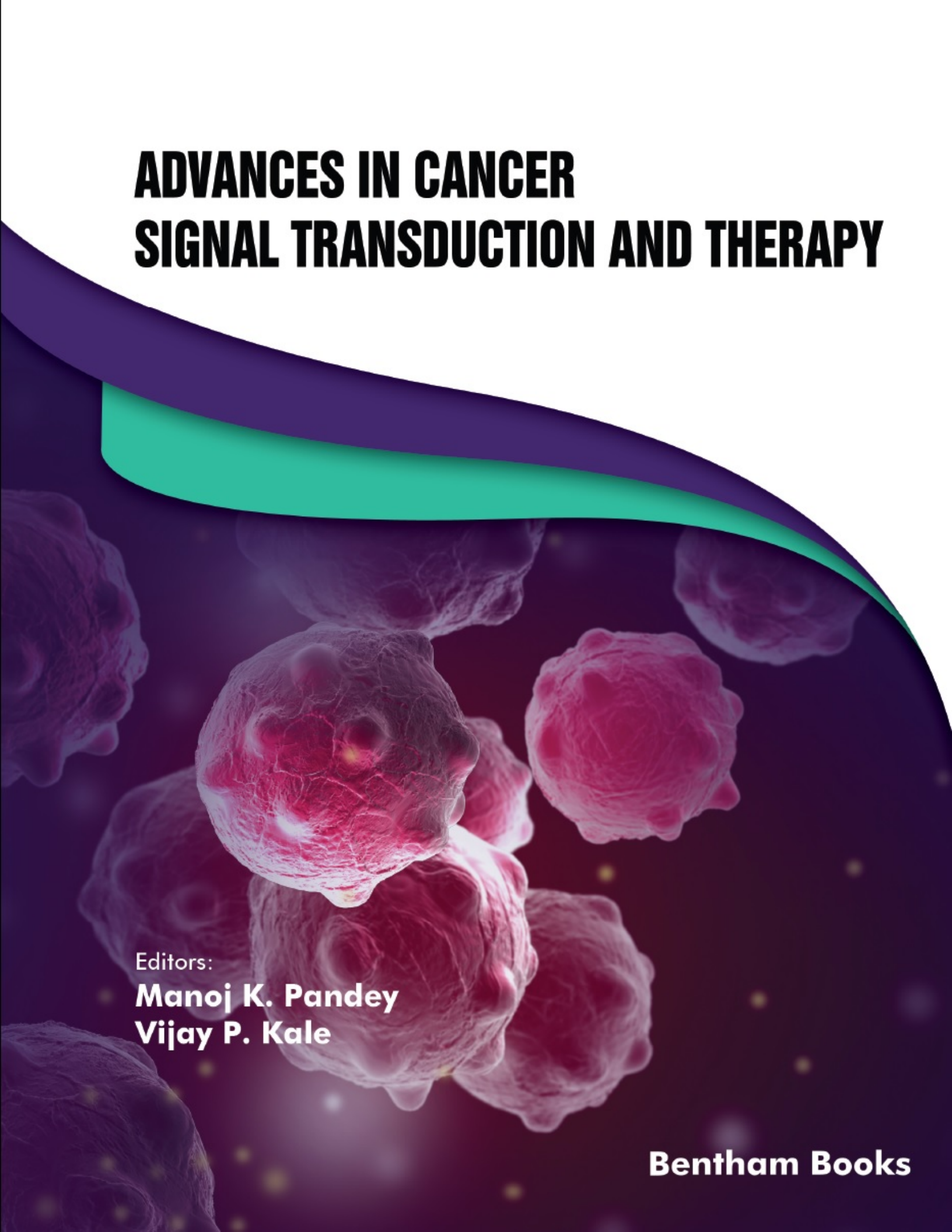
ADVANCES IN CANCER SIGNAL TRANSDUCTION AND THERAPY



Editors:
Manoj K. Pandey
Vijay P. Kale

Bentham Books

Recent Advances in Signal Transduction Research and Therapy

(Volume 1)

Edited by

Manoj K. Pandey

Department of Biomedical Sciences
Cooper Medical School of Rowan University
Camden, NJ 08103
USA

&

Vijay P. Kale

Translational Safety And Bioanalytical Sciences
Amgen Inc.
South San Francisco, CA 94080
USA

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Volume # 1

Advances in Cancer Signal Transduction and Therapy

Editors: Manoj K. Pandey & Vijay P. Kale

ISSN (Online): 2737-4432

ISSN (Print): 2737-4424

ISBN (Online): 978-981-14-5811-8

ISBN (Print): 978-981-14-5809-5

ISBN (Paperback): 978-981-14-5810-1

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PREFACE

Every year, almost 10 million people succumb to death around the world due to one or the other type of cancers. Hundreds of laboratories with thousands of scientists are trying to understand the ever-enigmatic biology of cancer cells, which is the basis for developing new therapies for cancer. Discovery of antifolates aminopterin in 1947 and methotrexate (aka amethopterin) in 1948 as potential anticancer agents by Drs. Yellapragada Subbarow and Sydney Farber not only initiated the era of chemotherapy but the discovery of these synthetic agents also infused the hope that cancers can be treated with synthetic chemicals. In the past more than seven decades, we have witnessed the journey of cancer therapy that started from nonspecific chemotherapy (*e.g.* antifolates) to targeted biologic agents (*e.g.* monoclonal antibodies) and now the era of personalized treatment (*e.g.* CART cells) and immunotherapy. However, deeper understanding of the signal transduction within the cancer cells, and between cancer cells and their surrounding tumor microenvironment has remained central to the development of therapies.

In the present volume 1 titled **'Advances in Cancer Signal Transduction and Therapy'** of the book series 'Recent advances in signal transduction research and therapy', we attempted to review recent advances in select cancer signal transduction pathways that have been targeted or could be potential targets for developing therapeutics for cancers. It would be too exhaustive to cover all the signaling pathways in all cancer types and we do not intend to do so, and due to the very rapidly progressing research on cancer signaling and therapy, we have no doubt that new discoveries will have been made by the time this book is published.

In the first chapter of this book, **Fultang *et al***., dive into the role of Wingless and Int-1 (Wnt) signaling in breast cancer oncogenesis. Moreover, the authors also review the current inhibitors of Wnt signaling that are under investigation. The C-X-C chemokine receptor type 4 (CXCR4) signaling is critical in hematological malignancies, while its role in breast cancer is still unraveling. In the second chapter **Raman *et al***., explain the intersection of CXCR4 signaling with other signaling pathways such as Phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), cellular Src (c-Src) and Janus kinases-signal transducer and activator of transcription proteins (JAK-STAT) in breast cancer progression and metastasis. A key role of epidermal growth factor receptor (EGFR) signaling in cell proliferation and survival in various cancers is now well known. Here in the third chapter, **Meena *et al***., discuss the role of EGFR in colon cancer and its prospective importance as a target for colon cancer therapy. The critical role of PI3K/AKT (protein kinase B)/mammalian target of rapamycin (mTOR) signaling pathway in various cancers has attracted cancer researchers for a long time. **Yadav and Mishra** elucidate the PI3K/AKT/mTOR signaling in hepatocellular carcinoma and review the various drugs under investigations that target this pathway in the fourth chapter. The MAPK pathway is one of the key survival pathways that have been targeted to develop cancer therapeutics. In chapter 5, **Prasad and Srivastava** review MAPK signaling in pancreatic cancer and therapeutic agents under investigation. Aberrant regulation and activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) has been implicated in several cancers, inflammatory and autoimmune disorders, and erroneous immune system development. In chapter 6, **Arora *et al***., summarize the role of NF-κB activation specifically in multiple myeloma and various strategies developed for its potential pharmacological intervention to abrogate the process of cancer cell proliferation. Recently, small-molecule inhibitors of Bruton's tyrosine kinase (BTK) such as ibrutinib have shown an impressive anti-tumor activity in clinical studies in patients with various B cell malignancies. BTK is crucial in B lymphocyte development, differentiation and oncogenic signaling. In chapter 7, **Gowda *et al***., elucidate the role of BTK in B cell malignancies and highlight the

current progress in the discovery of small molecule BTK inhibitors. Finally, in the last chapter of this book, **Pathania *et al***., discuss the interconnection between exosomes, tumor microenvironment and cMyc transcription factor, and therapeutic strategies to break that nexus.

We sincerely hope that you will enjoy diving into this book.

Manoj K. Pandey
Department of Biomedical Sciences,
Cooper Medical School of Rowan University,
Camden, NJ 08103,
USA

&

Vijay P. Kale
Translational Safety And Bioanalytical Sciences
Amgen Inc.
South San Francisco, CA 94080
USA

LIST OF CONTRIBUTORS

Alan Prem Kumar	Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
Anup S. Pathania	Department of Biochemistry and Molecular Biology & The Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, USA
Augustus M.C. Tilley	Department of Cancer Biology, University of Toledo Health Science Campus, Toledo, USA
Avtar S. Meena	Department of Pure and Applied Physics, CSIR-Centre for Cellular and Molecular Biology (CCMB) Habsiguda, Hyderabad, India
Bela Peethambaran	Department of Biological Sciences, University of the Sciences, Philadelphia, USA
Cory M. Howard	Department of Cancer Biology, University of Toledo Health Science Campus, Toledo, USA
Dayanidhi Raman	Department of Cancer Biology, University of Toledo Health Science Campus, Toledo, USA
Frank Arfuso	Stem Cell and Cancer Biology Laboratory, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Perth, Australia
Gautam Sethi	Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
Jennifer Dang	Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, USA
Krishne Gowda	Department of Pharmacology, Penn State College of Medicine, Hershey, USA
Kishore B. Challagundla	The Children's Health Research Institute, University of Nebraska Medical Center, Omaha, USA
Kuntal Bhowmick	Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, USA
Loukik Arora	Department of Pharmacology, Yong Loo Lin School of Medicine. National University of Singapore, Singapore
Manoj K. Pandey	Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, USA
Max Von Suskil	Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, USA
Neelam Yadav	Department of Biochemistry, Rammanohar Lohia Avadh University, Faizabad, India
Norman Fultang	Department of Biological Sciences, University of the Sciences, Philadelphia, USA
Omar S. Al-Odat	Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, USA
Prachi S. Narayan	Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, USA

Pradeep K. Shukla	Department of Physiology, University of Tennessee Health Science Center, Memphis, USA
Rachel Weber	Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, USA
Sahdeo Prasad	Department of Immunotherapeutics and Biotechnology, and Center for Tumor Immunology and Targeted Cancer Therapy, Texas Tech University Health Sciences Center, Abilene, USA
Sangita Sridharan	Department of Cancer Biology, University of Toledo Health Science Campus, Toledo, USA
Sanjay K. Srivastava	Department of Immunotherapeutics and Biotechnology, and Center for Tumor Immunology and Targeted Cancer Therapy, Texas Tech University Health Sciences Center, Abilene, USA
Shantu G Amin	Department of Pharmacology, Penn State College of Medicine, Hershey, USA
Vijay P. Kale	Translational Safety And Bioanalytical Sciences, Amgen Inc., South San Francisco, CA 94080, USA
Yoganchal Mishra	Department of Biochemistry, Rammanohar Lohia Avadh University, Faizabad, India

CHAPTER 1

Wnt Signaling in Breast Cancer Oncogenesis, Development and Progression

Norman Fultang and **Bela Peethambaran***

Department of Biological Sciences, University of the Sciences, Philadelphia, PA, USA

Abstract: Wnt signaling regulates several cellular processes, including differentiation, proliferation, and stem cell pluripotency. Mutations in Wnt signaling are known to lead to tumor initiation and progression. Wnt/ β-catenin signaling is dysregulated in breast cancer, where it has been shown to mediate oncogenic progression. In this review, the canonical and non-canonical pathways of Wnt/ β-catenin signaling, and their regulation of breast cancer oncogenesis and progression are described. During the last decade, several small molecules and natural compounds have shown to interfere with Wnt signaling and demonstrate potential as Wnt-targeting therapeutic agents. This review also highlights these molecules, some of which are in clinical trials. Finally, strategies of using these molecules in combination therapies with other drug agents are discussed.

Keywords: β-catenin, Breast cancer, Canonical and Non-canonical pathways, Mutations, Oncogenesis, Proliferation, Stem cell pluripotency, Wnt signaling.

INTRODUCTION

Overview

The Wnt/β-catenin pathway is a crucial and highly conserved pathway governing the processes of growth, development, and cell fate [1]. An ever-increasing body of evidence suggests a vital role for Wnt/β-catenin signaling in the oncogenesis, development and progression of cancer [2]. This review will summarize recent findings on the role of this pathway in breast cancer and discuss the emerging therapeutic approaches targeting Wnt signaling.

The Wnt signaling cascade is an evolutionarily conserved pathway. It was first discovered to be responsible for spontaneous mammary hyperplasia and tumor formation in mice after pro-viral insertion in the int-1 locus [3]. A few years later,

* **Corresponding author Bela Peethambaran:** Department of Biological Sciences, University of the Sciences, Philadelphia, PA, USA; Tel: 215-596-8923; Fax: 215-596-8710; E-mail: b.peethambaran@usciences.edu

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the Wingless gene responsible for segment polarity in *Drosophila melanogaster* was found to be a homolog of int-1. Hence the int/Wingless family was named *Wnt* [4, 5]. Wnt proteins are encoded by 19 different Wnt genes with sequences sharing a high degree of homology [6]. These proteins are known to bind to cellular receptors during embryonic development and mediate several processes, such as cell proliferation, survival, migration, polarity, cell fate, and self-renewal [7]. Wnt can activate distinct signaling pathways, which include a β-catenin dependent or canonical pathway, and a β-catenin independent or non-canonical pathway [4, 7]. Wnt ligands bind primarily to multiple Frizzled (Fzd) receptors [8], but other Wnt co-receptors include members of the low density lipo-protein related protein 5 and 6 (LRP5/6), receptor-like tyrosine kinase (RYK) and receptor tyrosine-kinase like orphan receptors (ROR) 1 and 2 [8, 9]. These co-receptors are important to regulate downstream signaling either through LRP5/6 co-receptors for the β-catenin dependent or through RYK/ROR1/2 for the β-catenin independent pathway.

Wnt Biosynthesis and Regulation

During its synthesis, Wnt has to undergo a palmitoylation step catalyzed by Porcupine (PORCN), which belongs to the membrane-associated O-acyltransferase family [10, 11]. This step is required for the interaction with Fzd receptors and promotes interaction with the multi-pass transmembrane protein, Wntless, which transports Wnt to the plasma membrane. Since PORCN is required for Wnt secretion, several small molecules have been developed to target it. There are 10 Wnt antagonists that also help mediate Wnt signaling. These antagonists include the secreted Fzd-related proteins (SFRPs), Wnt-inhibitory factor 1 (WIF-1), Wise/SOST, Dickkopf proteins (DKKs), insulin-like growth factor binding protein 4 (IGFBP4), Cereberus, Shisa, Wnt-activated inhibitory factor 1 (Waif1/5 T4), adenomatosis polypsosis coli down regulated 1 (APCDD1) and Tikil [9, 12]. Several of these regulatory factors have potential as therapeutic targets, but so far, only DKK1 has been evaluated clinically for drug development [13]. The challenge in using these regulatory proteins as targets is that they often regulate the activity of other important cellular pathways. For instance, SFRPs and WIF-1 can bind to Wnt in both canonical and non-canonical signaling depending on the cellular need. SFRPS, however, also regulates the Notch and Bone Morphogenic Protein (BMP) signaling cascade, a key developmental pathway [12, 14].

TYPES OF PATHWAYS

The Canonical Wnt Signaling Pathway in Breast Cancer

Canonical Wnt signaling plays an important role in cell fate decisions in early embryogenesis during the development of organs, such as the lungs, kidney, skin, and bone [8]. This pathway is also critical in neural patterning and stem cell renewal [15]. Canonical Wnt signaling is also called the β-catenin dependent pathway as it results in an accumulation of cytoplasmic β-catenin followed by the latter's translocation to the nucleus [8, 15, 16]. Genetic and biochemical evidence suggests that Wnt binds to Fzd receptors, which have seven transmembrane receptors with a cysteine-rich domain at the N-terminal. Fzd is required for multiple Wnt pathways, but another single-pass transmembrane receptor, LRP6/5 is specifically required for the Wnt/β-catenin canonical pathway [17].

Canonical Wnt signaling results in stabilization and nuclear translocation of ß-catenin [18, 19], which is degraded by a destruction complex consisting of AXIN, Protein phosphatase 2A (PP2A), Casein Kinase 1α (CK1α), and Glycogen synthase kinase 3 (GSK3) [19, 20]. In the absence of Wnt, this destruction complex phosphorylates ß-catenin, tagging it for ubiquitination and subsequent degradation by the proteasome. Wnt ligands, when bound to the Fzd receptor and the LRP 5/6 transmembrane co-receptor, trigger the recruitment of Disheveled (Dvl) to the plasma membrane [8, 21 - 24]. AXIN is also recruited to the phosphorylated cytoplasmic tail of LRP5/6 [17, 25]. Dvl forms a complex with AXIN, Fzd, and LRP5/6 [26]. Recruitment of AXIN and Dvl prevents the formation of the destruction complex leading to the stabilization of cytoplasmic ß-catenin [18, 19]. Dvl proteins also assemble the signalosome that is responsible for phosphorylation of multiple motifs of LFP5/6, one of which is phosphorylated-PPPSPXS/T. P-PPPSPXS/T acts as a competitive inhibitor of GSK3 [26], a kinase that phosphorylates and tags ß-catenin for degradation. The net result of these events is the accumulation of unphosphorylated β-catenin resulting in its stabilization. The stabilized β-catenin translocates to the nucleus, where it acts as a transcriptional co-activator in combination with the T-cell factor (TCF) and the lymphoid enhancer-binding factor (LEF) family of transcription factors. This leads to the recruitment of transcriptional Kat3 co-activators p300 and CREB binding protein (CBP) to transcribe Wnt target genes (Fig. **1A**) [27]. ß-catenin can also interact with other transcriptional co-activators, including BRG-1, a component of the SWI/SNF nuclear remodeling complex, Hsp90 co-chaperone Cdc37, and C-terminal-binding protein (CtBP) [28 - 31]. In the absence of Wnt signaling and nuclear ß-catenin, TCF forms a complex with Groucho proteins to recruit histone deacetylases (HDACs) and repress the transcription of Wnt target genes [32 - 34].

CHAPTER 2

CXCR4 Signaling and its Impact on Tumor Progression and Metastasis in Breast Cancer

Dayanidhi Raman*, **Cory M. Howard, Sangita Sridharan** and **Augustus M.C. Tilley**

Department of Cancer Biology, University of Toledo Health Science Campus, Toledo, OH, USA

Abstract: CXCR4 is a G_i-coupled chemokine receptor involved in chemotaxis (directed migration) of tumor and stromal cells into the primary tumor and the pro-metastatic niche that are enriched in CXCL12. In breast cancer, cell surface CXCR4 levels and activity are upregulated and play an important role in local invasion and metastasis. During cancer progression, the CXCL12-CXCR4 axis orchestrates infiltration of endothelial cells and a variety of leukocytes to drive an immuno-suppressive tumor microenvironment (TME). When CXCL12 from the TME activates plasma membrane-resident CXCR4 in tumor and stromal cells, a variety of pathways are activated involving signaling modules such as PI3K-AKT, MEK-ERK, and c-Sr--p130CAS-paxillin. This triggers a wide variety of cellular processes that drive breast cancer progression, chemoresistance, and metastasis. This provides an opportunity to intervene and target these signaling axes or nodes in clinical trials to antagonize tumor growth metastasis. Finally, careful selection of targeted therapies in combination with the standard of care therapy should be selected judiciously for each patient (precision medicine) with the aim of improving the longevity with minimal toxicity to metastatic breast cancer patients.

Keywords: Breast Cancer, CXCR4, Metastasis, Signaling, Tumor Progression.

INTRODUCTION

Longitudinal exposure to noxious agents such as non-infectious (environmental toxins, food additives, oxidative stress), infectious factors (certain bacteria and viruses), and infestations (parasites) contribute to chronic inflammation. The chronic inflammation is accompanied by the presence of pro-inflammatory cytokines such as interleukins [IL-1α/β [1], IL-6)], interferons (IFN-α), and tumor necrosis factor-α (TNF-α) [2 - 4]. These cytokines trigger the release of chemotactic cytokines or chemokines and the unresolved chronic inflammation

* **Corresponding author Dayanidhi Raman:** Department of Cancer Biology, University of Toledo Health Science Campus, Toledo, OH, USA; Tel: 419-383-4616, Fax: 419-383-6228 18; E-mail: dayanidhi.raman@utoledo.edu

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leads to the formation of a neoplastic foci. Chemokines are a family of small molecules (8-12 kDa) that have the ability to facilitate survival, proliferation, migration, local invasion, and eventually metastasis of the breast cancer cells to distant organs. This is important as it is the metastasis that is lethal and not the primary breast tumor. The intra- and inter-tumor heterogeneity observed in breast cancer supports tumor progression and facilitates metastasis. The stromal cells along with acellular matrices form the tumor microenvironment (TME) [5,6]. The stromal TME plays a key role in orchestrating tumor progression and also contributes to chemoresistance. Importantly, chemokines secreted into the TME recruits the pro-tumor stromal cells such as M2-like macrophages [7], N2 neutrophils [8 - 10], myeloid-derived suppressor cells (MDSCs) [11,12], endothelial progenitor cells (EPCs) [13], adipocytes, and endothelial cells [14]. The recruited and subverted stromal cells secrete many cytokines, chemokines and growth factors such as IL-6, CXCL8, CXCL12, and vascular endothelial growth factor (VEGF). They also secrete extracellular matrix proteins such as tenascin-C, collagen I, and matrix metalloproteinases (MMPs). This facilitates autocrine and paracrine signaling in the TME-resident tumor and stromal cells which recruits additional pro-tumorigenic cells to the developing TME. Carcinoma-associated fibroblasts (CAFs) are already present around the tumor cells and support tumor growth, chemoresistance, and metastasis [15 - 17]. The acellular matrix provides a desmoplastic microenvironment that makes the tumor denser and renders them more chemoresistant. Thus, the paracrine interactions between the breast cancer cells with the tumor-educated stromal cells facilitate all stages of breast cancer progression, survival, angiogenesis, and metastasis (Fig. **1**). The CXCL12-CXCR4 axis is intricately involved in many of these steps of tumorigenesis and metastasis.

CXCL12-CXCR4 AXIS

The chemokine stromal-derived factor-1α (SDF-1α) or CXCL12 is the ligand for the heterotrimeric, seven transmembrane G-protein coupled receptor (GPCR) CXCR4. There are six alternatively spliced isoforms of CXCL12 that have been identified [18]. CXCL12-α is made of 89 amino acid residues while CXCL12-β contains an additional four amino acid residues at the C-terminus [19]. The ligands, CXCL12-α and CXCL12-β, bind with comparable affinity to CXCR4 receptor (K_d of 7.5 and 13.7 nM, respectively) [20]. Four additional splice variants (CXCL12-γ, CXCL12-δ, CXCL12-ε, and CXCL12- Φ) have been identified that contain 30 additional amino acid residues at their C-termini as compared to CXCL12-α [21]. All of these isoforms are functional and have a differential tissue distribution, but CXCL12-α is the predominantly expressed form. CXCL12-γ has been detected in patients with advanced disease (stage IV) at the mRNA level

[22]. The cell migration induced by CXCL12-γ showed resistance to inhibition by common CXCR4 antagonists [22].

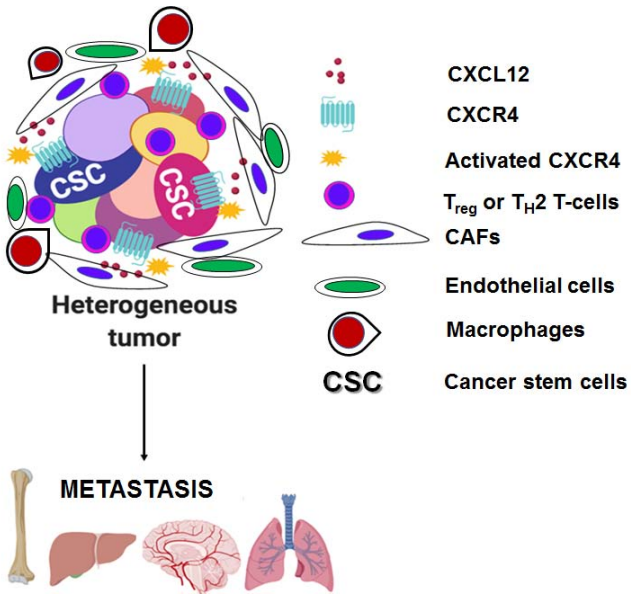

Fig. (1). Understanding the role of the heterogeneous breast tumor and the CXCL12-CXCR4 axis in the metastatic breast cancer cascade. The CXCL12 enriched microenvironment in distant organs such as the bone, lungs, liver or brain facilitates the metastatic spread of breast cancer cells expressing the cell surface CXCR4.

The CXCL12-CXCR4 axis is highly involved in primary tumor progression and metastasis in breast cancer [23 - 25]. Upon binding of CXCL12 to CXCR4, the receptor gets activated and triggers conformational changes in the heterotrimeric G-protein ($G\alpha\beta\gamma_i$, pertussis toxin-sensitive) initiating several divergent network of signaling pathways in breast cancer cells. Activation of CXCR4 signaling facilitates increase in intracellular calcium, cell survival and proliferation, gene transcription, cell adhesion, directional cell migration (chemotaxis), local invasion and metastasis [26 - 29]. Among them, the phosphatidylinositol 3'-kinase (PI3K), mitogen-activated protein kinase (MAPK), and c-*Src* signaling pathways tremendously contribute to tumorigenesis and subsequent metastasis [30] (Fig. **2**). CXCR4 also couples with $G\alpha_{13}$ to facilitate Rho GTPase-mediated transendothelial migration of basal-like breast cancer cells [31,32]

CHAPTER 3

Epidermal Growth Factor Receptor Signaling in Colon Cancer

Avtar S. Meena[1,*] and **Pradeep K. Shukla**[2,*]

[1] *CSIR-Centre for Cellular and Molecular Biology (CCMB) Habsiguda, Uppal Road Hyderabad - 500 007 Telangana, India*

[2] *Department of Physiology, College of Medicine, University of Tennessee Health Science Center, Memphis, TN, USA*

Abstract: Epidermal growth factor receptor (EGFR) expression regulates cancer cell proliferation, survival, and metastatic potential and is associated with the majority of human carcinomas, including colorectal carcinoma. The relationship between EGFR expression and its prognosis in cancer patients, however, has not been proven in clinical settings. Various preclinical studies suggest that the oncogenic potential of EGFR is associated with levels of EGFR ligands. Mutations in EGFR family ligands and their receptors are characteristic of many different kinds of tumors. Therefore, this signaling axis is an attractive target for the development of targeted therapies. Various small molecule inhibitors and antibodies are in clinical trials that specifically target EGFR.

Here, we will discuss the current literature's attempts to identify markers, which contribute resistance and sensitivity to small molecule EGFR inhibitors. Moreover, we will summarize the role of EGFR in the development of colon cancer. We will discuss the mechanistic basis for EGFR interaction with various molecules, its consequences for biology, and its prospective importance as a target for colon cancer therapy.

Keywords: Cancer therapy, Cancer cell proliferation, Colon cancer, EGFR ligands, Epidermal growth factor receptor (EGFR), Targeted therapies.

COLORECTAL CANCER AND RISK FACTORS

Colorectal cancer (CRC) is the second most prevalent human malignant disease. It is one of the prominent causes of cancer-related mortalities globally [1]. The development of CRC comprises a complex multistage process in which sequential

[*] **Corresponding authors Pradeep K. Shukla and Avtar S. Meena:** Department of Physiology, College of Medicine, University of Tennessee Health Science Center, Memphis, TN, USA; Tel: 38163-2113; (901) 448-3019 and CSIR-Centre for Cellular and Molecular Biology (CCMB), Habsiguda, Uppal Road, Hyderabad-500007 Telangana, India; Tel: +91-40-27160222; E-mails: pshukla2@uthsc.edu and avtarsingh@ccmb.res.in, respectively

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mutational events occur along with the progression of cancer. The 5 -year survival of patients diagnosed with metastatic colorectal cancer (mCRC) is approximately 13-18%. Early detection and treatments are essential for the prevention of colon cancer-related death. Approximately 60-80% of CRC patients exhibit overexpression of EGFR, which is associated with poor prognosis [2]. Various treatment agents have recently been established to demonstrate prominent efficacy in the treatment of mCRC.

For this reason, EGFR has been the central target for treatment with small-molecule inhibitor and monoclonal antibodies, cetuximab and panitumumab, which have been useful for patients with RAS wild type in randomized clinical trials [3 - 5]. There are multiple significant risk factors for the development of CRC, which are crucial for an increase in the rate of genetic mutation occurring in tumor suppressor genes and various oncogenes as well. These factors generally include environmental exposure, personal or family history of CRC, genetic mutation, location, and associated disease(s). Environmental exposures such as smoking, alcohol use, sedentary lifestyle, diet, and abdominal radiation are associated with an increased risk of CRC [6 - 12]. Supplementation of a high-fat Western diet promotes the development of colon cancer *via* an EGFR mediated mechanism [13]. Various habits or personal behaviors are major risk factors for the development of colorectal cancer (CRC). As with many cancers, the risk of CRC increases with age without gender differences [14]. Other than the age, people who have diabetes, obesity, and inflammatory bowel disease (IBD) are at higher risk of developing CRC, respectively [15 - 17]. We will discuss EGFR signaling, the development of anti-EGFR therapies, and mechanisms of resistance to EGFR therapies in CRC.

EGFR SIGNALING IN CRC

EGFR is one of the crucial targets to be exploited in cancer treatment, including CRC. The receptor, which belongs to the receptor tyrosine kinase (RTK) family, also known as HER1 (human EGFR receptor), is a 170-kDa transmembrane protein that acts as a receptor for the EGF family. In addition to EGFR, the receptors belonging to the RTK superfamily are ErbB- 2 (HER/c-neu), ErbB-3 (Her 3), and ErbB-4 (Her 4). All of these receptors are composed of an extracellular ligand-binding domain (cysteine-rich domain), a hydrophobic transmembrane region, a short juxta-membrane section, and an intracytoplasmic tyrosine kinase C-terminal domain (Fig. **1A**). EGFR and ErbB4 receptor exhibit a similar structure and are involved in ligand-dependent homo- or heterodimerization. In contrast, erbB2 lacks a ligand-binding domain and has been shown to elicit both ligand-dependent and independent dimerization [18, 19]. ErbB3 also undergoes ligand-dependent dimerization; however, these receptors have been

shown defective in their intrinsic tyrosine kinase activities. Therefore, they require heterodimerization with another member of the erbB family for the activation of signaling cascade [20]. Autophosphorylation of one intracellular kinase domain by the others promotes downstream signaling cascades. In general, there are seven combinations of receptor dimers and ten ligands binding partners that play a crucial role in the activation of downstream signaling cascade [21]. Various combinations of receptors and their ligands involved in signaling pathways summarized in Fig. (**1B**) and (**1C**). EGFR is expressed on all stromal and epithelial cells and is presented on various glial and smooth muscle cells as well [22]. Because of its involvement in cancers, EGFR has been a focus for the development of drug targets. Multiple studies suggest the role of EGFR in neoplastic progression. Three crucial mechanisms have been documented so far. First, a mutation in the EGFR gene, which leads to its constitutive activation even in the absence of ligand [23, 24]. Second, increased EGFR expression in breast, non-small cell lung, and colon cancer [25, 26]. However, it has remained unclear whether increased expression correlated with oncogenic activity. Third, EGFR signaling involves the binding of specific soluble ligands (*e.g.*, EGF, amphiregulin, epiregulin, betacellulin, or neuregulin), and mediate downstream signaling. Downstream signaling regulates cellular activities, including cell growth, survival, proliferation, inhibition of apoptosis, and differentiation in mammalian cells [27]. Binding of ligand to EGFR induces conformational changes in the receptor that promotes homo- or heterodimer formation between receptors. Receptor dimerization is crucial for the activation of intracellular tyrosine kinase and phosphorylation of the C-terminal domains, which provide a docking site for cytoplasmic proteins harboring phosphotyrosine-binding and Src homology-2 domains [28]. EGFR family activates Ras/Raf/MEK/MAPK, PLCγ-1/PKC, phosphatidylinositol-3 kinase/Akt, and STAT pathways (Fig. **2**). In the next section, we will discuss the role of the EGF-like growth factors, MAPK, and PI3K/ Akt signaling pathways, which have pivotal roles in CRC development and progression (Fig. **2**). Moreover, various signaling molecules and their mutation status involved in CRC progression are summarized in Table **1**.

Table 1. Summary of Signaling pathways altered in CRC.

Gene	Protein Function	Observed Alteration	Mutation	Ref.
EGFR	Transmembrane tyrosine kinase receptor	Mutation	Very Rare	[42]
		Protein Expression	30-90%	[148]
		Increased Copy Number	0-50%	[149]
KRAS	GDP/GTP binding protein facilitates EGFR downstream signaling	Activating Mutation	35-40%	[112, 113, 150]

CHAPTER 4

Targeting the PI3K/AKT/mTOR Signaling Pathway in Hepatocellular Carcinoma: Current State and Future Trends

Neelam Yadav[*] and **Yoganchal Mishra**[1]

[1] *Department of Biochemistry, Dr. Rammanohar Lohia Avadh University, Faizabad-224001, India*

Abstract: Hepatocellular carcinoma (HCC) is the most common liver cancer and the leading cause of cancer-related deaths. Advanced HCC has a poor prognosis with limited treatment option. Chronic liver diseases and cirrhosis are the main risk factors for the development of HCC. Phosphoinositide 3-kinase PI3K/AKT/mTOR, intracellular mediators play a very important role in the development and progression of HCC. This signaling pathway is frequently activated in HCC patients. Therefore, signaling pathways have become the main source of targets for new treatments in HCC patients.

In our chapter, we will discuss the role of PI3K/AKT/mTOR signaling pathway in the pathology of HCC and provide an update on the preclinical and clinical approaches for the development of various molecular agents targeting this proliferation/survival pathway, which include various PI3K/Akt/mTOR inhibitors and other agents for the treatment of HCC.

Keywords: Hepatocellular carcinoma, Inhibitors, Signaling pathways, Sorafenib, Therapy.

INTRODUCTION

Hepatocellular carcinoma (HCC) is an aggressive tumor of the liver and the third most common cause of death in human beings [1, 2]. Its poor prognosis is due to relapse and metastasis [3 - 5]. In HCC patients, metastasis is an essential cause of high mortality [6 - 8]. Ji *et al.* [9], reported that the clinical diagnosis and treatment of HCC patients have made great progress in the last few years. HCC occurs mainly in patients suffering with chronic liver disease and cirrhosis. Most of the mortality in HCC patients results from liver cirrhosis related problems like ascites, hepatic encephalopathy, hepatorenal syndrome and variceal hemorrhage.

[*] **Corresponding author Neelam Yadav:** Department of Biochemistry, Dr. Rammanohar Lohia Avadh University, Faizabad-224001, India; Tel: +91 9453731722; neelam2k4@gmail.com

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Several genes are responsible for the tumorigenesis and progression of HCC and many molecular changes play a crucial role in the development of this aggressive malignancy [10, 11].

Due to the activation of many signal transduction pathways that regulate cell proliferation and tumor progression and complex molecular alteration in hepatic tissue, treatment of HCC is not successful [12]. The phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathway is repeatedly activated in HCC.

Many patients with HCC are diagnosed at the advanced stage of the disease. Current treatment therapies for HCC are locoregional ablative approaches, surgical removal and interventional ablation techniques. Although these treatments could increase few year's survival rate in 70% patients of HCC, however, long-term survival remains low due to the high rate of recurrence and metastasis after surgical resection [13 - 15]. Therefore, novel evidence-based therapies for this aggressive malignancy is urgently needed.

Risk Factors for HCC

In HCC patients, no early noticeable symptoms appear during tumor development. Its occurrence is associated mainly with aflatoxin B1 contaminated food and endemic hepatitis C virus (HCV) and hepatitis B virus (HBV) infection, obesity and non-alcoholic steatohepatitis. Despite the food storage conditions and implementation of vaccinations to reduce the risk of aflatoxin B1 and contamination and hepatitis B infection respectively, the incidence of liver cancer is not decreasing. Early risk factors responsible for the development of HCC are immune-mediated chronic inflammation in hepatitis that results into progressive fibrosis and development of liver cirrhosis [16, 17].

Pathophysiology

The development of hepatocellular carcinoma is a complex process that involves many steps. It mainly occurs after liver cirrhosis and is associated with the diversity of aetiologies of the major chronic liver disease. In liver cirrhosis, HCC follows a sequence of events that involves pre-cancerous cirrhotic nodules development known as low-grade dysplastic nodules (LGDNs). High-grade dysplastic nodules (HGDNs) develop from LGDNs and convert into early-stage HCC and than more advanced HCC. Various cells, including mature hepatocytes and stem or progenitor cells, play an important role in malignant transformation into HCC [18]. The accumulation of somatic genomic alterations in driver and passenger cancer genes is responsible for HCC. However, genomic alterations are not accumulated, which suggests that many signaling pathways may involve in the promotion of carcinogenesis [19].

In HCC progression, several pathways and processes have been involved (Table **1**). A number of epigenetic and genetic changes like rearrangements and deletions of chromosomes, mutations, aneuploidy, gene amplification, and DNA methylations have been observed in HCC.

Table 1. Major gene alterations reported in advanced hepatocellular carcinoma.

Name of Pathway(s)	Gene(s) Name	Type of Alteration	Frequency Percentage (%) in HCC
AKT–mTOR– MAPK signalling	PTEN RPS6KA3 TSC1 and TSC2 FGF3, FGF4 and FGF19 P13KCA	Mutation or deletion Mutation Mutation or deletion Amplification Mutation	1-3% 2-9% 3-8% 4-6% 0-2%
WNT-β– catenin signalling	AXIN1 CTNNB1	Mutation or deletion Mutation	5-15% 11-37%
Angiogenesis	VEGFA	Amplification	3-7%
Oxidative stress	KEAP1 NFE2L2	Mutation Mutation	2-8% 3-6%
Telomere maintenance	TERT	Amplification Promoter mutation	5-6% 54-60%
Cell cycle control	RB1 CCND1 TP53 CDKN2A	Deletion or Mutation Amplification Mutation or Deletion Mutation or Deletion	3-8% 7% 12-48% 2-12%

AXIN1, axin 1; *CCND1*, cyclin D1; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CTNNB1*, β-catenin; *FGF*, fibroblast growth factor; *KEAP1*, kelch like ECH associated protein 1; *NFE2L2*, nuclear factor, *PI3K*, phosphoinositide 3-kinase; *PTEN*, phosphatase and tensin homologue; *RB1*, retinoblastoma 1; *RPS6KA3*, ribosomal protein S6 kinase, 90kDa *TSC*, tuberous sclerosis; *TERT*, telomerase reverse transcriptase; *TP53*, cellular tumor antigen p53; *VEGFA*, vascular endothelial growth factor A.

Basic Signaling Pathways

Llovet *et al.* [20], reported that the oxidative stress, chromatin remodeling, Wnt/ beta catenin and other signaling pathways including epidermal growth factor (EGF), insulin growth factor, vascular endothelial growth factor (VEGF), fibroblast-derived growth factor (FGF), platelet-derived growth factor (PDGF), and intracellular mediators RAS–RAF–MAPK (MAP kinase) and PI3/AKT are critical signaling pathways in HCC (Table **1**).

Many growth factors signaling (IGF, EGF, PDGF, FGF, and HGF), angiogenesis (VEGF) and cell differentiation (*e.g.*, WNT, Hedgehog, Notch) modules are de-

CHAPTER 5

MAPK Signaling Pathway: A Central Target in Pancreatic Cancer Therapeutics

Sahdeo Prasad and **Sanjay K. Srivastava***

Department of Immunotherapeutics and Biotechnology, and Center for Tumor Immunology and Targeted Cancer Therapy, Texas Tech University Health Sciences Center, Abilene, TX 79601, USA

Abstract: Pancreatic cancer remains one of the most clinically challenging cancer despite the advancement in molecular characterization of this disease. Malignancy of this disease is characterized by the constitutively activated mitogen-activated protein kinase (MAPK) pathway. The MAPK pathway is activated by growth factors, mitogens, hormones, cytokines and environmental factors. Activated MAPK induces expression of downstream genes and regulates cell proliferation, survival, differentiation, motility, receptor signaling, senescence and transport. Activation of MAPK in pancreatic cancer is associated with a poor prognosis and results in limited treatment options. This poor prognosis elicits a need for the development of effective therapeutic measures to treat and improve pancreatic cancer patient survival. MAPK targeted pancreatic cancer therapy has been developed in the last few decades with the use of a number of inhibitors. Inhibitors of RAS, MEK1/2 and ERK1/2 are the main drugs used pre-clinically and in clinical settings of pancreatic cancer treatment. Although these inhibitors have shown some clinical benefits, extensive research on the development of new MAPK signaling pathway inhibitors for the treatment of pancreatic cancer is warranted.

Keywords: Inhibitors, MAPK, Pancreatic cancer, Targeted therapy.

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related deaths and one of the most lethal malignant neoplasms across the world. According to the American Cancer Society, about 55,770 people will be diagnosed with pancreatic cancer and about 45,750 people will die in 2019. Pancreatic cancer accounts for about 7% of all cancer deaths in the USA and about 3% of all cancers. The 5-year survival rate of people with pancreatic cancer is also very low (8%). Incidence rates are also observed 25% higher in black people than in white people. Although tremendous

* **Corresponding author Sanjay K. Srivastava:** Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center, Suite 1305, 1718 Pine Street, Abilene, Texas 79601 USA; Tel: 325-696-0464; E-mail: sanjay.srivastava@ttuhsc.edu

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efforts have been made to improve therapeutic intervention, the prognosis remains poor because the diagnosis of pancreatic cancer at an early stage is very difficult due to the lack of specificity and cost-effective screening tests. This cancer is usually diagnosed at an advanced stage and is also resistant to chemotherapy [1]. Despite the recent advancement in therapeutic techniques and medical management, the median survival time of pancreatic cancer patients is only 5-8 months because of tumor cell invasion, early metastasis, and resistance to standard chemotherapy.

This poor prognosis, metastasis and resistance to therapy lead to target specific molecules for effective therapy and improvement of pancreatic patient survival. Advanced studies are needed to understand the pathogenesis, signaling network and molecular targets that may provide clues for the treatment of pancreatic cancer. Although pancreatic cancer is a multifactorial disease, the mitogen-activated protein kinase (MAPK) pathway is found to be constitutively activated in this cancer. In this chapter, the relevance and mechanism of MAPK activation in the pathobiology of pancreatic cancer will be explored. The therapeutic approach of pancreatic cancer by targeting MAPK will also be discussed.

Activation of the MAPK Pathway

MAPK proteins are Ser/Thr kinases, which coordinately regulate signal transduction, cell division, proliferation, gene expression, metabolism, motility, transport, survival, apoptosis, and differentiation. Typically MAPKs include the extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun amino (N)-terminal kinases 1/2/3 (JNK1/2/3), p38 [2 - 4], but recent studies suggest that atypically ERK3/4, ERK5, ERK7, and nemo-like kinase (NLK) are also included [5]. ERK, JNK, and p38 isoforms are known to form a group based on their activation motif, structure and function [6]. p38 MAPK is classified into four types such as p38α, p38β, p38γ, and p38δ [7].

MAPKs consist of three sets of kinases including MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). It has been found that the canonical MAPK/ERK pathway is composed of three types of MAPKKK that include A-RAF, B-RAF and RAF-1 or C-RAF kinases while MAPKK is composed of MAPK/ERK kinase (MEK)1 and MEK2. ERK1 and ERK2 are the downstream kinases and are the final effectors of the MAPK pathway [8]. MAPK is activated by a broad range of stimuli such as mitogens, growth factors, cytokines, environmental and other factors. Besides these, other factors such as chemokines, microRNA, kinases, and other proteins are associated with the activation of the MAPK signaling pathway (Fig. **1**). These stimuli first activate MAPKKK, which are protein Ser/Thr kinases, by phosphorylation. The activation of MAPKKK

occurs *via* receptor-dependent and -independent mechanisms. In receptor-dependent mechanism, activation is initiated by the binding of an inducing ligand to the receptor tyrosine kinase (RTK) residing on the plasma membrane of the cells, which leads to the activation of RAS G-protein. In turn, RAS recruits and activates the serine/threonine protein kinase, RAF, a MAPKKK [9]. The activated MAPKKK phosphorylates and activates its downstream kinase MAPKK, which in turn phosphorylates Thr and Tyr residues and activates MAPKs. Activation of MAPKs further leads to the activation of specific MAPK-activated protein kinases (MAPKAPKs). The activation of MAPKAPKs results in a broad range of fundamental cellular activities including stress response, growth, proliferation, differentiation, survival, motility and apoptosis. However, events of MAPK phosphorylation are found to be inactivated by MAPK protein phosphatases (MKPs) that dephosphorylate both threonine and tyrosine residues on MAPKs [10].

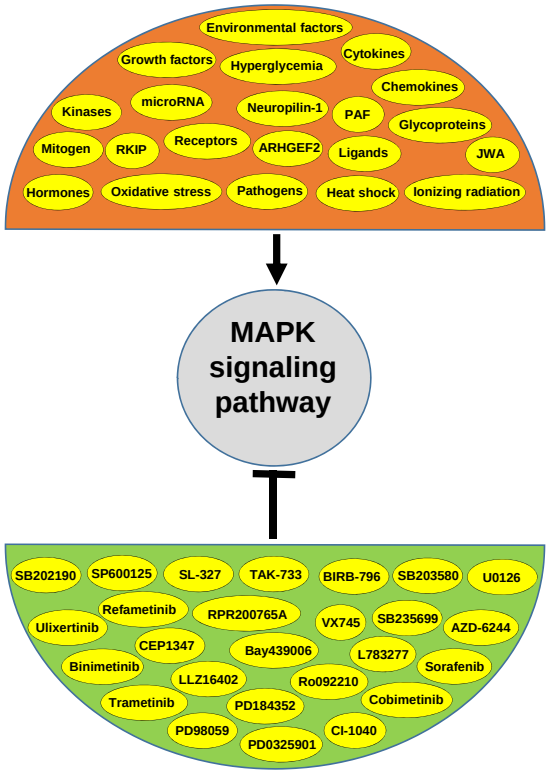

Fig. (1). Activators and inhibitors of the MAPK signaling pathway.

CHAPTER 6

Role of NF-κB Activation in Multiple Myeloma and Other Hematological Malignancies

Loukik Arora[1], Frank Arfuso[2], Alan Prem Kumar[1] and **Gautam Sethi[1,*]**

[1] *Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

[2] *Stem Cell and Cancer Biology Laboratory, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Australia*

Abstract: NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a rapid-acting transcription factor. It is present in almost all cell types and is one of the primary responders to several stimuli such as stress, cytokines, radiation, chemotherapeutic drugs, bacterial, and viral antigens. Aberrant regulation and activation of NF-κB have been implicated in several cancers, inflammatory and autoimmune disorders, viral infections, and erroneous immune system development. This chapter summarizes the role of NF-κB activation specifically in hematological malignancies and various strategies developed for its potential pharmacological intervention to abrogate the process of carcinogenesis.

Keywords: Leukemia, Lymphoma, Myeloma, NF-κB, IKKs.

INTRODUCTION

NF-κB (Nuclear factor kappa-light-chain-enhancer of B cells) is a nucleo-cytoplasmic protein complex involved in controlling transcription, production of cytokines, and regulating cell survival [1 - 5]. NF-κB is ubiquitously present in almost all human tissue types and responds to various stress stimuli such as cytokines, growth factors, radiation, oxidative stress, free radicals, *etc*. It also plays an important role in regulating the immune response to infections [2, 6 - 8]. Five members have discovered and classified in the mammalian NF-κB family so far. They are NF-κB1 NF-κB2, Rel A, Rel B, and c-Rel [9 - 11] (Table **1**). Fig. (**1**) shows the downstream target genes modulated by NF-κB to regulate a myriad of cellular responses.

* **Corresponding author Gautam Sethi:** Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117600; Tel: +65 65163267; Fax: +65 68737690; Email: phcgs@nus.edu.sg

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Table 1. Human NF-κB proteins.

Class	Protein	Gene
Class I	NF-κB1 (p50)	*NFKB1*
	NF-κB2 (p52)	*NFKB2*
Class II	RelA (p65)	*RELA*
	RelB	*RELB*
	c-Rel	*REL*

Theoretically, about 15 unique homo- and heterodimer combinations can be derived from the dimerization of five NF-κB subunits; of which 12 have been identified *in vivo*. The interactions between these subunits are based on the general principles of interaction between protein-protein complexes and this explains why RelA-p50 and RelB-p52 can form the most stable dimers among12 of them [12].

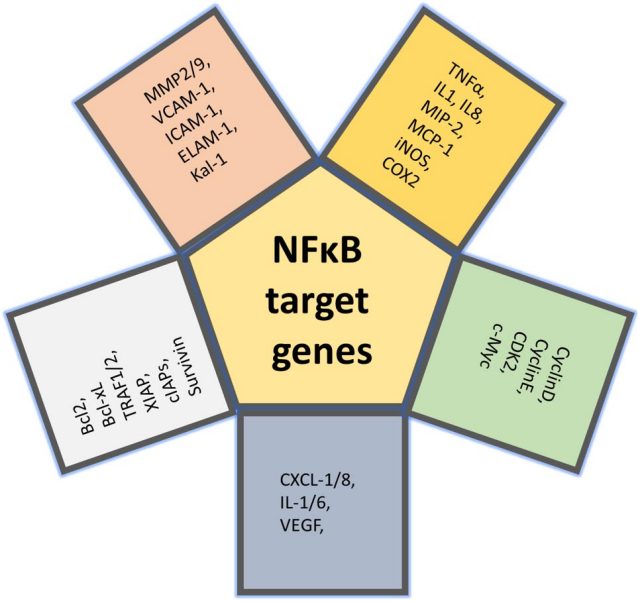

Fig. (1). A list of selected genes regulated by transcription factor NF-κB.

STRUCTURE AND SIGNALING CASCADE OF NF-κB PROTEINS

NF-κB Activation and Signaling

NF-κBs are classified as rapid-acting transcription factors; *i.e.* they are present in the cell in an inactive state and are not dependent on *de novo* protein synthesis for

their activation [13 - 15]. The NF-κB pathway can be primarily activated by two different processes, namely the canonical/classical pathway and the non-canonical pathway [16, 17] as briefly summarized in Fig. (**2**).

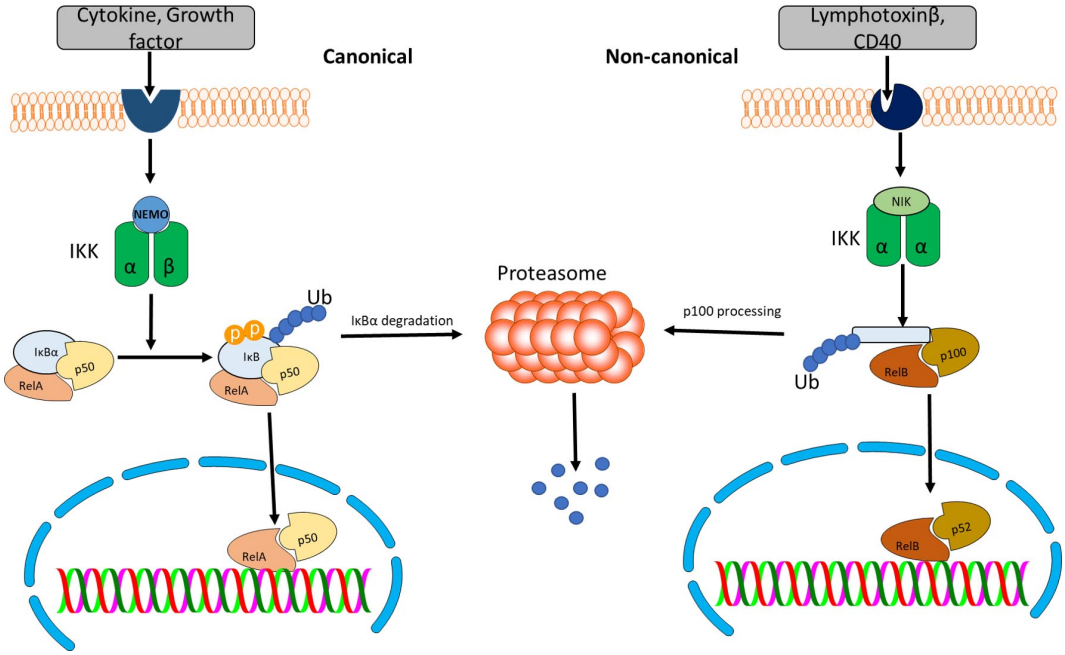

Fig. (2). A schematic representation of canonical and non-canonical NF-κB activation pathways.

Canonical or Classical Pathway

In the resting or unstimulated cell, the NF-κB heterodimer (RelA-p50) are sequestered in the cytoplasm as the nuclear localisation signal is masked by Inhibitors of κB (IκB). In canonical signaling, the ligand binding (cytokines, growth factors, lipopolysaccharides) to their respective receptors can induce the phosphorylation of the IKK (IκB Kinase) complex, which consists of IKKα, IKKβ, and NF-κB essential modulator (NEMO). The phosphorylation of IKKs leads them to phosphorylate IκBα. The phosphorylated IκBα after ubiquitination, undergoes proteasomal degradation, thereby freeing the p50-RelA heterodimer. This heterodimer translocates to the nucleus and initiates the transcription of NF-κB downstream effector genes [18]. It has been found that ubiquitination can activate NF-κB pathway independent of the proteasome. As the regulator of IKK complex, NEMO has been suggested to modulate this proteasome independent ubiquitination mediated by K63 polyubiquitin chains [19].

CHAPTER 7

Bruton's Tyrosine Kinase Signaling and Advancement in Multiple Myeloma Therapy

Krishne Gowda[1,*], Max Von Suskil[2], Omar S. Al-Odat[2], Jennifer Dang[2], Kuntal Bhowmick[2], Prachi S. Narayan[2], Shantu G Amin[1] and **Manoj K. Pandey[2,*]**

[1] *Department Pharmacology, Penn State College of Medicine, Hershey, PA, USA*

[2] *Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ 08103, USA*

Abstract: The Bruton's tyrosine kinase (BTK) is a non-receptor protein-tyrosine kinase (PTK) required for the growth and differentiation of B-lymphocytes, which play a critical role in the progression of numerous neoplasms. Therefore, BTK has emerged as an exciting and attractive target for inhibition of hematological malignancies. Various BTK inhibitors have already proved remarkable tumor suppressing ability in clinical studies. Ibrutinib was the trail blazing BTK inhibitor that first showed exceptional tumor inhibition in patients, this molecule showed excellent response in refractory/relapsed (R/R) conditions with high-risk genetic lesions patients, particularly among chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Based on ibrutinib's efficacy and tolerability, in 2016, the Food and Drug Administration (FDA) approved it as a first-line treatment for CLL patients. Ibrutinib occupies the ATP-binding active site of BTK, making salt bridges within the hinge that connects the two enzyme lobes followed by the unsaturated acrylamide group of ibrutinib covalently bonding with the BTK cysteine 481 residue to irreversibly form an inactive adduct. However, ibrutinib's irreversible binding mechanism leads to acquired resistance to the medication. Both resistance arising due to mutations that impair the affinity of ibrutinib for BTK and the undesirable side effects of the drug have led to the development of numerous second-generation inhibitors. The efficacy and specificity of novel BTK inhibiting agents such as Acalabrutinib, ONO/GS-4059, KS99, and other small molecules have substantiated solutions to ibrutinib's shortcomings. The detailed role of BTK signaling pathways, and its cross-talk between other signaling pathways, the significance of BTK inhibition in hematological malignancies, and the current progress in the discovery of small molecule BTK inhibitors are presented in this chapter.

Keywords: Multiple myeloma, Hematological malignancies, Bruton's tyrosine kinase, Myeloma stem cells, Drug development.

[*] **Corresponding author Krishne Gowda**: Department Pharmacology, Penn State College of Medicine, Hershey, PA, USA; Tel: 717-531-0003 Ext 285014; Fax: 717-531-0244; E-mail: kxg21@psu.edu

[*] **Corresponding author Manoj K Pandey**: Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ 08103, USA; Tel: 856-956-2751; E-mail: pandey@rowan.edu

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INTRODUCTION

Protein kinases (PKs) are enzymes that regulate the biological activities of proteins through mediating phosphorylation and play an essential role in every aspect of cellular functions [1]. PKs regulate cellular metabolism, transcription, division, migration, and apoptosis, as well as participate in immune responses and nervous system function. Protein phosphorylation involves the balanced action of PKs and phosphatases [2]. Phosphorylation plays a critical role in increasing or decreasing enzyme activities through post-transcriptional modification, thereby controlling signal transduction, gene expression, protein stabilization, enzyme affinity, and cellular location. Thus, the aberrant activation of PKs is associated with various disorders, especially neoplasms [3, 4].

BTK signaling was first discovered as the functional defect in the immune system condition X-linked agammaglobulinemia (XLA) in 1993. People with XLA suffer from marginal populations of B cells due to mutations in the BTK gene preventing either BTK production or function. Following the discovery of BTK in XLA, a number of studies have demonstrated conclusively that BTK is required for B-cell development, differentiation, and survival [5 - 8]. BTK knockdown studies in animal models suggest that B-lymphocytes deficient in BTK showed impaired signal transduction resulting in functional defects, inability to reach a mature state, and ultimately, high rates of apoptosis.

The B cell receptor (BCR) stimulation induces BTK phosphorylation and signal transduction [9, 10]. The BCR is a trans-membrane protein complex comprised of multiple subunits, including an immunoglobulin heavy chain (IgHC) covalently linked by disulfide bonds to an immunoglobulin light chain (IgLC) [11]. The B cell surface expresses BCR, which mediates antigen recognition. Besides the BCR pathway, BTK activation modulates various other receptor pathways vital to B cells, including chemokine-X-chemokine receptors (CXCR4 and CXCR5], toll-like receptor (TLR), and signaling mediated by Fc receptors [12 - 15]. Though in some cell types, such as in myeloid lineages, BTK acts as a downstream target of TLRs, FcεR, and FcɣRI signaling pathways [16 - 19]. Furthermore, BTK is shown to be linked in numerous additional signaling pathways, including receptor activator of nuclear factor-κB (RANK) in osteoclasts, collagen/CD32 signaling in platelets, and the NLRP3 (NOD-, LRR- and Pryin Domain-containing protein 3] inflammasome signaling in macrophages and neutrophils [20 - 22]. Based on various scientific reports, it is now established that most hematopoietic cells, including myeloid cells, express BTK, which has created significant interest among drug discovery and development groups in using BTK inhibition as an anti-cancer therapy against various malignancies (Fig. **1**) [23 - 25].

This chapter describes the significance of BTK signaling as well as the pathway cross talks. Also, how the small molecule inhibitors have clinical benefits of targeting BTK in B cell malignancies is described.

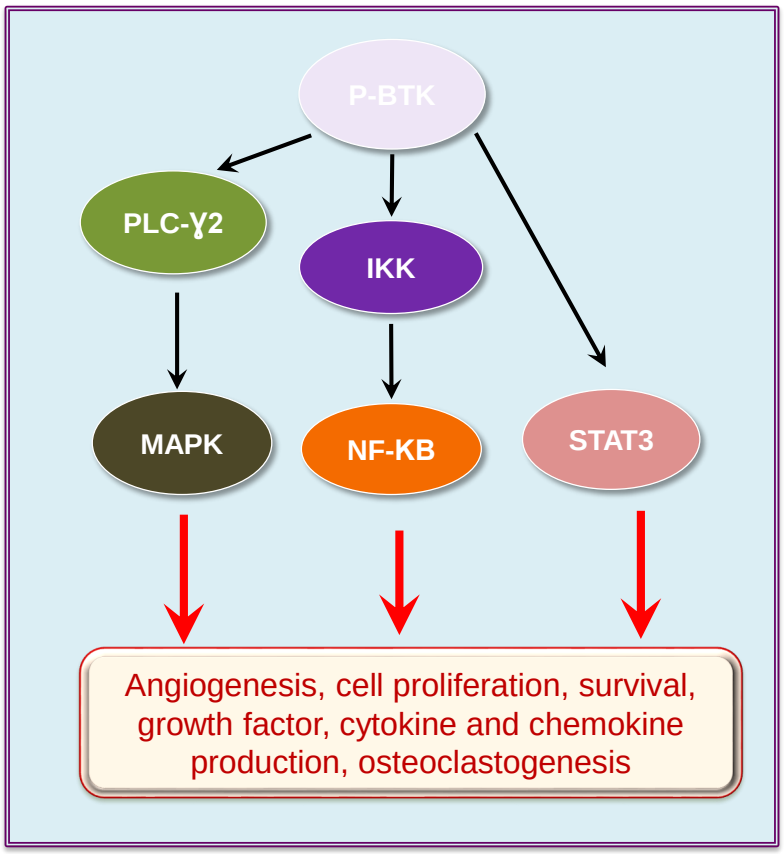

Fig. (1). The signaling of BTK pathways regulates various intermediate signaling. These signaling pathways individually contribute in the pathogenesis of malignancies.

THE BTK SIGNALING PATHWAY

Structure and Activation of BTK

BTK is one of the five members of TEC (Tec protein tyrosine kinase) family and consists of 659 amino acid residues [10]. The BTK domain structure is similar to that of the SRC family kinases in containing a C-terminal catalytic domain and the SRC homology domains (SH2 and SH3] (Fig. **2**). As compared to SRC, BTK lacks a negative regulatory tyrosine residue at the C-terminal and an N-terminal myristoylation signal [10]. Instead, BTK contains a proline-rich TEC homology (TH) domain and an N-terminal pleckstrin homology (PH) domain, which

CHAPTER 8

The Tumor Microenvironment Mediated Signaling Pathways in the Progression of Acute Myeloid Leukemia

Anup S. Pathania[1], Rachel Weber[2] and **Kishore B. Challagundla[1,3,*]**

[1] *Department of Biochemistry and Molecular Biology & The Fred and Pamela Buffett Cancer Center; University of Nebraska Medical Center, Omaha, NE, USA*

[2] *Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA*

[3] *The Children's Health Research Institute, University of Nebraska Medical Center, Omaha, NE, USA*

Abstract: Acute myeloid leukemia (AML) is a cancer of blood and bone marrow, caused by abnormal production of white blood cells. According to the recent 2020 statistics, an estimated number of 19,940 people in the United States will be diagnosed with AML. The hematologic tumor microenvironment plays a critical role in the progression of AML. Emerging evidence indicates that chemotherapy resistance and disease relapse are linked through the signaling pathways associated with the tumor microenvironment in AML. The leukemia cells communicate with the other non-cancerous cells of the tumor microenvironment through small vesicles that are within the size of 30-120nm called exosomes, a type of extracellular vesicles. Exosomes contain genetic information in their cargo, in the form of either protein, DNA, or non-coding RNAs and communicate to the distinct cells through various signaling pathways. The c-Myc oncogenic transcription factor protein is a master regulator of oncogenic signaling pathways in various cancers, including AML. C-Myc has been associated with the development of therapy resistance in AML, representing a key target. The interconnection between exosomes, tumor microenvironment, c-Myc and the development of progression, therapy resistance are discussed in this chapter and thus, represents a fundamental knowledge of the recent advances in cancer signal transduction and therapy.

Keywords: Acute myeloid leukemia, C-Myc, Drug resistance, Exosomes, Oncogene, Tumor microenvironment, Tumor suppressor gene.

* **Correspondence author Kishore B. Challagundla:** Department of Biochemistry & Molecular Biology, The Fred and Pamela Buffet Cancer Center, FPBCC 6.12.320, University of Nebraska Medical Center, 985870 Nebraska Medical Center, Omaha, NE 68198-5870, USA; Tel: 402.559.9032; Fax: 402.559.6650;
E-mail: kishore.challagundla@unmc.edu

# Anup S. Pathania & Rachel Weber share equal contribution

Manoj K. Pandey & Vijay P. Kale (Eds.)

INTRODUCTION

Leukemia is a type of cancer that forms in the blood and is typically characterized by an abnormal number of aberrant leukocytes. The bone marrow and lymphatic system are affected as that are where white blood cells are produced and found after maturation. Leukemia made up 30.4% of all blood cancers in 2017 and accounts for 2.9% of cancer cases overall [1]. This cancer is divided into two categories: acute and chronic. From there, it can be separated into subcategories based on the cell it affects, myeloid or lymphocytic. The five main types are acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL) [2]. Leukemia is the highest occurring cancer in children, with ALL making up the majority and AML is the second most common [3]. Acute leukemias tend to affect pediatric patients, whereas adults are more often seen with chronic leukemia. Major risk factors include smoking, family history, genetic disorders, exposure to chemicals, and other environmental factors [4, 5]. Chemotherapy, radiation, or bone marrow transplantation are typical treatment options, although most of these therapies are used in conjunction with another [6]. Cancer type and patient age affect the outcome of the treatment. It can be unsuccessful due to the leukemia cells communicating with the bone marrow microenvironment they reside in. During this exchange, cancer cells are able to develop the ability of self-renewal and chemoresistance [7 - 9]. Bone marrow is where hematopoietic stem cells (HSC) are located, which aids in the stemness of leukemia cells, thus leading to increased resistance to treatment [10, 11].

THE TUMOR MICROENVIRONMENT AND ACUTE MYELOID LEUKEMIA

The microenvironment cells, such as surrounding the blood, and lymph vessels, extracellular matrix, fibroblasts, and tumor-infiltrating immune cells, including B and T lymphocytes, natural killer cells, macrophages, neutrophils, dendritic cells, and mast cells are called tumor microenvironment. The tumor microenvironment plays a crucial role in the initiation and progression of leukemia. Many studies have extensively addressed the role of microenvironment in hematologic malignancies, including AML. One of the examples is retinoic acid receptor γ deficiency in bone marrow microenvironment, which can induce the development of myeloproliferative disorders, a group of slow-growing blood cancers in the bone marrow [12]. The genetic changes in specific mesenchymal cells of the hematopoietic microenvironment can induce myelodysplasia, a condition in which immature blood cells do not mature and later may progress to leukemia. Deletion of *Dicer1*, an RNAse III endonuclease essential for miRNA biogenesis and RNA

processing in mesenchymal osteolineage cells, induces myelodysplasia that later progression into AML. The transplantation of hematopoietic cells from *Dicer1* knock out mice into healthy mice does not induce myelodysplastic symptoms. Conversely, transplantation of hematopoietic cells from healthy mice into lethally irradiated mutant mice results in the development of leukemia like symptoms suggesting the role of microenvironment in determining leukemia induction [13]. Moreover, a constitutively active β-catenin mutation in mouse osteoblasts alters differentiation in myeloid and lymphoid progenitors, that can lead to the development of AML. β-catenin is the critical effector molecule that transduces Wnt singling to the nucleus and activate the transcription of Wnt specific genes involved in cell fate decisions [14].

Transplantation of bone marrow cells from constitutively active β-catenin mice into lethally irradiated wild-type mice induces hematopoietic dysfunction and AML [15]. β-catenin activation in osteoblasts stimulates expression of the Jagged 1, a Notch ligand that contributes to the deregulation of hematopoietic stem cells (HSC) lineage differentiation and AML development. Interestingly, β-catenin mutant and Jagged 1 knockdown mice do not show deregulated hematopoietic differentiation and AML development, suggesting that Notch signaling is required in β-catenin induced AML development. In patients with myelodysplastic syndrome or AML, 38.3% have increased β-catenin signaling and nuclear localization in osteoblasts. These patients also show a two-fold increase in NOTCH signaling in their hematopoietic cells compared to healthy controls. Mesenchymal stromal cells (MSCs) from AML patients have a higher level of Notch1 and jagged 1 expression compared to healthy controls. Pharmacological inhibitors of Notch significantly abrogate the survival effects of bonemarrow-derived stromal cells on leukemia cells from chemotherapy-induced apoptosis [16].

Activating mutations in *Ptpn11* gene (encodes protein tyrosine phosphatase SHP2) in the mouse bone marrow microenvironment hyperactivates cell proliferating pathways, including ERK, AKT, and NF-κB in HSCs that drives myeloproliferative neoplasm (NPM) development. Interestingly, *Ptpn11* mutations in mesenchymal stem/progenitor cells and osteoprogenitors and not in differentiated osteoblasts, or endothelial cells induces the development of NPM. *Ptpn11* mutated mesenchymal stem/progenitor cells, or osteoprogenitors secrete an excessive amount of inflammatory CC chemokine CCL3 and matrix metalloproteinase inhibitor TIMP-1 that attracts monocytes in the area. Activated monocytes secrete proinflammatory cytokines, including interleukin-1β, which hyperactivates HSCs residing in that area, resulting in the development of MPN [17]. Bone marrow MSCs secrete chemokine CXCL8 (also known as IL-8) that promotes AML cell proliferation and survival by activating the PI3/AKT survival

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Manoj K. Pandey

Manoj K. Pandey, Ph.D., is a faculty at Cooper Medical School of Rowan University (CMSRU), Camden, NJ, USA. Dr. Pandey earned his Ph.D. in Biochemistry from the Indian Institute of Toxicology Research (IITR) and Lucknow University, India. He accomplished his postdoctoral training at UT MD Anderson Cancer Center, Houston, TX. Dr. Pandey has authored more than 60 peer reviewed research articles, reviews, and book chapters in leading cancer biology and hematology oncology journals and served as Editor and Associate Editor of several journals. He is a member of the American Association of Cancer Research and American Society of Hematology.

Vijay P. Kale

Vijay P. Kale, B.V.Sc., M.V.Sc., Ph.D., DABT, ERT is a preclinical safety evaluation Scientist on anticancer drug development programs at Amgen Inc., California, USA. Dr. Kale has earned his Ph.D. from Penn State College of Medicine, Pennsylvania, USA. He has over 11 years of industrial experience in the drug discovery and development. Dr. Kale has authored several peer-reviewed research articles, abstracts at international scientific conferences, two book chapters, and delivered several oral presentations. He is a member of various scientific organizations such as AAAS, AACR, SOT, ACT, STP, STP-I and IAVP. He is certified as Diplomate of American Board of Toxicology (DABT) and is a European Registered Toxicologist (ERT).