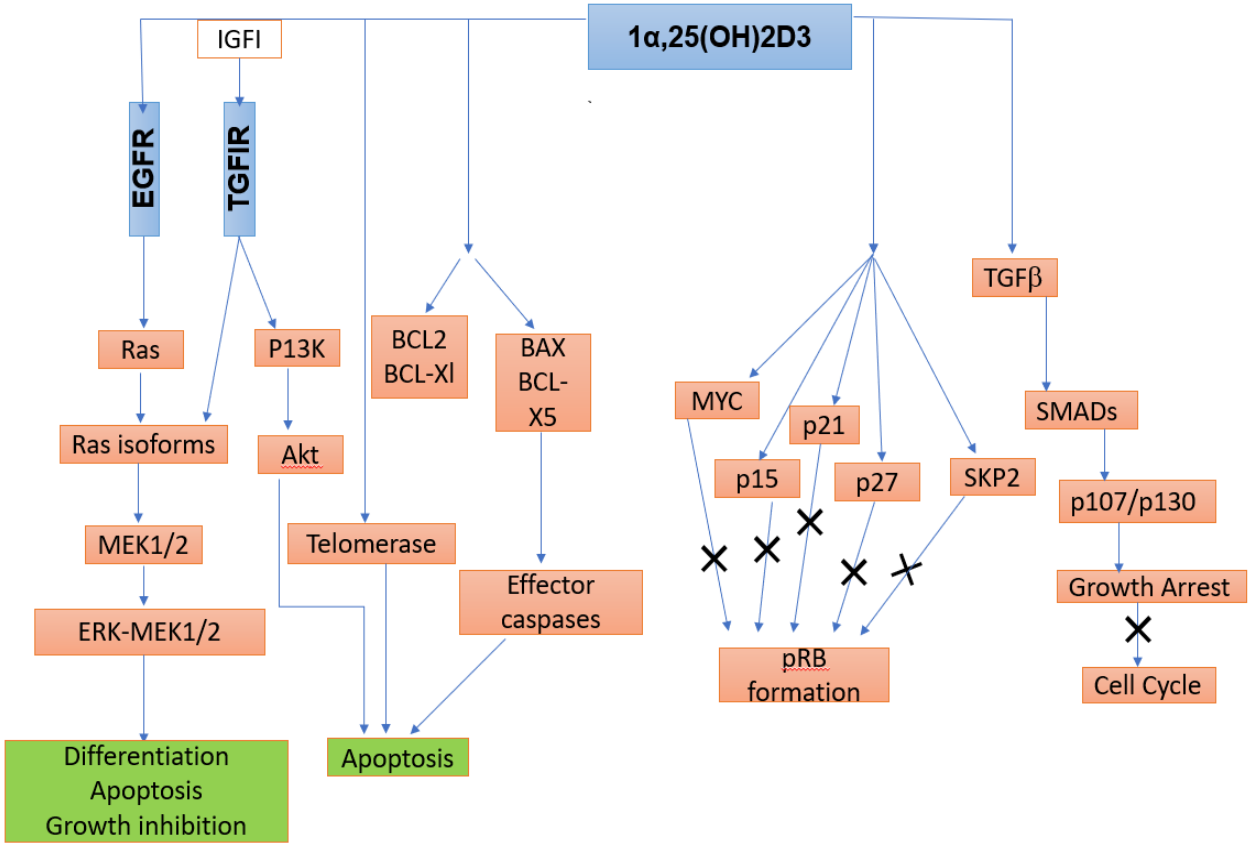

Figure 3: Signaling pathways targeted by 1α,25(OH)2D3 (adapted from ((Deeb et al., 2007))

The figure shows that 1α,25(OH)2D3 hinders mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase (ERK) 1 and 2 signaling through the destruction of epidermal growth factor (EGFR) and insulin-like growth factor 1 (IGF1), which both target Ras. 1α,25(OH)2D3 persuades apoptosis by the IGFR1−phosphatidylinositol 3-kinase (PI3K) − Akt-dependent signaling pathway (Erben et al., 2002). 1α,25(OH)2D3 inhibit telomerase, downregulates BCL2, produces BAX and activates caspase cleavage which causes apoptosis. Cell-cycle progression is disturbed by 1α,25(OH)2D3 through S-phase kinase-associated protein ubiquitin ligase (SKP2), and MYC, which causes pRB dephosphorylation. Transforming growth factor-β (TGFβ; f) affects the retinoblastoma pocket proteins (pRB, p107/p130) which causes growth arrest and blocks cell-cycle progression (Deeb et al., 2007).

1.5.3 PI3K/AKT pathway

PI3K (phosphatidylinositol 3-kinase)/Akt (protein kinase B) regulates cellular responses like cell growth, cell survival, or migration. Overactivation of this pathway often exists in human malignancies and significantly active mutation of these enzymes causes cancer progression. For the complete activation of this pathway, AKT phosphorylates various downstream effectors which are accountable for cell growth, survival, and proliferation (Manning & Cantley, 2007). Additionally, three different AKTs, (AKT1, AKT2, and AKT3) have different effects. Cell growth and cell survival are boosted by AKT1 whereas cellular invasiveness and mesenchymal characteristics are maintained by AKT2 (Irie et al., 2005; Maroulakou et al., 2007). The activation by tyrosine kinase receptors, PI3K catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate/ PtdIns(4,5)P2 to phosphatidylinositol 3,4,5-trisphosphate/ PtdIns(3,4,5)P3 and PDK1 and AKT to the plasma membrane requires which causes their activation. AKT then regulates multiple downstream effectors which ultimately results in cell growth, proliferation, and cell survival. However, PI3K-independent AKT activation is also seen in cancer. Several kinases such as

Ack I, IKKε, TBK1, Src, and PTK6 (protein tyrosine kinase 6) can also activate AKT independently which play important role in tumor growth (Faes & Dormond, 2015).

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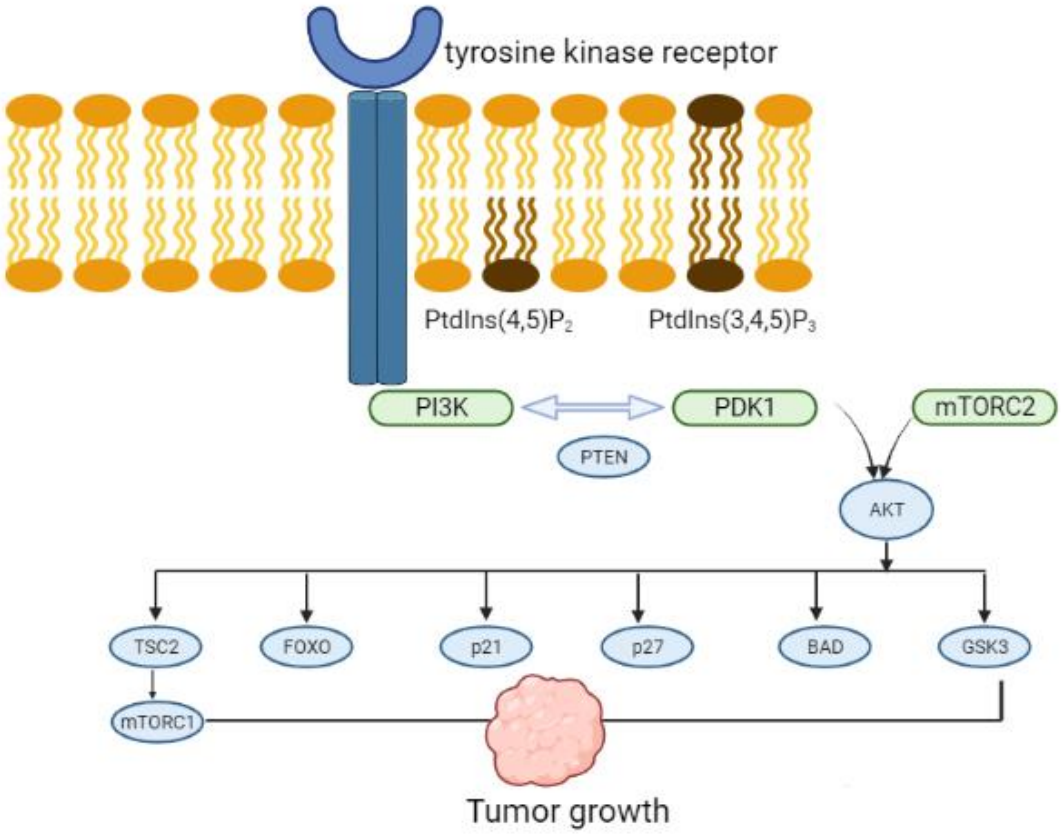

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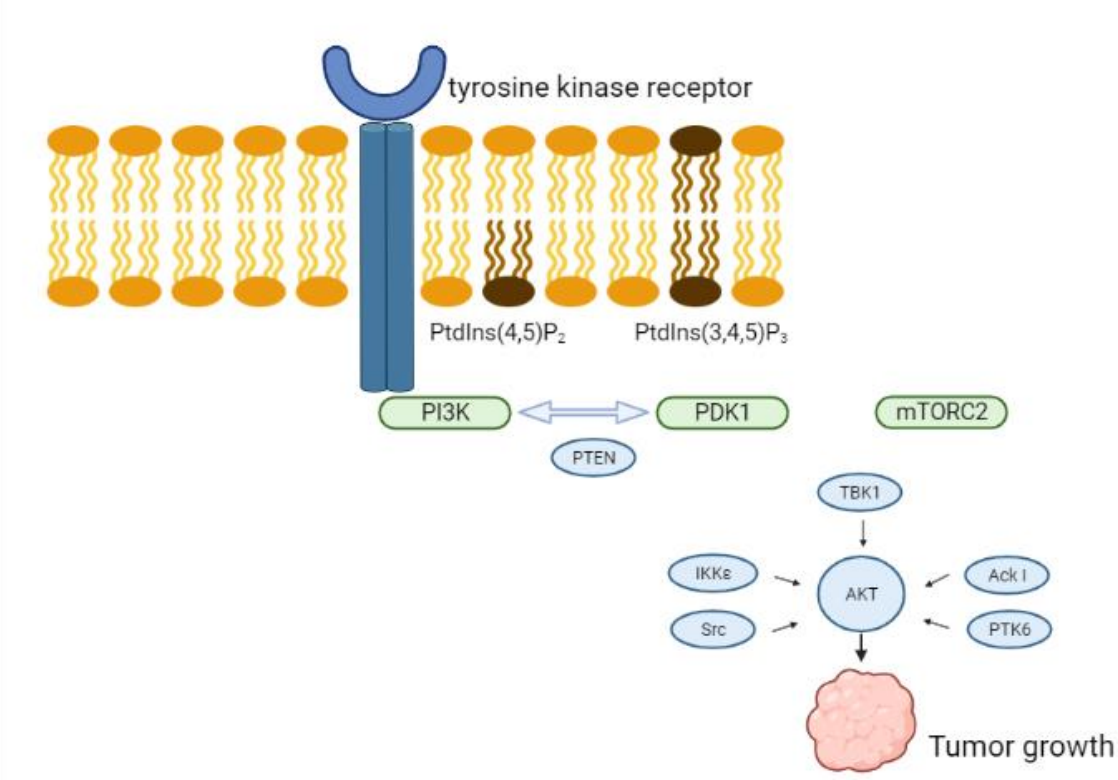


Figure 4: PI3K (phosphatidylinositol 3-kinase)/AKT signaling pathway (i.) PI3K dependent (ii.) PI3K independent pathway) (adapted from (Faes & Dormond, 2015))

1.5.4 ERK/MAPK signaling pathway

The extracellular signal-regulated kinase (ERK) signaling pathway maintains a wide range of cellular processes including cell proliferation, cell survival, cell differentiation, and cell motility (Pages et al., 1993; Seger & Krebs, 1995). This ERK pathway usually up-regulates the growth of human tumors and acts as a potential target for discovering anti-cancer drugs. Activation of the ERK pathway is frequently associated with cell proliferation, survival, and migration (Lewis et al., 1998).

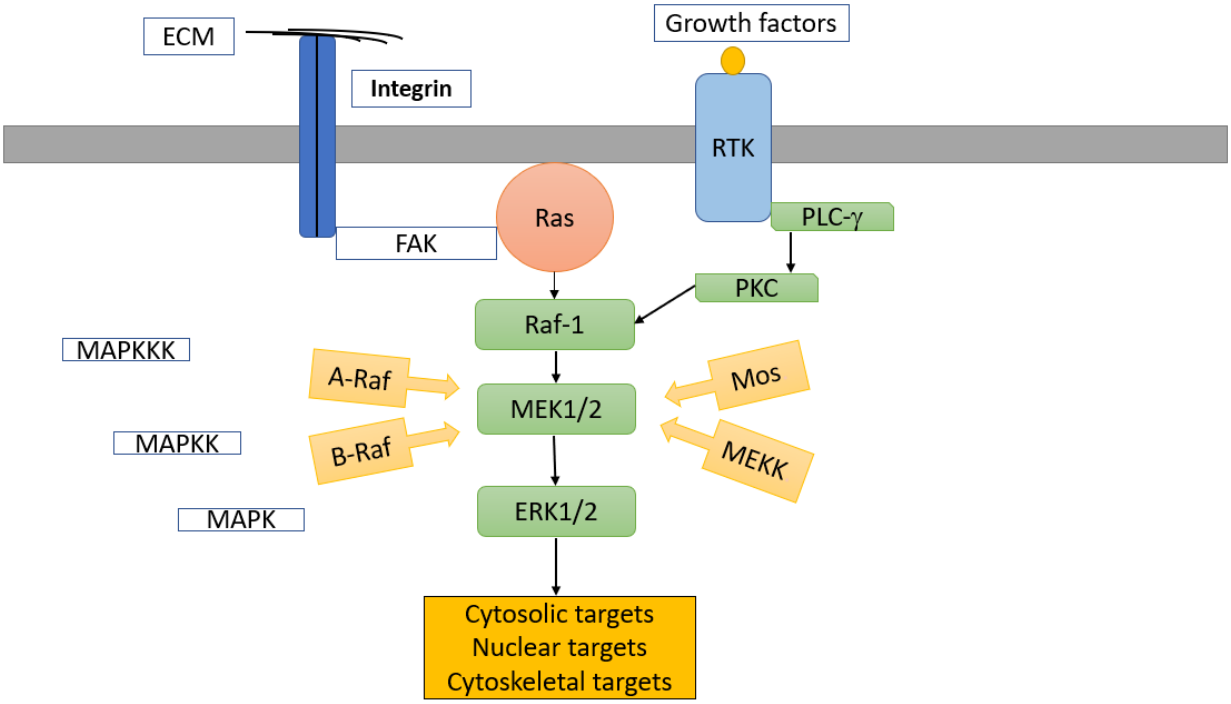


*Figure 5: The extracellular signal-regulated kinase (ERK) Signaling pathway (adapted from (*Kohno & Pouyssegur, 2006b))

The figure shows that ERK pathway activation is characterized by the guanosine triphosphate (GTP)-loading of Ras at the plasma membrane which eventually enrolls one of the Raf kinases e.g. Raf-1 to the plasma membrane which acts as a MAPK kinase and activates both of the MAPK/ERK kinase 1 and 2 by the phosphorylation of serine. MEK1/2, act as a catalyst here and does the phosphorylation of ERK1 and ERK2. Integrin-focal adhesion kinase (FAK) signaling pathway is also activated by the sticking of integrins to specific extracellular matrix (ECM) molecules and this pathway is also important for the activation of the ERK pathway. However, the unusual activation of any signaling molecule which causes the upregulation of ERK1/ERK2 would be responsible for the constitutive activation of these kinases and

results from tumorigenesis. Nevertheless, over-expression or activating mutations of epidermal growth factor (EGF) receptors is also linked to this pathway (Kohno & Pouyssegur, 2006a)

1.6 Potential Targets

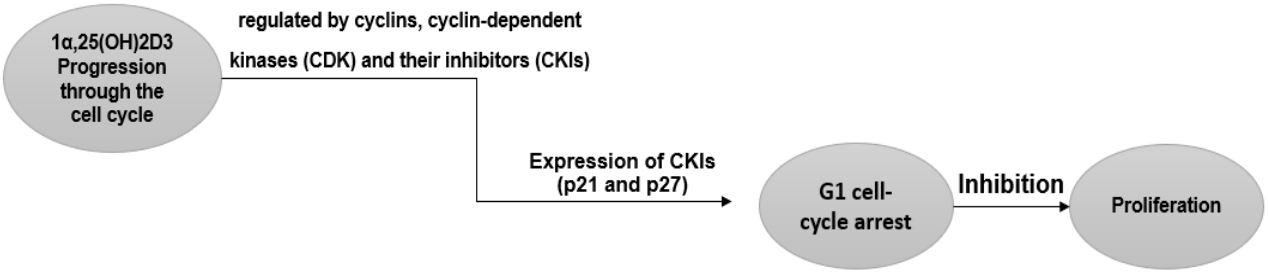
1.6.1 Through the epidermal growth factor receptor (EGFR) pathway

EGFR involves in diverse cellular processes; different techniques have been developed that target and interfere with EGFR-mediated effects. Some of them are the application of monoclonal antibodies and small-molecule tyrosine kinase inhibitors (TKIs) to develop anti-cancer drugs. Whereas anti-EGFR antibodies bind to extracellular domains, TKIs target the intracellular TK domains. However, several studies have described many noteworthy benefits of anti-EGFR agents in different solid tumors including colorectal, head and neck cancer, non-small cell lung cancer (NSCLC), and pancreatic cancer in terms of progression-free survival, total survival rate, and overall response rate in the whole life span (Seshacharyulu et al., 2012).

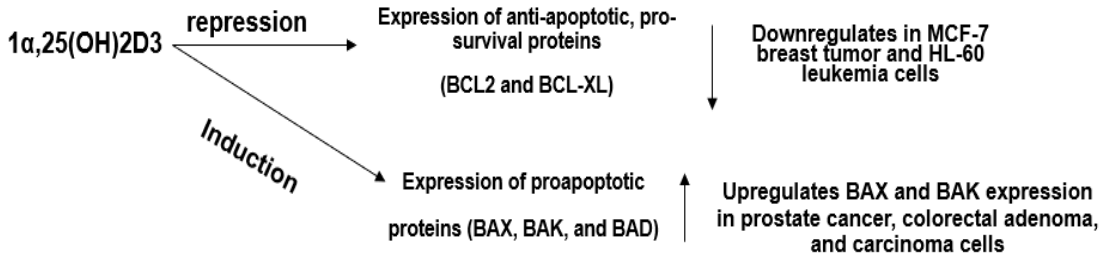
1.6.2 Through Vitamin D signaling pathway

Preclinical research specifies that the administration of the active metabolite of vitamin D, 1α,25(OH)2D3, vitamin D analogues which are also known as calcitriol was proved to have potential anticancer effects in squamous cell carcinoma (SCC), prostate adenocarcinoma, ovarian, breast and lung cancers as they can activate apoptotic pathways and inhibit angiogenesis. Calcitriol displays anti-inflammatory actions in several cancers by inhibiting prostaglandin synthesis and prostaglandin signaling (Deeb et al., 2007).

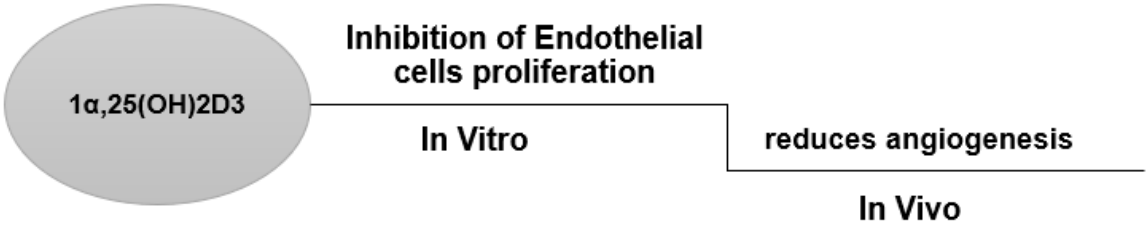
Antiproliferative effect



Apoptosis



Angiogenesis



1.6.3 Through PI3K/AKT pathway

There is a complex relationship between PI3K and AKT in human cancers. Studies showed that the PI3K inhibitors only instantly or inadequately block AKT activity. Very few PI3K inhibitors like CAL-101 (idelalisib, GS1101) are used to treat chronic lymphocytic leukemia, small lymphocytic lymphoma, and follicular lymphoma. It is the only FDA-approved agent and only a few agents have reached the phase III trial stage. Combined therapies are required for patients with advanced-stage cancer to yield a stronger anticancer effect. A combination of PI3K and AKT inhibitors is suggested by experts in cancer therapy(Faes & Dormond, 2015).

1.6.4 Through the ERK pathway

ERK inhibitors are expected to exhibit anti-proliferative, anti-metastatic, and anti-angiogenic effects in the tumor cells. Different inhibitors of Raf and MEK in the signaling pathway of ERK are used to develop anti-cancer drugs. Additionally, activated B-Raf is proven as an alluring target for cancer chemotherapy. Numerous small molecules such as BAY 43-9006 is reported as Raf inhibitor and PD98059, U0126, PD184352 (CI-1040), PD0325901, ARRY-14886, etc. are reported as MEK inhibitors. The MEK inhibitor PD98059 inhibits the proliferation of HT1080 fibrosarcoma cells and the ERK pathway cannot be activated (Kohno & Pouyssegur, 2006b).

1.7 Aim & Objectives

The main purpose of this literature review is to highlight the significance of using the RIPK pathway for cancer treatment. Other goals include advancing research and raising interest so that researchers may focus more on creating cancer treatments based on this Receptor interacting protein kinase (RIPK) pathway specifically RIP1 and RIP3 signaling pathways outlined in this review article.

Chapter 2

Research Methodology

A thorough literature research was carried out to gather appropriate information for this review study. A number of trustworthy sources, including peer-reviewed journals and an online scholarly database, were used to gather the information. Here is a list of a few of the several databases that have been searched for this study.

- Journal Database
- Newspaper Database
- Professional website
- Library Catalogue

In order to gather as much important information as possible regarding the RIP kinases in cancer diagnosis and treatment, a thorough search of several journals, review articles, and research papers from official websites and research databases were carried out. The data for this review study was collected using well-known and reliable sources including PubMed, SCOPUS, Google Scholar, and ScienceDirect. Relevant papers were gathered using suitable important keywords, such as cancer, signaling pathways, RIPK, and anti-cancer therapy. Around 78 articles have been assessed based on the title and keyword content. Then, 48 papers were reduced after reading the abstracts. The 30 papers that made up this review research were carefully selected and examined. Mendeley software was used for accurate and fair referencing in order to show respect for the writer's original works.

Chapter 3

RIPK in Cancer

Receptor Interacting Protein Kinases (RIPKs) is a 7-member family of Serine/Threonine/Tyrosine kinases (RIPK1–RIPK7) having significant characteristics like regulating inflammatory gene expression, functions of cutaneous and intestinal barrier along with necrosis.

3.1 Structures of RIP kinases

RIP family members (RIPK1-RIPK7) contain about 260-270 amino acids positioned at the N-terminus. There is a similarity in the structure of RIP1, RIP2, and RIP3. An N-terminal kinase domain and an RHIM domain are present in RIP1 and RIP3 which can interact with each other. However, necroptosis is triggered by other proteins containing the RHIM domain. The death domain is only present in RIPK1 having the ability to communicate with other proteins containing that specific domain. caspase-recruiting domain (CARD) is present in the RIP2 having the ability to activate the inflammatory caspase, caspase-1. The similarity is also seen in RIP4 and RIP5 (Sugen kinase 288 [SgK288]/ANKK1) as they both have ankyrin repeats which are non-kinase domains at the same position, C-terminus, and those repeats are also seen in RIP6 but at a different position, at the N-terminus. Ankyrin repeats ensure protein-protein interactions which is an important feature of RIP in regulating necrosis and immunity. Therefore, two types of RIP5s have been founded yet e.g. Dusty protein kinase and SgK288 (Sugen kinase 288) or ANKK1. Among RIP members RIP6 and RIP7 have the most distinctive characteristics. each contains leucine-rich repeats (LRRs) and a Ros of complex proteins (Roc)/C-terminus of Roc (COR) domain. RIP7 also contains WD40 repeats. The number of amino acids (AAs) in each protein is shown on the right. (Ermine et al., 2022).

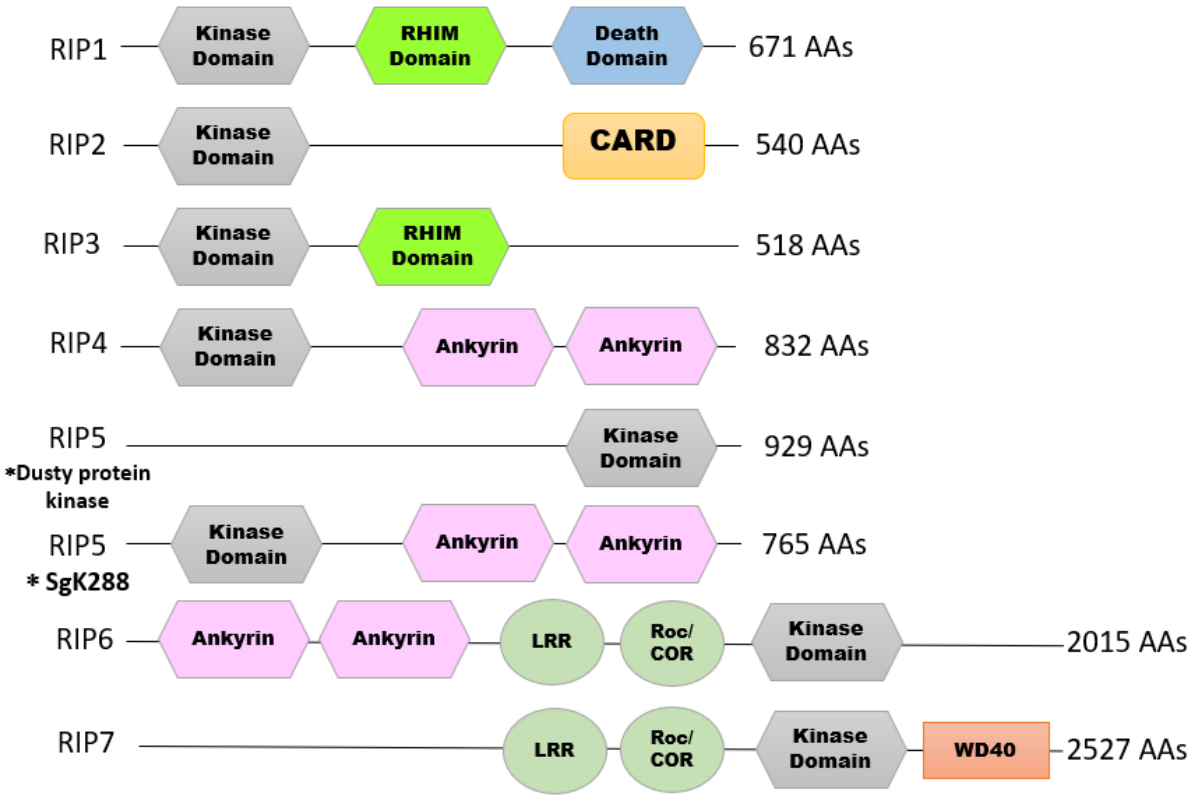


Figure 6: Structures of RIP Family members(Ermine et al., 2022)

RHIM= respective homotypic interaction motif

LRR=leucine-rich repeat

COR=C-terminus of Roc

Roc=Ros of complex proteins

ROS=reactive oxygen species

3.2 Functions of RIP kinases

Receptor-interacting protein kinases are proven to play remarkable roles in recent ages as they contribute to cell death, physiologic and pathologic inflammation, and other human diseases. Functions of RIPKs are listed below (Table 2):

Table 2: Functions of Receptor-interacting protein kinases.

RIP kinases	**Functions of Receptor-interacting protein kinases**
RIPK1	▪ RIPK1 is known as the "Swiss Army knife" as it regulates numerous innate immune receptors and sensors e.g. TNFRs, TLRs, IFNAR1, STING, MAVS, etc. ▪ RIPK1 is linked to regulating apoptosis, pyroptosis, necroptosis, transcription, and translation of inflammatory genes. ▪ Activate NF-κB in the TNFR1-bound Complex ▪ RIPK1 plays role in homeostatic control against improper activation of caspase-8 and RIPK3. ▪ RIPK1 has functions in inflammatory, autoimmune peripheral, degenerative, and CNS diseases. ▪ RIPK1 shields epithelial barrier integrity in the gut and epidermis from various damages during apoptosis and necroptosis. ▪ TAK1 and TBK1 kinases caused loss of RIPK1 inhibition which contribute to aging (Cuny & Degterev, 2021).

RIPK2	▪ Mainly activate the NF-κB ▪ RIPK2 showed catalytic activity. ▪ Catalytically inactive RIPK2 supported Nod receptor responses in cells while different RIPK2 kinase inhibitors were proved to block these responses. ▪ RIPK2 proved not to have any function in cell death (Cuny & Degterev, 2021).
RIPK3	▪ RIPK3 regulates necroptosis through a pseudokinase MLKL, pro-inflammatory gene expression, and sustained translation. ▪ RIPK3 regulates inflammasomes and apoptosis of cells. ▪ It protects epithelial barrier integrity in case of intestinal injury produced by severe dextran sodium sulfate (DSS) (Cuny & Degterev, 2021).
RIPK4	▪ RIPK4 activates the NF-κB. ▪ In mice, RIPK4 catalyzes normal epidermal differentiation and preserves the purpose of the skin barrier. ▪ Mutation of RIPK4 and IRF6 was found to develop Bartsocas-Papas syndrome and popliteal pterygium syndrome (Cuny & Degterev, 2021).

3.3 Role of RIP kinases in cancer development

RIP kinases can be tumor suppressive or oncogenic depending on the cancer type. Their dual function regulates inflammation, cell survival, and cell death. They greatly impact different types of cancer by altering the expression level of RIP kinases except for RIP5 and RIP7, outlined below (Table 3).

Table 3: Alterations of RIP kinase expression in different cancer.

RIP kinases	**Alterations in Different Types of Cancer**		**Tumor type**	**Frequency**	**Reference**
	Downregulation	Upregulation			
RIP1	breast cancer, colorectal cancer, head, and neck squamous cell carcinoma	gallbladder cancer, gastric cancer glioblastoma, lung cancer, melanoma, pancreatic adenocarcinoma.	Uterine Corpus Endometrial Carcinoma, Ovarian Serous Cystadenocarcinoma, Skin Cutaneous Melanoma, Liver Hepatocellular Carcinoma	6.19%	(Ermine et al., 2022)

RIP2	oral squamous cell carcinoma	breast cancer, colorectal cancer, gastric cancer, kidney renal clear cell carcinoma	Breast Invasive Carcinoma, Uterine Carcinosarcoma, Prostate Adenocarcinoma, Liver Hepatocellular Carcinoma	8.31%	(Ermine et al., 2022)
RIP3	acute myeloid leukemia, chronic lymphocytic leukemia, breast cancer, colorectal cancer, lung cancer, malignant mesothelioma, melanoma, prostate cancer	pancreatic adenocarcinoma	Skin Cutaneous Melanoma, Uterine Corpus Endometrial Carcinoma, Bladder Urothelial Carcinoma, Lung Adenocarcinoma	3.30%	(Ermine et al., 2022)

RIP4	hepatocellular carcinoma, tongue squamous cell carcinoma, lung cancer	bladder cancer, colorectal cancer, osteosarcoma, ovarian cancer, pancreatic cancer	Skin Cutaneous Melanoma, Stomach Adenocarcinoma, Bladder Urothelial Carcinoma, Uterine Corpus Endometrial Carcinoma	5.07%	(Ermine et al., 2022)
RIP5			Breast Invasive Carcinoma, Skin Cutaneous Melanoma, Uterine Corpus Endometrial Carcinoma, Liver Hepatocellular Carcinoma	6.59%	(Ermine et al., 2022)
RIP6	hepatocellular carcinoma		Sarcoma, Uterine Corpus Endometrial Carcinoma, Skin Cutaneous Melanoma,	9.81%	(Ermine et al., 2022)

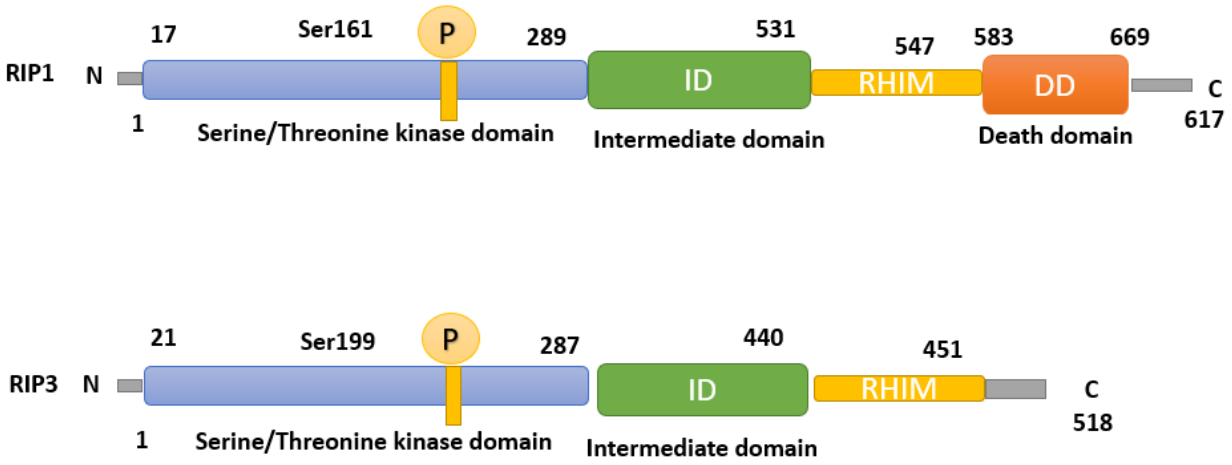
			Stomach Adenocarcinoma		
RIP7			Uterine Corpus Endometrial Carcinoma, Stomach Adenocarcinoma, Lung Squamous Cell Carcinoma, Skin Cutaneous Melanoma	14.37%	(Ermine et al., 2022)

Different types of RIP kinases are downregulated and upregulated in different types of cancers and their alteration can suppress or promote cancer metastasis in different types of cancer. In lung cancer activation of NF-kB and enhanced RIP1 expression increased metastasis. In an orthotopic nude mice model with gallbladder cancer disease, RIP1 boosted the metastasis in lymph nodes. Additionally, in triple-negative breast cancer (TNBC), metastatic properties were increased by RIP2. In contrast, RIP3 reduction in the model having acute myeloid leukemia (AML) and hepatocellular carcinoma (HCC) improved tumorigenesis while their overexpression in colorectal cancer cells reduced metastatic potential (Ermine et al., 2022).

3.4 RIP1, RIP3

The receptor-interacting serine/threonine kinase (RIP) gene is situated on the human chromosome namely 6p25.2. Seven splicing isoforms are encoded by the RIP gene such as RIP1, RIP2, RIP3, RIP4, RIP5, RIP6, and RIP7. In 1995, RIP1 was primarily recognized as a protein that interacted with the death domain (DD) of receptor Fas (CD 95) and caused a distinctive programmed death response in susceptible cells. RIP3 was invented in 1997 and was found to lessen both RIP1 and tumor necrosis factor receptor 1 (TNFR1) induced NF-κB activation. RIP1 and RIP3 have many structural similarities and they share almost half of the amino acid sequences. Both of the kinases are composed of an N-terminal serine/threonine kinase domain, an intermediate domain (ID), and RIP homotypic interaction motif (RHIM) domain. RIP1 specifically contains a C-terminal death domain (DD). Whereas in humans, RIP1 comprises 671 amino acids, RIP3 comprises 518 amino acids. RIP3 does not possess any death domain (DD). However, RIP1, a multifunctional adaptor protein, responds and its death domain (DD) binds to the activated signal of death receptors (DRs) of TNFR1, Fas, and TNF-related apoptosis-tempting ligand (TRAIL) which is not required by RIP3. RIP1 regulates NF-κB activation and mediates apoptosis and necroptosis which is caspase and RIP kinase-dependent respectively. For RHIM, the RIP1 protein interacts with RIP3 and the latter binds to RIP1 for inhibiting NF-κB activation which is mediated by RIP1 and TNFR1(Y. Liu et al., 2019a).

(A)



(B)

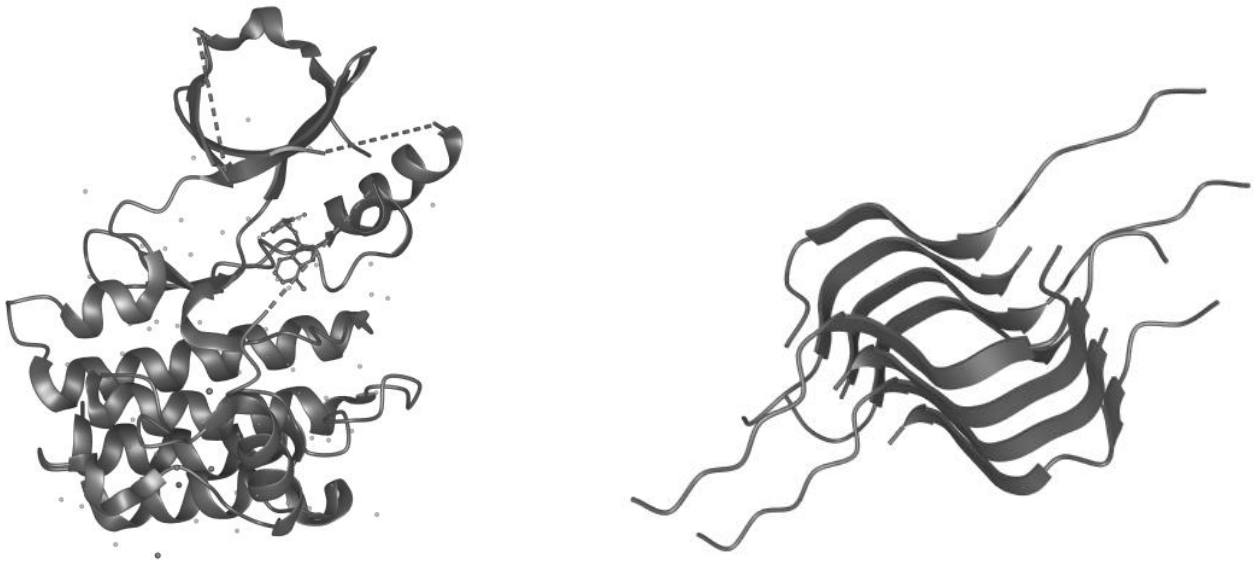


Figure 7: Structural diagrams of RIP1 and RIP3. (A) Schematic of functional domains of RIP1 and RIP3. (B) Protein tertiary structures of RIP1 and RIP3 respectively

RIP = receptor-interacting serine/threonine kinase

ID = intermediate domain

RHIM = RIP homotypic interaction motif

DD = death domain

3.5 Role of RIP kinases in anti-cancer therapies

In necroptosis, among the two types of kinases RIP1 and RIP3, RIP1 is not crucial like RIP3. The signaling pathway of RIP1 mediates cell death whereas RIP3 facilitates necroptosis and apoptosis. Different studies also highlighted tumorigenesis activity of both RIPK1 and RIPK3. Additionally, this theory acts behind the development of many anti-cancer therapies nowadays. The RIP1/RIP3 signaling pathway is basically planned by caspase activation and ubiquitination. Where apoptosis is blocked, necroptosis alternatively is carried cell death programs for cancer prevention. Escalation of the RIP3 level can develop colon and lung cancers, nasopharyngeal carcinoma, and non-Hodgkin lymphoma. The topoisomerase inhibitor SN38, a cytotoxic mediator, promotes necroptosis progression and inhibits cell proliferation. This SN38 activates RIP1 and following necroptosis can exhibit therapeutic efficacy in colorectal carcinoma. Furthermore, different studies suggested that a RIP1/RIP3 inhibitor-IFN-γ combination act as a novel strategy that is used to inhibit necroptosis in the treatment of acute myeloid leukemia. Besides, bufalin, one of the most effective anti-cancer agents, upsurges necroptosis mediators RIP1/RIP3 and ROS expression which leads to tumor cell death and also inhibits tumor growth in human breast cancer cells (Y. Liu et al., 2019b). RIPK3 signaling pathway suppresses tumor development by killing cancer cells through necroptosis, T cell-mediated cancer immune surveillance, secreting tumor-repressive cytokines, and limiting tumor expansion through mitochondrial metabolism and the production of ROS. Again, diminished RIPK3 expression can cause disease progression and metastasis and reduced overall survival of the patients

affected with different types of cancers e.g. breast, colorectal cancer, acute myeloid leukemia (AML), head and neck squamous cell carcinoma, melanoma, primary malignant mesotheliomas, prostate tumors, liver cancer. On the contrary, the RIPK3 signaling pathway also aids tumor progression by the accumulation of immune-repressive myeloid cells to promote cancer cells' escape from the immune surveillance, secrets tumor-promoting cytokines that induce angiogenesis, promotes tumor metastasis causing vascular endothelial cell death and also cause tumor relapse. Specifically, inhibition of the both RIPK1 and RIPK3 signaling pathway can be used as novel strategies in different anti-cancer therapies. Though tumorigenesis with the RIPK1 signaling pathway is not well established yet. However, future research should focus on the promising application of RIP1/RIP3-dependent necroptosis in anticancer therapy (S. Liu et al., 2021).

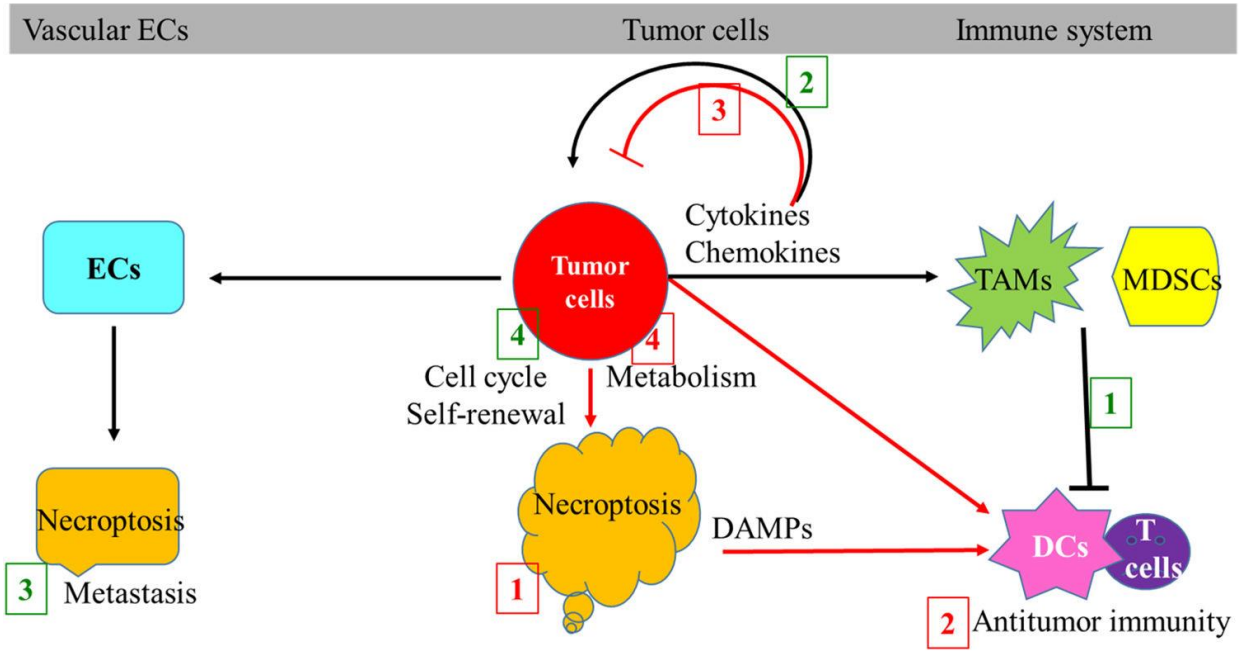


Figure 8: Tumor promoting and tumor-repressing activity of RIPK3 (adapted from (S. Liu et al., 2021))

Chapter 4

Current Therapeutic prospect

At present, various types of selective inhibitors such as inhibitors for the RIPK3, GSK'840, GSK'843, GSK'872, GW440139B, HS-1371, ponatinib, and dabrafenib (a BRAF inhibitor) and inhibitors for the RIPK1, necrostatins, GSK2982772, GSK963, GSK3145095, RIPA-56, DNL747, ponatinib, and pazopanib have been developed and advanced for future studies to assess whether those inhibitors can selectively block the specific cancer types when combined with other chemotherapies and inhibitors (S. Liu et al., 2021). Additionally, based on their pharmacodynamic (PD) type I, II, and III binding mode, RIPK1 inhibitors can be classified as Type I (Tozasertib, Sunitinib), type II (GSK2606414, GSK2656157, PN10, Foretinib), type III (Nec-1s, GSK3145095, Benzoxazeoinone (BOA)) and unclassified inhibitors (KW-2449, AST-487, Phenytoin) whereas RIPK3 inhibitors can be classified as Type I (Dabrafenib, GSK’843), type II (GSK’067, GSK’074, Sorafenib), type III and unclassified inhibitors (DCC-2036, GSK’840, GSK’872) (Martens et al., 2020). Different studies reported that a small-molecule PK6 and its derivatives PK68 block cellular activation of RIPK1 and RIPK3 signaling which is used to develop the treatment option for inflammatory disorders and cancer metastasis (Hou et al., 2019).

Chapter 5

Future Perspectives

538 different protein kinases are encoded by the human genome involving the transformation of the γ-phosphate group from ATP and ATP to serine, threonine, or tyrosine residues. Most of the kinases are linked with the initiation and advancement of human cancers. Several kinase inhibitors successfully have passed clinical trials in recent days whereas 150 kinase-targeted drugs are still in the clinical and some are in the preclinical stage of drug discovery. Moreover, kinase inhibitor drug discovery has proceeded dramatically in the past few decades. Remarkably, protein kinases, the second most targeted group, in the development of numerous drug targets used in cancer treatment. in earlier 1980, FDA approved about 37 kinase-specific inhibitors to treat malignancies of breast and lung cancer. Numerous pieces of research have demonstrated promising outcomes for RIP1/RIP3 signaling-based anti-cancer treatment. Inhibitors' selectivity and their combinatorial uses with other anti-cancer agents as well as other therapies yet need to be studied. Overcoming unexpected toxicities associated with kinase inhibitors as well as better selectivity to facilitate the vast patient population demands further studies. Furthermore, the development of sophisticated modeling of chemotherapy to overcome kinase resistance for facilitating the combined application of kinase inhibitors to successfully treat cancer is a need (Bhullar et al., 2018). Therefore, RIP1 and RIP3 kinases are one of the most significant tools in a variety of different biological sectors as well as cancer therapy with further research improving these formulation techniques for practical application.

Chapter 6

Conclusion

Receptor-interacting protein kinases (RIPK) have grabbed a lot of attention in the biomedical areas because of their distinguishing structures and functional characteristics of their signaling pathways in cancer development and its successful treatment. Proteins kinases mediate tumor cell functions through signaling pathways and their deregulation causes cancer cell proliferation and metastases. On the other hand, anti-apoptotic effects are boosted by the hyperactivation of protein kinases. Presently, pharmaceutical industries are conducting their research with one-third of kinase-based protein targets. Based on molecular genetics and molecular signaling pathway, kinase inhibitors are used in targeted therapies that are more effective than conventional cancer therapies. Kinase inhibitors approved by FDA basically choose ATP-binding sites and exert therapeutic efficacy against carcinogenesis. The main issue and practical limitation that needs to be addressed is the potential toxicity along with effective combined use with other anti-cancer agents. Although many efforts have been made to address this problem. Further studies and proper execution of receptor protein kinases (RIPKs) signaling pathways are necessary to develop potential anti-cancer therapies. To conclude, due to remarkable features, kinase inhibitors especially for RIP1 and RIP3 kinases denote a novel and promising aspect of cancer therapy having valuable clinical effects.

References

Bhullar, K. S., Lagarón, N. O., McGowan, E. M., Parmar, I., Jha, A., Hubbard, B. P., & Rupasinghe, H. P. V. (2018). Kinase-targeted cancer therapies: Progress, challenges and future directions. In *Molecular Cancer* (Vol. 17, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12943-018-0804-2

Cancers by Body Location/System - NCI. (n.d.). Retrieved January 19, 2023, from https://www.cancer.gov/types/by-body-location

Citri, A., & Yarden, Y. (2006). EGF–ERBB signalling: towards the systems level. *Nature Reviews Molecular Cell Biology 2006 7:7*, *7*(7), 505–516. https://doi.org/10.1038/nrm1962

Cuny, G. D., & Degterev, A. (2021). RIPK protein kinase family: Atypical lives of typical kinases. *Seminars in Cell & Developmental Biology*, *109*, 96. https://doi.org/10.1016/J.SEMCDB.2020.06.014

Danaei, G., vander Hoorn, S., Lopez, A. D., Murray, C. J. L., & Ezzati, M. (2005). Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *Lancet (London, England)*, *366*(9499), 1784–1793. https://doi.org/10.1016/S0140-6736(05)67725-2

Deeb, K. K., Trump, D. L., & Johnson, C. S. (2007). Vitamin D signalling pathways in cancer: Potential for anticancer therapeutics. In *Nature Reviews Cancer* (Vol. 7, Issue 9, pp. 684–700). https://doi.org/10.1038/nrc2196

Erben, R. G., Soegiarto, D. W., Weber, K., Zeitz, U., Lieberherr, M., Gniadecki, R., Möller, G., Adamski, J., & Balling, R. (2002). Deletion of deoxyribonucleic acid binding domain of the vitamin D receptor abrogates genomic and nongenomic functions of vitamin D. *Molecular Endocrinology (Baltimore, Md.)*, *16*(7), 1524–1537. https://doi.org/10.1210/MEND.16.7.0866

Ermine, K., Yu, J., & Zhang, L. (2022). Role of Receptor Interacting Protein (RIP) kinases in cancer. *Genes & Diseases*, *9*(6), 1579. https://doi.org/10.1016/J.GENDIS.2021.10.007

Faes, S., & Dormond, O. (2015). PI3K and AKT: Unfaithful Partners in Cancer. *International Journal of Molecular Sciences*, *16*(9), 21138. https://doi.org/10.3390/IJMS160921138

Hou, J., Ju, J., Zhang, Z., Zhao, C., Li, Z., Zheng, J., Sheng, T., Zhang, H., Hu, L., Yu, X., Zhang, W., Li, Y., Wu, M., Ma, H., Zhang, X., & He, S. (2019). Discovery of potent necroptosis inhibitors targeting RIPK1 kinase activity for the treatment of inflammatory disorder and cancer metastasis. *Cell Death and Disease*, *10*(7). https://doi.org/10.1038/s41419-019-1735-6

Irie, H. Y., Pearline, R. v., Grueneberg, D., Hsia, M., Ravichandran, P., Kothari, N., Natesan, S., & Brugge, J. S. (2005). Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial-mesenchymal transition. *The Journal of Cell Biology*, *171*(6), 1023–1034. https://doi.org/10.1083/JCB.200505087

Kohno, M., & Pouyssegur, J. (2006a). Targeting the ERK signaling pathway in cancer therapy. *Annals of Medicine*, *38*(3), 200–211. https://doi.org/10.1080/07853890600551037

Kohno, M., & Pouyssegur, J. (2006b). Targeting the ERK signaling pathway in cancer therapy. *Annals of Medicine*, *38*(3), 200–211. https://doi.org/10.1080/07853890600551037

Krasinskas, A. M. (2011). EGFR Signaling in Colorectal Carcinoma. *Pathology Research International*, *2011*, 1–6. https://doi.org/10.4061/2011/932932

Lewis, T. S., Shapiro, P. S., & Ahn, N. G. (1998). Signal transduction through MAP kinase cascades. *Advances in Cancer Research*, *74*, 137–139. https://doi.org/10.1016/S0065-230X(08)60765-4

Liu, S., Joshi, K., Denning, M. F., & Zhang, J. (2021). RIPK3 signaling and its role in the pathogenesis of cancers. *Cellular and Molecular Life Sciences : CMLS*, *78*(23), 7199. https://doi.org/10.1007/S00018-021-03947-Y

Liu, Y., Liu, T., Lei, T., Zhang, D., Du, S., Girani, L., Qi, D., Lin, C., Tong, R., & Wang, Y. (2019a). RIP1/RIP3-regulated necroptosis as a target for multifaceted disease therapy (Review). *International Journal of Molecular Medicine*, *44*(3), 771–786. https://doi.org/10.3892/ijmm.2019.4244

Liu, Y., Liu, T., Lei, T., Zhang, D., Du, S., Girani, L., Qi, D., Lin, C., Tong, R., & Wang, Y. (2019b). RIP1/RIP3-regulated necroptosis as a target for multifaceted disease therapy (Review). *International Journal of Molecular Medicine*, *44*(3), 771–786. https://doi.org/10.3892/ijmm.2019.4244

Manning, B. D., & Cantley, L. C. (2007). AKT/PKB signaling: navigating downstream. *Cell*, *129*(7), 1261–1274. https://doi.org/10.1016/J.CELL.2007.06.009

Maroulakou, I. G., Oemler, W., Naber, S. P., & Tsichlis, P. N. (2007). Akt1 ablation inhibits, whereas Akt2 ablation accelerates, the development of mammary adenocarcinomas in mouse mammary tumor virus (MMTV)-ErbB2/neu and MMTV-polyoma middle T transgenic mice. *Cancer Research*, *67*(1), 167–177. https://doi.org/10.1158/0008-5472.CAN-06-3782

Martens, S., Hofmans, S., Declercq, W., Augustyns, K., & Vandenabeele, P. (2020). Inhibitors Targeting RIPK1/RIPK3: Old and New Drugs. In *Trends in Pharmacological Sciences* (Vol. 41, Issue 3, pp. 209–224). Elsevier Ltd. https://doi.org/10.1016/j.tips.2020.01.002

Mitsudomi, T., & Yatabe, Y. (2010). Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *The FEBS Journal*, *277*(2), 301–308. https://doi.org/10.1111/J.1742-4658.2009.07448.X

Pages, G., Lenormand, P., L'Allemain, G., Chambard, J. C., Meloche, S., & Pouyssegur, J. (1993). Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation. *Proceedings of the National Academy of Sciences*, *90*(18), 8319–8323. https://doi.org/10.1073/PNAS.90.18.8319

Saini A, Kumar M, et all. (2020). *Cancer Causes And Treatments. International Journal of Pharmaceutical Sciences and Research*. *Vol. 11(7).*

Seger, R., & Krebs, E. G. (1995). The MAPK signaling cascade. *The FASEB Journal*, *9*(9), 726–735. https://doi.org/10.1096/FASEBJ.9.9.7601337

Seshacharyulu, P., Ponnusamy, M. P., Haridas, D., Jain, M., Ganti, A. K., & Batra, S. K. (2012). Targeting the EGFR signaling pathway in cancer therapy. In *Expert Opinion on Therapeutic Targets* (Vol. 16, Issue 1, pp. 15–31). https://doi.org/10.1517/14728222.2011.648617

Spano, J. P., Fagard, R., Soria, J. C., Rixe, O., Khayat, D., & Milano, G. (2005). Epidermal growth factor receptor signaling in colorectal cancer: preclinical data and therapeutic perspectives. *Annals of Oncology*, *16*(2), 189–194. https://doi.org/10.1093/ANNONC/MDI057

The Economic Burden of Cancer | The Cancer Atlas. (n.d.). Retrieved January 19, 2023, from https://canceratlas.cancer.org/taking-action/economic-burden/

The top 10 causes of death. (n.d.). Retrieved January 19, 2023, from https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

What Is Cancer? - *NCI*. (n.d.). Retrieved January 19, 2023, from https://www.cancer.gov/about-cancer/understanding/what-is-cancer#definition