Chapter
Role of Activated Cdc42-Associated Kinase 1 (ACK1/TNK2)-Inhibitors in Precision Oncology
Ruby Srivastava

Abstract
Activated Cdc42-associated kinase 1 (ACK1) is an intracellular non-receptor tyrosine kinase referred to as TNK2, which is considered as an oncogene and therapeutic target in various cancers including breast cancer, non-small-cell lung cancer (NSCLC), hepatocellular carcinoma (HCC), and many others. Oncogenic non-receptor tyrosine kinase mutations occur either due to point mutations, duplications or insertions and deletions, or by involving in the development of a fusion gene resulting from a chromosomal rearrangement. ACK1 is involved with multiple signaling pathways of tumor progression. With these signaling networks, ACK1 participates in cell survival, invasion, migration, and tumorigenesis that are strongly related to the prognosis and clinicopathology of cancers. Previous studies predicted that ACK1 is a carcinogenic factor and blockage of ACK1 inhibits cancer cell survival, proliferation, migration, and radiation resistance. FDA has approved many multi-kinase inhibitors as therapeutic drugs that show good inhibitory activity not against ACK1 but also towards multiple targets. As ACK1 is a key target for other neurological diseases, inflammation, and immunological diseases also, so the studies on these inhibitors not only provide potential strategies for the treatment of cancers that require simultaneous targeting of multiple targets but also can be used in drug repurposing for other diseases.

Keywords: inhibitors, therapeutics, signaling pathway, prognosis, clinicopathology

1. Introduction

Tyrosine kinases are enzyme family member which interpose the movement of the phosphate group to tyrosine residues of target protein, thus transmitting signals from the cell surface to cytoplasmic proteins and the nucleus to regulate physiological processes. TKs are divided in two sub groups: receptor and non-receptor proteins. Receptor tyrosine kinases (RTKs) include Platelet-derived growth factor receptors (PDGFR), Fibroblast growth factor receptor (FGFR), Epidermal growth factor receptor (EGFR), and Insulin receptor (IR). The Non-receptor TKs (NRTK) are divided in 9 sub-families based on the sequence similarities which included Abl, FES, JAK, ACK, SYK, TEC, FAK, SRC, and CSK. Activated Cdc42-associated kinase 1 (ACK1/TNK2) (PDB code-6VQM) is a non-receptor tyrosine kinase, which belongs to VIII tyrosine kinase family. There are seven different types of ACKs as, ACK1/TNK2, ACK2, DACK, TNK1, ARK1, DPR2 and KOS1 [1]. ACK1
was identified as first effector protein of Cdc42 [2, 3], and was cloned in hippocampus of the human brain that binds to GTP-bound form of Cdc42 [4] and inhibits its GTPase activity. ACK regulates about 147 proteins expression which is strongly connected with cell survival mechanisms [5]. The crystal structure of ACK1 is given in Figure 1. ACK1 is an approximately 114 kDa protein and have 1038 amino acids. ACK1 consists of 8 domains; sterile α motif domain (SAM), tyrosine kinase domain (TKD), Src homology 3 domain (SH3), Cdc42/Rac-interactive binding motif (CRIB), clathrin-binding region (CLATH), PPXY motif or WW domain-interacting region, epidermal growth factor receptor-binding domain (EBD) or Mig-6-homology region (MHR), and ubiquitin association domain (UBA). The SAM domain is related to membrane localization, dimerization, and activation of ACK1 (Figure 2) [7].

Its coding gene TNK2 is located on 3q29. The main function of TNK2 is to regulate the cell cycle by binding to CDC42 [8]. TNK2 can also act as an effector of Cdc42 to regulate cellular attachment and migration [9]. The CRIB domain is important for ACK1 activation and its cytoskeletal functions. ACK1 is more specified for Cdc42 activation over other GTPases (Rac and Rho) [10]. The second half of ACK1 has GRB2 [2], Sortin nexin 9 (SNX9) [10], and cortactin [11] as SH3 domain-containing binding partners. The frequent amplification and mutations of ACK1 leads to the abnormal activity of the ACK1 signaling cascades [12]. TNK2 is related to the hematological malignancies and other types of cancers [5, 13–16]. The structure of ACK family includes ACK1, 38-negative kinase 1 (TNK1), their splicing variants, activated Cdc42-associated kinase 2 (ACK2), kinase of embryonic stem cells (Kos1), and homologous proteins. It can be easily identified in mice, cows and fruit flies (ACK (Dack)) and A Ras-regulating kinase 1 (Ark-1). TNK1 is the first tyrosine kinase in which the tumor suppressor activity is found. TNK1 participates in inflammatory responses and promotes apoptosis. Its genetic variation is related to the Alzheimer’s disease. ACK1 has a special structure, which gives it unique regulatory functions. ACK1 can integrate many RTK signals and proved to be associated with cancer cell survival, proliferation, migration, and radiation resistance. It is used for cancer prediction and prognosis also. The multidomain structure of ACK1 has ability to bind to a variety of proteins, which is not only conductive to the precise location of ACK1, but also promotes its various diversified functions. ACK1 act as an important transducer of variety of extracellular signals [11]. The amplification of ACK1 gene can cause ACK1 phosphorylation (p-ACK1) and

Figure 1. The crystal structure of ACK1/TNK2 protein with loop (A, C), lobe (C, N) and helix (C) with PDB code-6VQM. The DLG, gate keeper and hinge is represented in a separate box. Adapted from Aoxue Wang et al. [6].
auto-activation, which results in the activation of ACK1 signal transduction [15, 17]. Activated ACK1 senses extracellular signals while interacting with activated receptor-tyrosine kinases including AKT, EGFR, HER2 and MERTK [18], clathrin, WW domain-containing oxidoreductase (Wwox), Grb2, AKT1, ubiquitin, androgen receptor, and Nedd4-1/2 E3 ligases [19–23]. Further studies indicated that tyrosine kinases directly regulate the activity of DNA repair and cell cycle checkpoint proteins by tyrosine phosphorylation. ACK1 as an oncoprotein which act as an epigenetic regulator. Tyrosine kinases epigenetically regulate DNA damage signaling pathways by modifying the core histones as well as chromatin modifiers at critical tyrosine residues. The deregulated tyrosine kinase driven epigenomic alterations have intense inferences in malignancies, aging and genetic abnormalities (Figure 3).

ACK1 phosphorylates and activates key survival-promoting kinase receptors on different tyrosine residues and eliminates tumor suppressors through similar mechanisms, resulting in cell survival, proliferation, and migration. ACK1 can interact with several components of vesicle dynamics in cell endocytosis and trafficking. ACK1 plays an important role in promoting extrinsic apoptosis, intervene in mechanically-induced inhibition of growth and weaken mitogenic signals to avert the abnormal growth of tissues.

The physiological roles of ACK1 include both the cancer and the normal tissues. In cancer, ACK1 participates in the regulation of many signaling pathways and exerts corresponding physiological functions, which include proliferation, differentiation, survival, apoptosis, migration, and epidermal-mesenchymal transition (EMT) and influences several important cellular processes. ACK1 is frequently overexpressed in various aggressive tumors also. It was found that ACK1 is a molecular component of the signaling cascade of neurotrophins. It is highly expressed in human brain and plays important physiological function in inflammation and immune system.

![Diagram of downstream signaling pathways of ACK1](image-url)
There are three ways to activate ACK1, which are RTK interaction, somatic cell missense mutation, and gene amplification. In previous studies, mutations in ACK1 genes have been observed in 21 kinds of cancers. 131 missense mutations, 39 nonsense mutations, and 3 fusion mutations are found in different regions of ACK1 [6]. The gene amplification of ACK1 is also observed in approximately 20 types of cancers. In cancers ACK1 is a key drug target of approximately 24 types of cancers as Metastatic Colorectal Cancer, Breast Cancer, Leukemia, Prostate Cancer, Melanoma, Gastric cancer, Lung cancer and many more. In one of RNA sequencing studies on Non-small Cell lung cancer (NSCLC) it was found that silencing of ACK1 upregulated several immune pathways as T cell receptor signaling, PI3K-Akt, Ras signaling pathways, MAPK, cAMP, Wnt signaling pathways. It was observed that ACK1 gene copy numbers were inversely linked with the infiltration levels of B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil, and dendritic cells in NSCLC [25]. Studies showed that many ACK1 tyrosine kinase signaling proteins in many tumor cells are activated repeatedly in breast cancers and the expression of ACK1 is positively correlated to the disease
severity progression and negatively correlated to the survival rate in breast cancer patients. [4, 12, 26–30] However clinical trials of targeting ACK1 in triple negative breast cancers (TNBCs) have not shown any promising results with specific inhibitors. Many tyrosine kinases (EGFR), oncoproteins (AKT), tumor suppressor proteins (Wwox), and epigenetic modification regulatory proteins (KDM3A) interacts with ACK1 in breast cancer [4, 28–32]. The clinical trials in hepatocellular carcinoma (HCC) studies predicted that ACK1 was highly expressed to the HCC tissues than in non–HCC tissues and further analysis indicated that ACK1 is positively correlated with p-ACK1 and negatively correlated with WWOX expression in HCC. The investigation revealed that ACK1 can act as potential prognostic biomarker and therapeutic target in HCC [33]. TNK2 and miR-125a-3p are considered as potential diagnostic and therapeutic targets in Colon cancer [34]. TNK2 drives the malignant state via a feed-forward ACK1/pY88-H4/WDR5/MLL2/AR epigenetic circuit in castration-resistant prostate cancers [35] and prostate cancer survival [36].

As ACK1 is highly expressed in many cancers and play a major role in tumor occurrence, targeting ACK1 gives a promising strategy for tumor treatment. Interestingly, increased Cdc42-dependent Ack1 phosphorylation has been observed in cells depleted of dynamin, and in these cells, ACK1 showed enhanced binding of both endocytic and ubiquitylated proteins [37]. ACK1 has shown potential to overcome drug resistance and provide novel possibilities of drug combination schemes for targeted therapies in cancer treatment. Kinase Inhibitors as a major drug class were emerged after the FDA approval of imtinib in 2001. Till now there are 71 small-molecule FDA approved kinase inhibitors (SMKIs) and additional 16 SMKIs which are approved by other government authorities. In oncology, 110 novel kinases as a target are explored, for which 45 targets of approved kinase inhibitors are developed so far [38]. Small molecule inhibitors are discovered, designed and synthesized by researchers to target ACK1. Various methods as fragment-based drug design, high-throughput screening, repurposing, and skeleton transitions are used for this purpose. Many inhibitors exhibited favorable pharmacokinetic activities and good anticancer activity, which can be used for clinical treatment of cancers. These drugs can be divided as (a) Selective Inhibitors, (b) Multikinase Inhibitors, and (c) Combination Drugs.

The chemical structures of few selective drugs are given in Figure 4. Compound 1 having IC$_{50}$ (24 nM), is used to suppress pan cancer cells [39] through PTEN/AKT/mTOR signaling pathways [40]. Compound 2 and 3 has hindrance activity for ACK1. It was observed that the ACK1 inhibitory ability was not higher in Compound 4. Compound 5 is also a suitable drug with good pharmacokinetic properties. Compound 6 is a fragment based drug design with low water solubility. Though Compound 7, 8, 9, 10 have low pharmacokinetic activities, they can be used to provide reference to develop novel inhibitors for mutations in ACK1 tumors. Many other studied inhibitors are Pyrrolo [2,3-d]pyrimidine, Pyrazolopyrimidine, Imidazopyrazine and their derivatives [6].

We have used in silico approaches to study the pharmacokinetic properties of 14 multikinase inhibitors and its interaction to activated Cdc42-associated Kinase 1 (ACK1/TNK2) [41]. Many of these multikinase inhibitors are FDA approved therapeutic drugs targeting multiple targets for disease treatments. These drugs included the third generation dasatinib (5) [42], and bosutinib (6) [43] as an Abelson leukemia virus (ABL) and proto-oncogene tyrosine protein kinase Src kinase inhibitor. ADZ9291 (10) [44], Sunitinib (11), flavopiridol (12), gefitinib (13) [42] and compound 14 [45] has inhibitory effects on ACK1 (Figure 5).
As these drugs alone are not enough for the survival, so advances are made on the combination therapies for effective cancer treatment. The studied inhibitors showed better results when used in combination with other drugs.

2. Challenges

Though ACK1 is a therapeutic target for cancers, inflammation, immune and neurological diseases, very few inhibitors have entered the clinical trials. Hence there is urgent need to develop potential inhibitors. The in vivo pharmacokinetic properties of inhibitors also need to be improved. Some inhibitors have limited solubility in water which restricts the studies to be carried out on the animal models only. Due to the large distribution and participation of ACK1 in regulation of many signaling pathways, high specificity and precise positioning of inhibitors to diseased tissues are required, which increases the difficulty in drug designing. So, it is necessary to explore more biological functions of ACK1 and to verify the effectiveness of drugs in vivo and in vitro. As the inhibitors are developed by only limited methods (screening small molecules and fragment libraries), it have weak affinities which makes the selection of drug candidates difficult and time consuming. Further the development of allosteric inhibitors of ACK1 is also difficult as
it need full-length proteins in the biochemical analysis of ACK1, which is a great challenge as these proteins may exhibit aggregation, conformational changes and other phenomena, which are not possible for the in vivo and in vitro studies. In last 10 years, innovative immunochemotherapies have shown promising results in disease control rates but not survival. So, there is an acute need to develop novel drugs that can target dysregulated pathways in malignant tumors. Several functional challenges include the description of genetic abnormalities in the cancer kinomes and the recognition of accurate drivers which are accountable for tumor development. Only the precise analysis of the therapeutic involvement will indicate the clear role of kinases; as a tumor suppressor in non-cancer cells or a tumor mediator in cancer cells.

3. Application of inhibitors in drug repurposing

In oncology, repurposing of drugs means the reuse of already existing drugs to treat cancer rather than testing new drugs for the existing symptoms with malignancies. Introducing new drugs is a very time-consuming and costly process which requires many pre-clinical trials before its use for the commercial purposes. The existing drugs have a huge potential with untapped agents, which are clinically more relevant for disease treatment. More than 200 existing used off patent drugs have shown some evidence for anti-cancer treatment. Since these FDA approved drugs are not in larger number, it is better to repurpose the existing drugs for therapeutic purposes. These drugs can be repurposed for not only cancer treatment but also in rheumatoid arthritis and other disorders. Interestingly multikinase inhibitors are used to interact simultaneously many targets, these drugs can play an important role in drug repurposing for treatment of different diseases.

4. Future perspectives

Now it is well established that ACK1 is a promising target for tumor therapy and the clinical studies show that there is a strong correlation between the expression level of activated ACK1 and prognosis and progression of cancers. Six specific inhibitors with high affinity for ACK1 has been identified which showed potential inhibitory activity. Some inhibitors also showed good pharmacokinetics properties in vivo. It has been observed that light-controlled PROTACs degrade specific proteins at certain locations in the body, so novel ACK1 inhibitors could have a local impact on pathologic tissues by light control. Fortunately, immunotherapy has been considered as an alternative tool for cancer patients. The treatment included many checkpoint inhibitors as nivolumab, pembrolizumab, and atezolizumab. Many other inhibitors as dasatinib, nilotinib, bosutinib along with imatinib mesylate has also used as chemotherapeutic agent for treatment in chronic myeloid leukemia (CML) patients. Considering these problems, Allosteric inhibitors, inhibitors targeting different structural domains of ACK1, inhibitors having blocking interactions within proteins, Proteolysis targeting chimeras (PROTACs), Combination therapies and dual-target drug complexes need to be develop in future. Moreover, many ACK1 interacted proteins or substrates need to be identified which can be utilized for precision medicine in cancer patients. The implementation of bioinformatics based methodologies as structure based drug designing can definitely help in drug delivery precision medicine for cancers. Refinement of effective compound screening and profiling technologies, and natural compounds need to be explored to reduce the off-target toxicity. Allosteric and covalent inhibitors, and targeted
degraders such as PROTACs and molecular glues will be the next players of kinase drug discovery in future.

Acknowledgements

RS acknowledges the financial assistance by the DST WOS-A (SR/WOS-A/CS-69/2018). RS is also thankful to her mentor Dr. Shrish Tiwari, Bioinformatics Department, CSIR—Centre for Cellular and Molecular Biology, Hyderabad and Prof. G. Narahari Sastry, Director, NEIST for the technical support.

Author details

Ruby Srivastava
Bioinformatics, CSIR—Centre for Cellular and Molecular Biology, Hyderabad, India

*Address all correspondence to: amitruby1@gmail.com

IntechOpen

© 2022 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Prieto-Echague V, Miller WT. Regulation of ack-family nonreceptor tyrosine kinases. Journal of Signal Transduction. 2011;2011:742372. DOI: 10.1155/2011/742372

[2] Lin Q, Wang J, Childress C, Yang W. The activation mechanism of ACK1 (activated Cdc42-associated tyrosine kinase 1). The Biochemical Journal. 2012;445:255-264. DOI: 10.1042/BJ20111575

[3] Manser E, Leung T, Salihuddin H, Tan L, Lim L. A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42. Nature. 1993;363:364-367. DOI: 10.1038/363364a0

[4] Mahajan K, Mahajan NP. ACK1/TNK2 tyrosine kinase: Molecular signaling and evolving role in cancers. Oncogene. 2015;34:4162-4167. DOI: 10.1038/onc.2014.350

[5] Xu SH, Huang JZ, Xu ML, Yu G, Yin XF, Chen D, et al. ACK1 promotes gastric cancer epithelial-mesenchymal transition and metastasis through AKT-POU2F1-ECD signalling. The Journal of Pathology. 2015;236:175-185. DOI: 10.1002/path.4515

[6] Wang A, Pei J, Shuai W, Lin C, Lu F, Wang Y, et al. Small molecules targeting activated Cdc42-associated kinase 1 (ACK1/TNK2) for the treatment of cancers. Journal of Medicinal Chemistry. 2021;64(22):16328-16348. DOI: 10.1021/acs.jmedchem.1c01030

[7] Liu X, Wang X, Li L, Han B. Research progress of the functional role of ACK1 in breast cancer. BioMed Research International. 2019;2019:1018034. DOI: 10.1155/2019/1018034

[8] Modzelewksa K, Newman LP, Desai R, Keely PJ. Ack1 mediates Cdc42-dependent cell migration and signaling to p130Cas. The Journal of Biological Chemistry. 2006;281:37527-37535. DOI: 10.1074/jbc.M6043 42200

[9] Satoh T, Kato J, Nishida K, Kaziro Y. Tyrosine phosphorylation of ACK in response to temperature shift-down, hyperosmotic shock, and epidermal growth factor stimulation. FEBS Letters. 1996;386:230-234. DOI: 10.1016/0014-5793(96)00449-8

[10] Yeow-Fong L, Lim L, Manser E. SNX9 as an adaptor for linking synaptojanin-1 to the Cdc42 effector ACK1. FEBS Letters. 2005;579:5040-5048. DOI: 10.1016/j.febslet.2005.07.093

[11] Kelley LC, Weed SA. Cortactin is a substrate of activated Cdc42-associated kinase 1 (ACK1) during ligand-induced epidermal growth factor receptor downregulation. PLoS One. 2012;7:e44363. DOI: 10.1371/journal.pone.0044363

[12] Mahajan K, Mahajan NP. PI3K-independent AKT activation in cancers: A treasure trove for novel therapeutics. Journal of Cellular Physiology. 2012;227:3178-3184. DOI: 10.1002/jcp.24065

[13] Qi L, Ding Y. TNK2 as a key drug target for the treatment of metastatic colorectal cancer. International Journal of Biological Macromolecules. 2018;119:48-52. DOI: 10.1016/j.ijbiomac.2018.07.124

[14] Lei X, Li YF, Chen GD, Ou DP, Qiu XX, Zuo CH, et al. Ack1 overexpression promotes metastasis and indicates poor prognosis of hepatocellular carcinoma. Oncotarget. 2015;6:40622-40641. DOI: 10.18632/oncotarget.5872

[15] Shinmura K, Kiyose S, Nagura K, Igarashi H, Inoue Y, Nakamura S, et al. TNK2 gene amplification is a novel predictor of a poor prognosis in patients with gastric cancer. Journal of Surgical...
10

[10] Xu SH, Huang JZ, Chen M, Zeng M, Zou FY, Chen D, et al. Amplification of ACK1 promotes gastric tumorigenesis via ECD-dependent p53 ubiquitination degradation. Oncotarget. 2017;8:12705-12716. DOI: 10.18632/oncotarget.6194

[11] Gajiwala KS, Maegley K, Ferre R, He YA, Yu X. Ack1: Activation and regulation by allosteroy. PLoS One. 2013;8:e53994. DOI: 10.1371/journal.pone.0053994

[12] Howlin J, Rosenkvist J, Andersson T. TNK2 preserves epidermal growth factor receptor expression on the cell surface and enhances migration and invasion of human breast cancer cells. Breast Cancer Research. 2008;10:R36. DOI: 10.1186/bcr2087

[13] Shen F, Lin Q, Gu Y, Childress C, Yang W. Activated Cdc42-associated kinase 1 is a component of EGF receptor signaling complex and regulates EGF receptor degradation. Molecular Biology of the Cell. 2007;18:732-742. DOI: 10.1091/mbc.e06-02-0142

[14] Chan W, Tian R, Lee YF, Sit ST, Lin L, Manser E. Down-regulation of active ACK1 is mediated by association with the E3 ubiquitin ligase Nedd4-2. The Journal of Biological Chemistry. 2009;284:8185-8194. DOI: 10.1074/jbc.M806877200

[15] Lin Q, Wang J, Childress C, Sudol M, Carey DJ, Yang W. HECT E3 ubiquitin ligase Nedd4-1 ubiquitinates ACK and regulates epidermal growth factor (EGF)-induced degradation of EGF receptor and ACK. Molecular and Cellular Biology. 2010;30:1541-1554. DOI: 10.1128/MCB.00013-10

[16] Tan DS, Haaland B, Gan JM, Tham SC, Sinha I, Tan EH, et al. Bosutinib inhibits migration and invasion via ack1 in kras mutant non-small cell lung cancer. Molecular Cancer. 2014;13:13. DOI: 10.1186/1476-4598-13-13

[17] Hu F, Liu H, Xie X, Mei J, Wang M. Activated cdc42-associated kinase is upregulated in non-small-cell lung cancer and necessary for FGFR-mediated AKT activation. Molecular Carcinogenesis. 2016;55:853-863. DOI: 10.1002/mc.22327

[18] Mahajan K, Mahajan NP. Shepherding AKT and androgen receptor by ACK1 tyrosine kinase. Journal of Cellular Physiology. 2010;224(2):327-333. DOI: 10.1002/jcp.22162

[19] Mahajan K, Coppola D, Challa S, et al. ACK1 mediated AKT/PKB tyrosine 176 phosphorylation regulates its activation. PLoS One. 2010;5(3):e9646. DOI: 10.1371/journal.pone.0009646

[20] Wu X, Zahari MS, Renuse S, et al. The non-receptor tyrosine kinase TNK2/ACK1 is a novel therapeutic target in
Role of Activated Cdc42-Associated Kinase 1 (ACK1/TNK2)-Inhibitors in Precision Oncology
DOI: http://dx.doi.org/10.5772/intechopen.102343

triple negative breast cancer.
Oncotarget. 2017;8(2):2971-2983.
DOI: 10.18632/oncotarget.13579

[31] Yoo J, Jeon YH, Cho HY, et al. Advances in histone demethylase KDM3A as a cancer therapeutic target. Cancers (Basel). 2020;12(5):1098. DOI: 10.3390/cancers12051098

[32] Mahajan K, Lawrence HR, Lawrence NJ, Mahajan NP. ACK1 tyrosine kinase interacts with histone demethylase KDM3A to regulate the mammary tumor oncogene HOXA1. Journal of Biological Chemistry. 2014;289(41):28179-28191. DOI: 10.1074/jbc.M114.584425

[33] Xie B, Zen Q, Wang X, He X, Xie Y, Zhang Z, et al. ACK1 promotes hepatocellular carcinoma progression via downregulating WWOX and activating AKT signaling. International Journal of Oncology. 2015;46:2057-2066. DOI: 10.3892/ijo.2015.2910

[34] Ling S, He Y, Li X, Ma Y, Li Y, Kong B, et al. Significant gene biomarker tyrosine kinase non-receptor 2 mediated cell proliferation and invasion in colon cancer. Frontiers in Genetics. 2021;12:653657. DOI: 10.3389/fgene.2021.653657

[35] Mahajan K, Malla P, Lawrence HR, Chen Z, Kumar-Sinha C, Malik R, et al. ACK1/TNK2 regulates histone H4 Tyr88-phosphorylation and AR gene expression in castration-resistant prostate cancer. Cancer Cell. 2017;31:790-803. DOI: 10.1016/j.ccell.2017.05.003

[36] Mahajan NP, Coppola D, Kim J, Lawrence HR, Lawrence NJ, Mahajan K. Blockade of ACK1/TNK2 to squelch the survival of prostate cancer stem-like cells. Scientific Reports. 2018;8:1954. DOI: 10.1038/s41598-018-20172-z

[37] Shen H, Ferguson SM, Dephoure N, Park R, Yang Y, Volpicelli-Daley L, et al. Constitutive activated Cdc42-associated kinase (Ack) phosphorylation at arrested endocytic clathrin-coated pits of cells that lack dynamin. Molecular Biology of the Cell. 2011;22(4):493-502. DOI: 10.1091/mbc.e10-07-0637

[38] Zhang B, Kirov S, Snoddy J. WebGestalt: An integrated system for exploring gene sets in various biological contexts. Nucleic Acids Research. 2005;33:W741-W748. DOI: 10.1093/nar/gki475

[39] Mahajan K, Coppola D, Chen YA, Zhu W, Lawrence HR, Lawrence NJ, et al. Ack1 tyrosine kinase activation correlates with pancreatic cancer progression. The American Journal of Pathology. 2012;180:1386-1393. DOI: 10.1016/j.ajpath.2011.12.028

[40] Wang B, Song K, Chen L, Su H, Gao L, Liu J, et al. Targeted inhibition of ACK1 can inhibit the proliferation of hepatocellular carcinoma cells through the PTEN/AKT/mTOR pathway. Cell Biochemistry and Function. 2020;38:642-650. DOI: 10.1002/cbf.3522

[41] Srivastava R. Molecular and Biological efficacy of Multikinase Inhibitors and interaction to Activated Cdc42-Associated Kinase 1 (ACK1/TNK2). Communicated to Frontier in Chemistry

[42] Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, et al. Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. Journal of Medicinal Chemistry. 2004;47:6658-6661. DOI: 10.1021/jm049486a

[43] Stansfield L, Hughes TE, Walsh-Chocolaad TL. Bosutinib: A second-generation tyrosine kinase inhibitor for chronic myelogenous
leukemia. The Annals of Pharmacotherapy. 2013;47:1703-1711. DOI: 10.1177/1060028013503124

[44] Remsing Rix LL, Rix U, Colinge J, Hantschel O, Bennett KL, Stranzl T, et al. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia. 2009;23:477-485. DOI: 10.1038/leu.2008.334

[45] Song D, Lee M, Park CH, Ahn S, Yun CS, Lee CO, et al. Novel 2,4-diaminopyrimidines bearing tetrahydronaphthalenyl moiety against anaplastic lymphoma kinase (ALK): Synthesis, in vitro, ex vivo, and in vivo efficacy studies. Bioorganic & Medicinal Chemistry Letters. 2016;26:1720-1735. DOI: 10.1016/j.bmcl.2016.02.052