

REVIEW

ACK1/TNK2 tyrosine kinase: molecular signaling and evolving role in cancers

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Deregulated tyrosine kinase signaling alters cellular homeostasis to drive cancer progression. The emergence of a non-receptor tyrosine kinase (non-RTK), ACK1 (also known as activated Cdc42-associated kinase 1 or TNK2) as an oncogenic kinase, has uncovered novel mechanisms by which tyrosine kinase signaling promotes cancer progression. Although early studies focused on ACK1 as a cytosolic effector of activated transmembrane RTKs, wherein it shuttles between the cytosol and the nucleus to rapidly transduce extracellular signals from the RTKs to the intracellular effectors, recent data unfold a new aspect of its functionality as an epigenetic regulator. ACK1 interacts with the estrogen receptor (ER)/histone demethylase KDM3A (JHDM2a) complex, which modifies KDM3A by tyrosine phosphorylation to regulate the transcriptional outcome at *HOXA1* locus to promote the growth of tamoxifen-resistant breast cancer. It is also well established that ACK1 regulates the activity of androgen receptor (AR) by tyrosine phosphorylation to fuel the growth of hormone-refractory prostate cancers. Further, recent explosion in genomic sequencing has revealed recurrent *ACK1* gene amplification and somatic mutations in a variety of human malignancies, providing a molecular basis for its role in neoplastic transformation. In this review, we will discuss the various facets of ACK1 signaling, including its newly uncovered epigenetic regulator function, which enables cells to bypass the blockade to major survival pathways to promote resistance to standard cancer treatments. Not surprisingly, cancer cells appear to acquire an 'addiction' to ACK1-mediated survival, particularly under stress conditions, such as growth factor deprivation or genotoxic insults or hormone deprivation. With the accelerated development of potent and selective ACK1 inhibitors, targeted treatment for cancers harboring aberrant ACK1 activity may soon become a clinical reality.

Oncogene (2015) 34, 4162–4167; doi:10.1038/onc.2014.350; published online 27 October 2014

INTRODUCTION

Oncogenomics and oncoproteomics has not only lead to unearthing of biomarkers specific to each cancer type and disease stage but has also expedited our understanding of the molecular alterations/characteristic of each individual tumor. These approaches have heightened the significance of many understudied signaling proteins that promote tumor progression in less than favorable environments. A signaling protein that appears to be recurrently activated in cancer cells is the ACK1 tyrosine kinase.^{1,2} Although originally identified as a non-receptor tyrosine kinase (non-RTK) that specifically binds to the GTP-bound form of Cdc42 and inhibits its GTPase activity,³ its distinct signaling in cancer cells has rekindled interest in this structurally unique kinase—as a molecular entity for targeted therapeutics.

ACK1 gene encodes a large protein of 1038 amino acids (140 kDa) that is posttranslationally modified by multiple tyrosine phosphorylations that regulate its kinase activation.^{4,5} The multidomain structure of ACK1 sets it apart from other non-RTKs and includes at least eight distinct domains; the sterile α motif (SAM), kinase or catalytic domain, Src homology domain 3 (SH3) domain, GTPase-binding domain (also known as Cdc42-binding domain or CRIB domain), clathrin-interacting region, PPXY motif or WW domain-interacting region, a MIG6 homology region (MHR), also known as epidermal growth factor receptor (EGFR)-binding domain and an ubiquitin association (UBA) domain (Figure 1). It is the only kinase known to possess an SH3 domain carboxy terminal to the kinase domain.⁶ A most recognizable outcome of

this multidomain structure appears to be to not only facilitate ACK1 localization to different cellular compartments but to also promote its association with disparate proteins fostering its functional diversity.¹

Being an intermediary kinase that bridges the RTKs and effector proteins, it became quickly apparent that the ACK1 was providing a niche for intracellular communication. Crystal structure analysis and extensive biochemical studies now provide a glimpse into the carefully orchestrated process of ACK1 activation in normal as well as cancer cells. Within ACK1, an insertion of a 10-amino-acid sequence that is rich in proline residues has been identified upstream of the kinase recognition segment of the MHR. It has been suggested that this proline-rich sequence interacts with the SH3 domain-facilitating orientation of the MHR for inhibitory interactions with the C-terminal region of the kinase domain, stabilizing an autoinhibited state of the ACK1 kinase.^{7–9} Activation of ACK1 is achieved when ligand-activated RTKs interact with the MHR of ACK1 overcoming an autoinhibitory interaction of the MHR with its own kinase domain.^{7,9,10} In addition, another step may be involved in accomplishing the complete ACK1 activation. Recently, it has been demonstrated that the amino-terminal SAM domain facilitates membrane localization and symmetric dimerization leading to transphosphorylation and activation.¹¹ Thus, ACK1 may be switching to different modes of kinase activation, to adapt rapidly to cellular requirements.

Multiple ligands such as EGF, GAS6 (ligand binding to Mer receptor tyrosine kinase and activating ACK1 downstream),

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Received 11 August 2014; revised 17 September 2014; accepted 22 September 2014; published online 27 October 2014

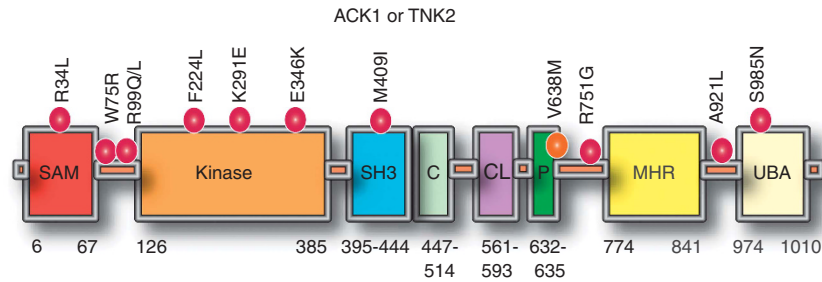


Figure 1. A schematic representation of ACK1 domain architecture. Different structural domains present in ACK1 are shown. C, Cdc42-binding domain; CL, clathrin-interacting domain; kinase, tyrosine kinase domain; P, PPXY motif or WW domain-interacting region and SAM, sterile α motif. Various mutations identified in ACK1 have been shown on the top.

heregulin, insulin growth factor and insulin cause a rapid albeit temporal activation of the ACK1, suggesting that ACK1 acts as a major integrator of RTK signaling.^{4,5,12,13} Interestingly, investigation into the consequences of EGFR-mediated ACK1 activation not only revealed rapid Tyr phosphorylation (and activation) of ACK1, followed by the loss of ACK1 levels, but also that the EGFR protein itself was significantly downregulated.¹² These data indicate that not only EGFR-mediated ACK1 activation may play a role in maintaining ACK1 homeostasis, but ACK1 in turn could also regulate EGFR levels in ligand-activated cells, thus switching off EGFR-ACK1 signaling. Accordingly, suppression of ACK1 expression by small interfering RNA resulted in the inhibition of EGF-induced degradation of EGFR.¹⁴ Although EGFR-ACK1 cross-talk provides clues to the delicate balance that exist within cells, whether other proteins could compensate for the loss of ACK1 function to regulate EGFR levels remain to be seen. At the molecular level, the loss of UBA domain of ACK1 blocked the ligand-dependent degradation of EGFR, suggesting that ACK1 may regulate both EGFR and its own degradation via its UBA domain. Consistent with these findings, some of the key players required to turn off ACK1 expression in cells are the ubiquitin ligases. In particular, ACK1 has been shown to interact with the WW2 and WW3 domains of E3 ubiquitin ligase Nedd4-2, via a conserved PPXY-containing region, resulting in ACK1 polyubiquitination and degradation.¹⁵ Interestingly, ACK1 interacts with the WW domain of tumor suppressor WWOX using the same region to cause its polyubiquitination and degradation.^{4,15} It is plausible that in cancer cells, ACK1 interaction with WWOX may obstruct interaction with the E3 ubiquitin ligase Nedd4-2, and thus limit ACK1 degradation, stabilizing the ACK1 protein levels.

Recently, ACK1 was shown to interact with sequestosome 1 (p62/SQSTM1), an autophagy receptor, through an UBA domain in an EGF-dependent manner, indicating that ACK1 diverted activated EGFR into a degradative pathway by its association with p62/SQSTM1, NBR1 and Atg16L.¹⁶ A second mechanism that has recently been uncovered involves ACK1 interaction with Seven in absentia homolog (SIAH) ubiquitin ligases, SIAH1 and SIAH2, via a conserved SIAH-binding motif located in the far carboxy terminus of ACK1.¹⁷ Interestingly, the association of ACK1 with SIAHs was independent of its kinase activity. These include ER-dependent signaling; interestingly, EGFR and ER signaling cascades are often regulated antagonistically.¹⁷ Moreover, SIAHs can restrict oncogenesis.¹⁸ Taken together, these data indicate that apart from EGFR activation, other pathways could also induce proteasomal degradation of ACK1 and play role in tumor initiation.

ACK1 IN NEURONAL SIGNALING

ACK1 expression is detectable at fairly high levels in the developing and adult brain, with the highest expression seen in the hippocampus, neocortex and cerebellum.¹⁹ These expression studies lead to uncovering the role for ACK1 in synaptic function;

ACK1 mRNA levels were significantly upregulated by increased neural activity. ACK1 kinase was also shown to be expressed in proliferative areas and in migratory pathways in the developing brain.²⁰ The mechanistic details of ACK1 signaling in the brain became recently available, wherein in response to neurotrophins, ACK1 was shown to interact with Trk receptors leading to its activation, which in turn activated AKT.²¹ Overall, these data indicate that ACK1 is an important player in neurotrophin signaling, neuronal extension and branching; however, the pathological significance of ACK1 signaling in the brain was not entirely clear, till a Belgian-Italian family was identified where all the three siblings presented with autosomal recessive, infantile onset epilepsy and intellectual disability.²² The exome sequencing identified a homozygous missense variant, V638M, in the ACK1 (Figure 1). Unlike WT ACK1 protein, the EGF ligand treatment of V638M variant protein did not result in its degradation, primarily because of its failure to associate with E3 ubiquitin ligases, NEDD4-1 and NEDD4-2. These data indicate that V638M is a gain-of-function mechanism of upregulation of ACK1 levels. Further, these data open up the possibility of ACK1's role in intellectual disability and the onset of epilepsy, which would need further examination.

ONCOGENIC ACTIVATION OF ACK1

The oncogenic properties of ACK1 kinase first became evident when activated ACK1 was shown to promote anchorage-independent growth and tumor growth *in vivo*.⁴ Since this discovery, significant new information has become available, which has established that aberrant ACK1 activation leading to its oncogenicity may occur by at least three distinct mechanisms, (a) deregulated RTK activation feeding into ACK1, (b) gene amplification and (c) somatic missense mutations.¹

An intriguing aspect of ACK1 activation is its tight temporal activation; stimulation of cells treated with growth factors not only exhibited rapid activation of their respective RTKs but also led to ACK1 activation, as seen by Tyr phosphorylation, in a time-dependent manner.^{4,5,12} The precision with which the ligand mediated ACK1 activation occurred provided two major clues: (1) RTKs directly activate ACK1 and (2) the specificity of the ligand/RTK-mediated ACK1 activation is cell type-dependent. While multiple RTKs can potentially interact with ACK1 resulting in its activation, there are distinct differences in the kinetics of when and how long ACK1 remains activated in cell types of different origins.^{4,5,12,23}

ACK1 gene is located on human chromosome 3q29. Advances in genome sequencing has revealed ACK1 gene amplification, consequently causing mRNA overexpression, as another mechanism of RTK-independent ACK1 activation in a variety of cancer cells (Figure 2). Notably, ACK1 gene amplification has been observed in cervical, ovarian, lung squamous, head and neck squamous cell carcinomas, breast, prostate and gastric cancers (cBioPortal; <http://www.cbioportal.org>).²⁴⁻²⁶

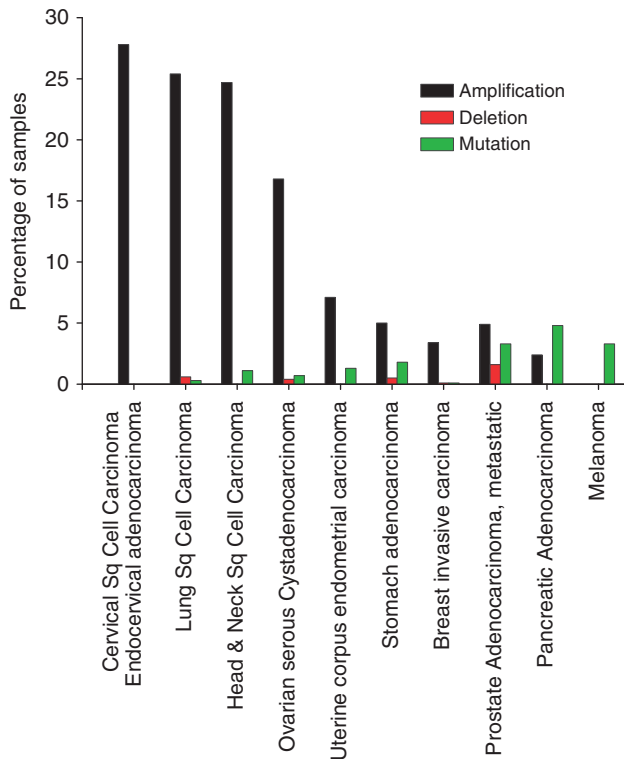


Figure 2. *ACK1* gene alterations in human cancers. A percentage of patient population of each tumor type exhibiting gene amplification, homozygous deletion or somatic mutations are shown. Data are derived from cBioPortal for Cancer Genomic, Memorial Sloan-Kettering Cancer Center (<http://www.cbioportal.org>).

Exome sequencing has also revealed recurrent somatic mutations in the *ACK1* gene. Seventy-one distinct missense substitutions and 10 nonsense mutations were identified within the various domains of the *ACK1* gene (COSMIC and cBioPortal databases). Of these, four missense mutations, R34L, R99Q, E346K and M409I (Figure 1), have been analyzed in detail because of their location within distinct domains of *ACK1*.¹² The *ACK1*-E346K mutation identified in ovarian cancer exhibits significant increase in *ACK1* autoactivation.^{9,12} R34L, located in SAM domain, and R99Q and M409I, located within the SH3 domain, too display significant *ACK1* autoactivation.⁹ Overall, unlike *ACK1* amplification that occurs in almost a quarter of the cervical, ovarian and lung cancer patients, missense substitutions in *ACK1* occurs with a significantly lower frequency (Figure 2), suggesting that gene amplification underlies the etiology of deregulated *ACK1* activation in the majority of the cancers.

MODULATION OF AKT ACTIVATION: ANTIAPOPTOTIC AND PROLIFERATIVE FUNCTIONS OF *ACK1*

ACK1 can transduce extracellular signals to cytosolic and nuclear effectors, distinguishing it from many of the other non-RTKs (Figure 3). The cellular function and role of the multiple *ACK1*-interacting proteins has been reviewed earlier.¹ Here, we will discuss in detail some of the effectors of *ACK1* signaling, for example, AKT, androgen receptor (AR), ER and KDM3A, that have a crucial role in prostate and breast cancers.

AKT activation is critically dependent on its phosphorylation. A predominant mode of activation is the RTK/phosphoinositide 3-kinase (PI3K) signaling pathway, which facilitates membrane recruitment of AKT, as a consequence of the binding of the pleckstrin homology domain of AKT to phosphatidylinositol 3,4,5

trisphosphate, followed by phosphorylation at two sites, Thr308 and Ser473, leading to its kinase activation.^{27–29} Recently, a novel mechanism of AKT activation has been uncovered that is phosphatidylinositol 3,4,5 trisphosphate- and PI3K-independent, and requires interaction with a non-RTK. Serendipitously, we discovered that *ACK1* directly interacts with the oncogene AKT and phosphorylates it at an evolutionary conserved tyrosine residue at the 176th position. Tyrosine 176 (Tyr176)-phosphorylated AKT localized to the plasma membrane owing to its preferential binding to the phospholipid, phosphatidic acid (PA), and was further phosphorylated at Ser473 and Thr308 sites leading to its activation.¹² Subsequently, AKT translocated to the nucleus to suppress the expression of proapoptotic and cell cycle arrest genes, promoting mitotic progression.^{2,12,30} Significantly, somatic autoactivating mutation in *ACK1*, for example, E346K, also catalyzes robust AKT Tyr176 phosphorylation and activation, suggesting that cancer cells could bypass blockade to RTK/PI3K-dependent AKT activation by using alternate survival pathways, such as those mediated by the oncogenic *ACK1*.

In addition to AKT activation, *ACK1* may also overcome cell death by interacting with other survival pathways. One such example has recently been uncovered in *Drosophila*, wherein *ACK1* was shown to possess potent antiapoptotic activity that is dependent on its interaction with two effector proteins, Drk, the *Drosophila* homolog of GRB2, and yorkie, a transcriptional coactivator that is downstream of the Salvador-Hippo-Warts pathway, promoting transcription of proliferative and antiapoptotic genes.³¹

AR REGULATION: *ACK1* SIGNALING IN PROSTATE CANCER

AR is a crucial regulator of prostate cancer growth and has a paramount role in the progression of cancer to the highly metastatic disease, often termed as castration-resistant prostate cancer (CRPC).^{32–34} AR upon binding to its cognate ligand, androgen, translocates to the nucleus and regulates transcriptional activation of a large number of genes. This androgen-dependent transcriptional activity has been the cornerstone of the androgen deprivation therapy, a preferred treatment to negate AR transcriptional coactivator activity for prostate cancer. While chemical treatment with antiandrogens or surgical treatment by orchiectomy provides immediate palliative benefits, these androgen deprivation therapies are ineffective in long term, as the recalcitrant disease recurs within 2–3 years. In spite of the 'castrate' levels of androgens, prostate cancer cells still accomplished AR activation and were able to drive disease to the CRPC stage. Consequently, resistance to androgen deprivation therapy has become one of the most perplexing problems in prostate cancer therapy.^{32,35,36} The role of *ACK1* in AR regulation first became clear when treatment of prostate cancer-derived cell lines with ligands such as EGF, heregulin and GAS6 resulted in not only robust *ACK1* activation but, surprisingly, also lead to Tyr phosphorylation of AR.⁵ *ACK1* bound AR and directly phosphorylated AR at two distinct sites, Tyr267 and Tyr-363, both located within the transactivation domain, facilitating androgen-independent transcriptional activation of AR. The significance of *ACK1*/AR signaling nexus became obvious in xenograft tumor studies; castrated mice injected with cells expressing activated *ACK1* not only formed androgen-independent tumors but also exhibited compromised tumor growth when mutant AR (Y267F mutation) was coexpressed with activated *ACK1*.⁵

Over the past few years, a critical role for *ACK1* in prostate cancer pathogenesis is further underscored by several observations; namely, *ACK1* mRNA is not only upregulated in prostate cancers but activated *ACK1* expression also correlates positively with the progression to the malignant CRPC stage.^{5,13,24} Indeed, 10 out of 13 CRPC tumors exhibited 5- to >100-fold *ACK1* overexpression.²⁴ Notably, alterations in *ACK1* is associated with

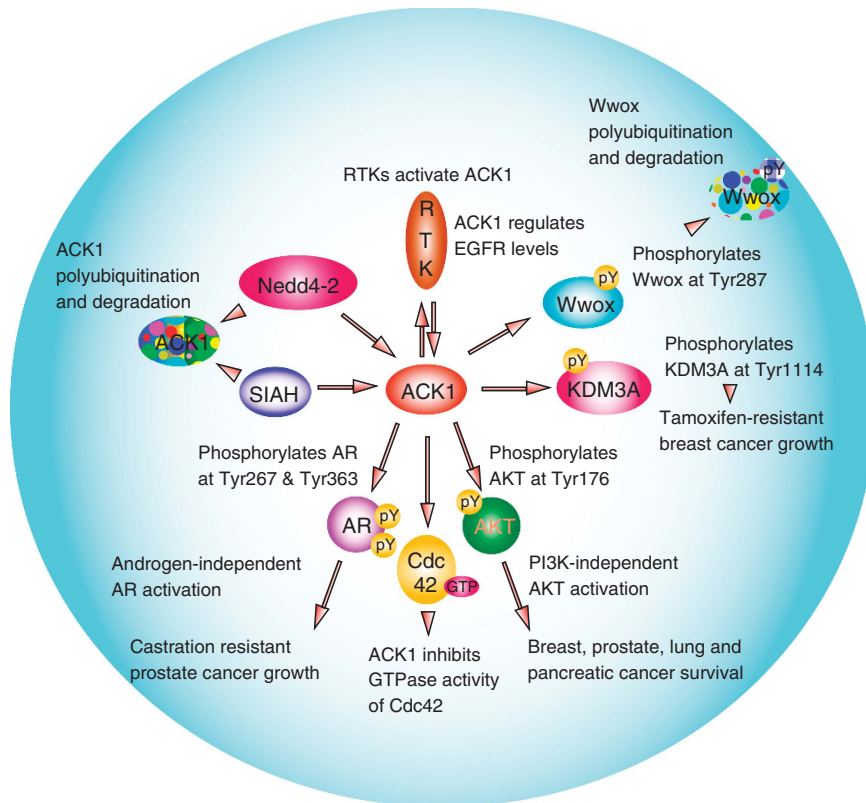


Figure 3. ACK1 interactome. ACK1 kinase network where it functions as a conveyer of signals from a diverse group of activated RTKs to transmit cell survival, growth and proliferative signals via modification of multiple downstream effectors by unique tyrosine phosphorylation events.

median disease-free state of only 1.3 months compared with 110 months for PC patients without the ACK1 alteration (cBioPortal). One of the most compelling evidence came from transgenic mice studies wherein Probasin-ACK1 transgenic mice that express activated ACK1 specifically in prostates developed prostatic intraepithelial neoplasias and rare carcinomas.^{12,37} The prostates of ACK1 transgenic mice exhibited significant increase in not only AR Tyr267 phosphorylation but also in AKT Tyr176 phosphorylation leading to AKT substrate FOXO3a Ser318/321 phosphorylation, suggesting that both biochemical events may be needed for the neoplastic transformation of prostates.¹² ACK1-mediated AKT Tyr176 phosphorylation and activation may be more proximal stage-initiating processes in neoplastic progression that serve as an alternative to PTEN loss, which has been well studied in the mouse models of prostate cancer,³⁸ whereas AR Tyr267 phosphorylation paving the way for the progression of disease.

Overall, it appears that CRPC tumors may have hijacked the ACK1 oncogenic activity to phosphorylate AR causing transcriptional activation of AR regulatory genes in a low androgen environment; however, could Tyr267-phosphorylated AR regulate transcription of the genes that are not regulated by androgen-bound AR? A large-scale chromatin immunoprecipitation followed by hybridization to genomic chip revealed that, indeed, such a program may be operational in CRPC tumors. Tyr267-phosphorylated AR initiated a distinct androgen-independent transcription program in prostate cancer cells.³⁷ Interestingly, many of the genes that are regulated by pTyr267-AR were the members of the p53-dependent DNA damage signaling pathway, for example, p300, MDM2 and ATM (ataxia telangiectasia mutated). Being a critical upstream regulator of the DNA damage signaling and checkpoint pathways that maintain genetic integrity

and facilitate DNA repair in response to DNA damage, ATM expression was studied in greater detail. pTyr267-AR not only bound upstream of the ATM gene transcription start site facilitating increased ATM mRNA expression, but the primary human CRPCs with upregulated activated ACK1 and pTyr267-AR also exhibited significant increase in ATM expression.³⁷ Overall, these findings underscore a dominant role for ACK1 in hormone-refractory prostate cancer.

ACK1 AS AN EPIGENETIC MODULATOR IN BREAST CANCER

The regulation of AR by ACK1 kinase has opened doors for an obvious question: can ACK1 be relevant in other hormonally regulated cancers? Estrogen receptor (ER) is an important nuclear hormone receptor for the normal physiology of mammary cells.³⁹ The ER protein binds to its cognate ligand estrogen (E₂) with high affinity to form a functionally active complex that differentially interacts with coregulator proteins to modify histones within the chromatin to modulate target gene expression.⁴⁰ Similar to prostate cancer cells, the breast cancer cells are also critically dependent on estrogen for their growth, which has led to the successful use of a selective ER modulator, tamoxifen in the treatment of ER-positive breast cancer.^{41,42} Although a large number of the ER-positive breast tumors are sensitive to tamoxifen therapy, within 15–60 months, tumors become refractory to tamoxifen.^{43,44} As ER is expressed and functional in tamoxifen-resistant breast cancers, paradoxically, it may be the estrogen-independent or ER-dependent signaling that promulgates tamoxifen resistance. The potential role of ACK1 in ER signaling to facilitate the growth and survival of breast cancers seemed plausible, specially based on its robust interaction with another related nuclear hormone receptor—the AR. Moreover, not

only does heregulin-mediated HER-2 activation cause robust activation of ACK1 in breast cancer cell lines,¹² but overexpression of ACK1 in human breast cancer cell lines followed by injection into immunocompromised mice induced tumor development.²⁴ Further, activated ACK1 (and pTyr176-AKT) expression correlated with breast cancer progression and the Kaplan–Meier analyses revealed that the breast cancer patients with high expression of activated ACK1 (and AKT Tyr176 phosphorylation) are at a higher risk for cancer-related deaths.¹²

Although ACK1 has emerged to be an important player in breast cancer, the precise mechanistic details of its *modus operandi* have become clear only recently. ACK1 phosphorylates the ER coactivator, KDM3A, also known as JHDM2A or Jmjd1a, an H3K9 demethylase at an evolutionarily conserved tyrosine 1114 site in a heregulin-dependent manner.⁴⁵ Accordingly, ACK1 activation resulted in significant decrease in dimethyl-H3K9 epigenetic marks, facilitating transcriptional upregulation of a critical ER-regulated mammary tumor oncogene, *HOXA1*, even in the presence of tamoxifen.⁴⁵ Overall, these data indicate that by its ability to regulate the epigenetic activity of an ER coactivator, ACK1 modulated the expression of ER target genes in the absence of E₂, conferring tamoxifen resistance.⁴⁵ As two-thirds of breast cancers are ER positive and are initially expected to be responsive to selective ER modulators, ACK1 inhibitors may be of crucial importance to counter tamoxifen resistance, particularly in those breast tumors that aberrantly activate ACK1.

Buchwald *et al.*¹⁷ have demonstrated that ACK1 levels are E₂/ER-dependent in ER-positive breast cancer cells. Taken together, it opens up a new mechanism of ACK1 role in breast cancer; when E₂/ER signaling is inhibited by Tamoxifen, loss of SIAH expression could lead to increased ACK1 levels, driving Tamoxifen resistance. Mechanistically, ACK1 can promote drug resistance in breast cancer by directly activating a second target, the serine-threonine kinase AKT.¹² Ligand-bound HER-2/ErbB-2 or EGFR activates ACK1, which in turn phosphorylates and activates AKT at Tyr176 that is not hindered by PI3K inhibition or knockdown of PI3K expression by RNA interference.¹² Thus, a subset of drug-resistant breast cancer cells may be resensitized to undergo apoptosis by treatment with the ACK1 inhibitors. Overall, RTK/ACK1 signaling nexus explains alternate modes of AKT activation in those tumors that display amplification/activation of RTKs and non-RTKs and therefore uncovers a new arsenal in the form of ACK1 inhibitors.

ACK1 SIGNALING IN THE PATHOGENESIS OF PANCREATIC AND LUNG CANCERS

The rapid activation of ACK1 in a variety of pancreatic-, ovarian- and lung cancer-derived cells suggests that cancer cells are primed to use this pathway for survival.^{12,30} Conforming to this regulatory paradigm, AKT is also frequently activated in pancreatic cancer, which has been shown to be highly correlated to HER-2/*neu* overexpression.⁴⁶ Moreover, many of the pancreatic cell lines and tumors expressing activated AKT were not mutated for the tumor suppressor, PTEN.^{47,48} We reported that PanIN and pancreatic adenocarcinomas exhibit significantly higher levels of activated ACK1 (phosphorylated at Tyr284) and Tyr176-phosphorylated AKT.³⁰ Conversely, pancreatic cancer cell lines treated with ACK1 inhibitors displayed inhibition of cell growth and apoptosis, indicating that AKT activation in the absence of PTEN mutations may be driven by RTK/ACK1 signaling axis.^{2,30}

TNK2/ACK1 AS AN EFFECTOR OF MUTANT COLONY-STIMULATING FACTOR 3 IN CHRONIC NEUTROPHILIC LEUKEMIA

A tyrosine kinase-specific small interfering RNA screen for genetic drivers of leukemia cells with activating mutations in the gene encoding the receptor for colony-stimulating factor 3 revealed

that the mutations segregate within two distinct regions of colony-stimulating factor 3 and lead to preferential downstream kinase signaling through either the ACK1 or JAK kinases. These cells also displayed differential sensitivity to kinase inhibitors.⁴⁹ A patient with chronic neutrophilic leukemia or atypical CML, carrying a JAK-activating colony-stimulating factor 3 mutation, had marked clinical improvement after the administration of the JAK1/2 inhibitor ruxolitinib; however, the effectiveness of ACK1 inhibitors in this patient population remains to be seen.

ACK1 SMALL-MOLECULE INHIBITORS

Realizing the significance of ACK1 in cancer pathogenesis, as a quick route to clinic, previously characterized kinase inhibitors were reassessed for their inhibitory activities against ACK1. In addition, new ACK1-specific kinase inhibitors were developed after screening small-molecule compound libraries. Detailed information about discovery and characterization of multiple ACK1 inhibitors has recently been reviewed.⁵⁰ Two inhibitors, Dasatinib (BMS-354825 or Sprycel) and AIM-100, have primarily been assessed for the inhibition of ACK1 signaling *in vitro* and *in vivo*. Dasatinib not only inhibited ACK1 phosphorylation but also its substrate AR Tyr phosphorylation and KDM3A Tyr phosphorylation.^{45,51} However, because of the multitarget activity of Dasatinib against many tyrosine kinases, it is difficult to interpret whether the cellular toxicity is specifically because of inhibition of ACK1, and thus limiting its use *in vivo* to target ACK1 activated tumors. A critical step in the progression of localized indolent cancer to the more aggressive and metastatic stage is the ability to invade into neighboring tissues and enter the blood stream. Recent reports reveal that Bosutinib a small-molecule kinase inhibitor that targets Src also inhibits ACK1-dependent migration and invasion of the KRAS mutant non-small-cell lung cancer.⁵²

In contrast to Dasatinib and Bosutinib, AIM-100 has emerged to be specific and the best-studied ACK1 inhibitor.^{13,30,37,45,53} AIM-100 prevented AKT Tyr176 phosphorylation and AR Tyr267 phosphorylation and function in breast and CRPC cells in which ACK1 is activated because of RTK activation or when ACK1 is autoactivated by somatic mutation (for example E346K-ACK1).^{13,30,37} In spite of its ability to inhibit cancer cell proliferation, because of its limited solubility in aqueous environment, AIM-100 has not progressed further as a prospective therapeutic agent.

FUTURE PERSPECTIVE

The cross-talk of tyrosine kinases with the epigenetic machinery has opened a new chapter into how activated kinases are directly modulating gene expression programs within cells. Past research has focused on the cytosolic function of ACK1; however, with the recent findings that ACK1 interacts with a histone demethylase, it opens hitherto unknown aspect of ACK1 functionality in the nucleus. Importantly, the epigenetic alterations are reversible, and thus targeting ACK1 may be critical to reverse epigenetic changes and thus proliferative gene expression programs. Future studies into the epigenetic regulatory roles of ACK1 in the pathophysiology of cancers, the availability of mouse tumors models in an ACK1-deficient background and the development of selective and potent inhibitors will allow us to precisely target individual tumors to have better success with ACK1 inhibitors for the treatment of a variety of cancers.

CONFLICT OF INTEREST

The authors are named as inventors on a US patent application no. 8 557 516, titled 'AKT tyrosine 176 phosphorylation as cancer biomarker'.

ACKNOWLEDGEMENTS

This work was supported in part by Department of Defense (W81XWH-12-1-0248 and W81XWH-14-1-0251) to KM and by the National Cancer Institute, NIH (1R01CA135328), Department of Defense (W81XWH-14-1-0002 and W81XWH-14-1-0003) and Career Development Award by Moffitt Lung Cancer SPORE to NPM.

REFERENCES

- Mahajan K, Mahajan NP. Shepherding AKT and androgen receptor by Ack1 tyrosine kinase. *J Cell Physiol* 2010; **224**: 327–333.
- Mahajan K, Mahajan NP. PI3K-independent AKT activation in cancers: a treasure trove for novel therapeutics. *J Cell Physiol* 2012; **227**: 3178–3184.
- 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.
- Mahajan NP, Whang YE, Mohler JL, Earp HS. Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: role of Ack1 in polyubiquitination of tumor suppressor Wwox. *Cancer Res* 2005; **65**: 10514–10523.
- Mahajan NP, Liu Y, Majumder S, Warren MR, Parker CE, Mohler JL *et al.* Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. *Proc Natl Acad Sci USA* 2007; **104**: 8438–8443.
- Yokoyama N, Miller WT. Biochemical properties of the Cdc42-associated tyrosine kinase ACK1. Substrate specificity, autophosphorylation, and interaction with Hck. *J Biol Chem* 2003; **278**: 47713–47723.
- Gajiwala KS, Maegley K, Ferre R, He YA, Yu X. Ack1: activation and regulation by allostery. *PLoS ONE* 2013; **8**: e53994.
- Prieto-Echague V, Miller WT. Regulation of ack-family nonreceptor tyrosine kinases. *J Signal Transduct* 2011; **2011**: 742372.
- Prieto-Echague V, Guzca A, Craddock BP, Brown DA, Miller WT. Cancer-associated mutations activate the nonreceptor tyrosine kinase Ack1. *J Biol Chem* 2010; **285**: 10605–10615.
- Lin Q, Wang J, Childress C, Yang W. The activation mechanism of ACK1 (activated Cdc42-associated tyrosine kinase 1). *Biochem J* 2012; **445**: 255–264.
- Prieto-Echague V, Guzca A, Brown DA, Miller WT. Regulation of Ack1 localization and activity by the amino-terminal SAM domain. *BMC Biochem* 2010; **11**: 42.
- Mahajan K, Coppola D, Challa S, Fang B, Chen YA, Zhu W *et al.* Ack1 mediated AKT/PKB tyrosine 176 phosphorylation regulates its activation. *PLoS One* 2010; **5**: e9646.
- Mahajan K, Challa S, Coppola D, Lawrence H, Luo Y, Gevariya H *et al.* Effect of Ack1 tyrosine kinase inhibitor on ligand-independent androgen receptor activity. *Prostate* 2010; **70**: 1274–1285.
- 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. *Mol Biol Cell* 2007; **18**: 732–742.
- Chan W, Tian R, Lee YF, Sit ST, Lim L, Manser E. Down-regulation of active ACK1 is mediated by association with the E3 ubiquitin ligase Nedd4-2. *J Biol Chem* 2009; **284**: 8185–8194.
- Jones S, Cunningham DL, Rappoport JZ, Heath JK. The non-receptor tyrosine kinase Ack1 regulates the fate of activated EGFR by inducing trafficking to the p62/NBR1 pre-autophagosome. *J Cell Sci* 2014; **127**: 994–1006.
- Buchwald M, Pietschmann K, Brand P, Gunther A, Mahajan NP, Heinzel T *et al.* SHAH ubiquitin ligases target the nonreceptor tyrosine kinase ACK1 for ubiquitinylation and proteasomal degradation. *Oncogene* 2012; **32**: 4913–4920.
- Kramer OH, Stauber RH, Bug G, Hartkamp J, Knauer SK. SHAH proteins: critical roles in leukemogenesis. *Leukemia* 2013; **27**: 792–802.
- Urena JM, La Torre A, Martinez A, Lowenstein E, Franco N, Winsky-Sommerer R *et al.* Expression, synaptic localization, and developmental regulation of Ack1/Pyk1, a cytoplasmic tyrosine kinase highly expressed in the developing and adult brain. *J Comp Neurol* 2005; **490**: 119–132.
- La Torre A, del Rio JA, Soriano E, Urena JM. Expression pattern of ACK1 tyrosine kinase during brain development in the mouse. *Gene Expr Patterns* 2006; **6**: 886–892.
- La Torre A, del Mar Masdeu M, Cotrufo T, Moubarak RS, del Rio JA, Comella JX *et al.* A role for the tyrosine kinase ACK1 in neurotrophin signaling and neuronal extension and branching. *Cell Death Dis* 2013; **4**: e602.
- Hitomi Y, Heinzen EL, Donatello S, Dahl HH, Damiano JA, McMahon JM *et al.* Mutations in TNK2 in severe autosomal recessive infantile onset epilepsy. *Ann Neurol* 2013; **74**: 496–501.
- Galisteo ML, Yang Y, Urena J, Schlessinger J. Activation of the nonreceptor protein tyrosine kinase Ack by multiple extracellular stimuli. *Proc Natl Acad Sci USA* 2006; **103**: 9796–9801.
- van der Horst EH, Degenhardt YY, Strelow A, Slavin A, Chinn L, Orf J *et al.* Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. *Proc Natl Acad Sci USA* 2005; **102**: 15901–15906.
- Mahajan K, Mahajan NP. ACK1/TNK2 tyrosine kinase: An emerging target for cancer therapeutics. *AACR Educ Book* 2014; **2014**: 6.
- Shimura 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. *J Surg Oncol* 2013; **109**: 189–197.
- Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007; **129**: 1261–1274.
- Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* 2005; **24**: 7410–7425.
- Huang H, Tindall DJ. Dynamic FoxO transcription factors. *J Cell Sci* 2007; **120**: 2479–2487.
- Mahajan K, Coppola D, Chen YA, Zhu W, Lawrence HR, Lawrence NJ *et al.* Ack1 tyrosine kinase activation correlates with pancreatic cancer progression. *Am J Pathol* 2012; **180**: 1386–1393.
- Schoenherr JA, Drennan JM, Martinez JS, Chikka MR, Hall MC, Chang HC *et al.* Drosophila activated Cdc42 kinase has an anti-apoptotic function. *PLoS Genet* 2012; **8**: e1002725.
- Burnstein KL. Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. *J Cell Biochem* 2005; **95**: 657–669.
- Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R *et al.* Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004; **10**: 33–39.
- Lupien M, Brown M. Cistromics of hormone-dependent cancer. *Endocr Relat Cancer* 2009; **16**: 381–389.
- Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat Rev* 2001; **1**: 34–45.
- Edwards J, Bartlett JM. The androgen receptor and signal-transduction pathways in hormone-refractory prostate cancer. Part 1: Modifications to the androgen receptor. *BJU Int* 2005; **95**: 1320–1326.
- Mahajan K, Coppola D, Rawal B, Chen YA, Lawrence HR, Engelman RW *et al.* Ack1-mediated androgen receptor phosphorylation modulates radiation resistance in castration-resistant prostate cancer. *J Biol Chem* 2012; **287**: 22112–22122.
- Blanco-Aparicio C, Renner O, Leal JF, Carnero A. PTEN, more than the AKT pathway. *Carcinogenesis* 2007; **28**: 1379–1386.
- Liang J, Shang Y. Estrogen and cancer. *Annu Rev Physiol* 2013; **75**: 225–240.
- Green KA, Carroll JS. Oestrogen-receptor-mediated transcription and the influence of co-factors and chromatin state. *Nat Rev Cancer* 2007; **7**: 713–722.
- Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM *et al.* Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 1998; **90**: 1371–1388.
- Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A *et al.* Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 2005; **97**: 1652–1662.
- Howell A, DeFriend D, Robertson J, Blamey R, Walton P. Response to a specific antiestrogen (ICI 182780) in tamoxifen-resistant breast cancer. *Lancet* 1995; **345**: 29–30.
- Clarke R, Leonessa F, Welch JN, Skaar TC. Cellular and molecular pharmacology of antiestrogen action and resistance. *Pharmacol Rev* 2001; **53**: 25–71.
- Mahajan K, Lawrence HR, Lawrence NJ, Mahajan NP. ACK1 tyrosine kinase interacts with histone demethylase KDM3A to regulate the mammary tumor oncogene HOXA1. *J Biol Chem* 2014; **289**: 28179–28191.
- Schlieman MG, Fahy BN, Ramsamooj R, Beckett L, Bold RJ. Incidence, mechanism and prognostic value of activated AKT in pancreatic cancer. *Br J Cancer* 2003; **89**: 2110–2115.
- Matsumoto J, Kaneda M, Tada M, Hamada J, Okushiba S, Kondo S *et al.* Differential mechanisms of constitutive Akt/PKB activation and its influence on gene expression in pancreatic cancer cells. *Jpn J Cancer Res* 2002; **93**: 1317–1326.
- Sakurada A, Suzuki A, Sato M, Yamakawa H, Orikasa K, Uyeno S *et al.* Infrequent genetic alterations of the PTEN/MMAC1 gene in Japanese patients with primary cancers of the breast, lung, pancreas, kidney, and ovary. *Jpn J Cancer Res* 1997; **88**: 1025–1028.
- Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA *et al.* Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. *N Engl J Med* 2013; **368**: 1781–1790.
- Mahajan K, Mahajan NP. ACK1 tyrosine kinase: targeted inhibition to block cancer cell proliferation. *Cancer Lett* 2013; **338**: 185–192.
- Liu Y, Karaca M, Zhang Z, Gioeli D, Earp HS, Whang YE. Dasatinib inhibits site-specific tyrosine phosphorylation of androgen receptor by Ack1 and Src kinases. *Oncogene* 2010; **29**: 3208–3216.
- 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. *Mol Cancer* 2014; **13**: 13.
- DiMauro EF, Newcomb J, Nunes JJ, Bemis JE, Boucher C, Buchanan JL *et al.* Discovery of 4-amino-5,6-biaryl-furo[2,3-d]pyrimidines as inhibitors of Lck: development of an expedient and divergent synthetic route and preliminary SAR. *Bioorg Med Chem Lett* 2007; **17**: 2305–2309.