Review Article

The non-receptor tyrosine kinase ACK: regulatory mechanisms, signalling pathways and opportunities for attACKing cancer

Millie Fox[1], Claire Crafter[2] and Darerca Owen[1]

[1]Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, U.K.
[2] Bioscience, Research and Early Development, Oncology R&D, AstraZeneca, Cambridge, U.K.

Correspondence: Darerca Owen (do202@cam.ac.uk)

Abstract

Activated Cdc42-associated kinase or ACK, is a non-receptor tyrosine kinase and an effector protein for the small G protein Cdc42. A substantial body of evidence has accumulated in the past few years heavily implicating ACK as a driver of oncogenic processes. Concomitantly, more is also being revealed regarding the signalling pathways involving ACK and molecular details of its modes of action. Some details are also available regarding the regulatory mechanisms of this kinase, including activation and regulation of its catalytic activity, however a full understanding of these aspects remains elusive. This review considers the current knowledge base concerning ACK and summarises efforts and future prospects to target ACK therapeutically in cancer.

Abbreviations

AR, androgen receptor; AREs, Androgen Response Elements; ChIP, chromatin immunoprecipitation; CRPC, castration resistant prostate cancer; DHT, dihydrotestosterone; DSB, double strand breaks; ECM, extracellular matrix; EBD, Epidermal growth factor Binding Domain; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; $GI_{50,}$ half maximal growth inhibitory concentration; GPCR; G-protein coupled receptor; IC_{50}, half maximal inhibitory concentration, MCSP, melanoma chondroitin sulphate proteoglycan; MVBs, multi-vesicular bodies; NRTK, non-receptor tyrosine kinase; PIN, prostatic intraepithelial neoplasia; PIP_2, phosphatidylinositol 4,5-bisphosphate; PRR, proline rich region; RTK, receptor tyrosine kinase; SAM, sterile alpha motif; VCA, verprolin, cofilin, acidic.

Introduction

The tyrosine kinase ACK (also known as ACK1 and TNK2) was identified over 25 years ago as the first effector protein described for Cdc42, a member of the Rho family of small G proteins. ACK belongs to a family of non-receptor

tyrosine kinases (NRTKs) and has been implicated in diverse cellular functions, including survival, proliferation and migration. In humans there are three ACK-related proteins, ACK (the product of *TNK2*), TNK1 (*TNK1*) and Mig-6 (*ERRFI1*) (Figure 1) and homologues have been identified in mouse, *Drosophila melanogaster* and *Caenorhabditis elegans*. ACK and TNK1 are members of the same family of non-receptor tyrosine kinases based on the similarity of their kinase domains, while the adapter protein Mig-6 shares Cdc42/Rac and EGFR binding regions with ACK. Splice variants of both ACK and TNK1 have been reported. ACK2, a C-terminally truncated form of ACK was first identified in bovine brain (1), while Kos1, a C-terminally truncated version of TNK1 has been isolated from mouse stem cells (2).

Clues to the normal physiological roles of the ACK related proteins have come from studies in model organisms. *Drosophila* possess two ACK related proteins: DACK and DRP2. DACK is more similar in primary sequence to ACK, however it lacks a Cdc42/Rac interacting motif (CRIB). DPR2 has less similarity at the primary sequence level to ACK but does contain a CRIB motif, making it difficult to assign orthologues between humans and mice. DACK is critical for sperm formation (3) and has been shown to function in dorsal closure during embryogenesis (4). Although DACK lacks a CRIB motif, its functional similarity to ACK is supported by rescue experiments where mouse ACK could rescue sterility caused by a mutation in DACK, whereas DPR2 could not (3). Many interacting proteins are also shared between ACK and DACK, further suggesting functional homology. Interestingly DACK has been shown to suppress apoptosis downstream of EGFR and to also function in regulating EGFR signalling via endocytosis, suggesting functional conservation with the mammalian system (see below) (5).

Additional work in *Caenorhabditis elegans* has studied the role of ARK-1 in vulval development, a process regulated through the RTK/Ras/MAPK signalling pathway. ARK-1 (Ack-related tyrosine kinase) was shown to be a novel inhibitor of *let-23* EGFR signalling (6) paralleling its role in higher eukaryotes (see below).

All ACK family members have been implicated in cancer progression. ACK gene amplification and protein overexpression correlates with poor prognosis in a wide variety of cancers: somatic mutations have also been identified in ACK, which act as cancer drivers and have been shown to possess increased kinase activity (7-9). In contrast, TNK1 is thought to act as a tumour suppressor since *TNK1* knockout mice develop spontaneous lymphomas and carcinomas upon ageing (10). Similarly, Mig-6 knockout mice develop tumors in various tissues (11-15).

Given the prominence of ACK in the initiation and maintenance of various cancer types it is timely to reflect on our current understanding of the regulation of this kinase, its impact on cellular signalling pathways and the progress that has been made in targeting this specific protein for therapeutic purposes in cancer.

The activation and regulation of ACK

ACK comprises 1038 amino acids and is made up of multiple domains, as befits a signalling protein (Figure 1A). The N-terminus of ACK comprises a sterile alpha motif (SAM) domain, nuclear export signal (NES), tyrosine kinase domain, Src homology-3 (SH3) domain and a Cdc42/Rac-interactive binding (CRIB) motif. The proline-rich C-terminus of ACK includes a clathrin binding region, an epidermal growth factor receptor-binding domain (EBD, also referred to as the MHR, Mig6-homology region) and a ubiquitin association domain (UBA). In fact the domain organization of ACK is unique amongst mammalian tyrosine kinases with the SH3 domain C-terminal to the kinase domain (16) and the presence of a CRIB motif.

ACK has been reported to be stimulated downstream of various major classes of cell surface receptors: RTKs, GPCRs and Integrins (Figure 2). Despite this, the specific details of how this activation occurs remain elusive. ACK has been shown to respond to a wide variety of extracellular stimuli including growth factors such as EGF, heregulin, PDGF, Gas6, IGF, Insulin and neutrophins (17-19), suggesting activation downstream of RTKs. The interaction between ACK and RTKs is often direct but ACK has also been shown to associate with adaptor proteins including Grb2, Nck (17) and HSH2 (20) facilitating indirect RTK association (21). Apart from RTKs, ACK responds to cell adhesion via integrin stimulation (17,22,23) and also sits downstream of GPCRs for example muscarinic receptors (24). It has been suggested that the involvement of Src (see below) in the activation of ACK facilitates this wide-ranging response.

Perhaps understandably, most of the efforts directed towards investigating the activity and regulation of ACK have concentrated on its kinase activity. Initial investigations using purified protein reported that ACK undergoes autophosphorylation on Tyr284 in the activation loop but this only modestly enhances kinase activity (16). The activity of ACK towards test substrates was also assessed to be much lower than other NRTKs. Interestingly binding of ligands to neither the SH3 domain nor the CRIB region activated ACK. Initial structural studies confirmed the early biochemical data. The ACK kinase domain adopts a classic kinase fold and comparison of the non-phosphorylated and phosphorylated forms showed few differences, corroborating the kinase activity data and suggesting that ACK is constitutively active (25). The ACK kinase domain is closest in structure to the EGRF kinase domain, which equally does not require phosphorylation for activity.

Later work added detail to the regulation of ACK kinase activity (26). Analysis of the ACK dimers observed in the crystal lattices formed by ACK revealed that active ACK adopts a symmetrical dimer. This is now thought to be a major mechanism behind the activation of ACK, in a manner analogous to the

autoactivation of EGFR via dimerization. Furthermore experimental dimerization, mediated by the addition of a GST tag, increased the activity of ACK constructs significantly. These data fitted nicely with previous work indicating that the SAM domain at the N-terminus of ACK was required for activation of ACK (17,27). SAM domains are well characterized as mediating dimerization (28) and the SAM domain of ACK is implicated in its self-association (27).

Further work shed light on the different intramolecular interactions in ACK that likely regulate its kinase activity (Figure 1B). The SH3 domain appears to bind a proline rich sequence (PRR) C-terminal to the EBD (17,26). The structure of the SH3 revealed it is actually rather unusual, with a non-canonical peptide-binding site (26). This probably explains the failure of early studies to see any effects with representative peptide substrates (16). The SH3-PRR interaction is thought to orientate the EBD permitting inhibitory interactions with the kinase domain (8). Binding of the EBD to RTKs relieves this inhibition and frees the kinase domain. Dimerization of ACK via the SAM domain orientates the free kinase domain to allow autoactivation (26). Slight variations around this theme have also been proposed (29).

Although these studies do not include a role for phosphorylation in the activation of ACK, ACK had been shown to interact with, and be a substrate for, Src and Hck (16). Later work in cells identified Src as the major kinase acting at ACK Tyr284 (30). Thus it appears that ACK activity can be increased by phosphorylation but this is probably mediated by Src or a Src family kinase. In summary, it appears that autoactivation by dimerization plays the major role in ACK activation but phosphorylation at Tyr284 by Src contributes either to full activation or represents an alternative mechanism to regulate ACK.

Despite its initial discovery as a binding partner for Cdc42, the role of Cdc42 and its binding to the CRIB region in ACK activation remain elusive. Initial studies with purified protein ruled out a direct role for Cdc42 in the activation of ACK kinase activity (16). Studies using an ACK mutant (H464D) incapable of binding Cdc42 have shown inconsistent results, with both a severe reduction in phosphorylation reported (17) and no changes observed (30). As small G proteins often act to localize their effectors to membranes, it is possible that the interaction between ACK and Cdc42 may be required for correct localization in the cell that then facilitates activation. In fact cellular localization, by whatever means, appears crucial for the activity of ACK. The SAM domain has been postulated as a membrane association domain and deletion of the SAM domain in ACK perturbs phosphorylation of the kinase (17). Whilst localization to membranes may be important for ACK activity, ACK is increasingly being shown to have crucial functions in the nucleus and the Cdc42 interaction may facilitate directing ACK to the nucleus, although no details are currently available (31).

The nuclear functions of ACK: ACK is an epigenetic modifier and transcriptional activator

ACK shuttles between the cytoplasm and nucleus transducing extracellular signals to both cytosolic and nuclear effectors. The androgen receptor (AR), which plays a pivotal role in the normal growth and development of the prostate gland as well as in prostate carcinogenesis, is a major nuclear target for ACK and is heavily implicated in prostate cancer. The AR binds to its natural ligand, an androgenic hormone, in the cytoplasm, leading to dimerization and subsequent translocation to the nucleus. Upon binding of the AR to androgen response elements (AREs) in the promoter and enhancer regions of target genes, the AR-ligand complex recruits additional machinery to initiate transcription (32). Uncontrolled activation of the AR is a prerequisite for the progression of prostate cancer to the hormone refractory stage also known as castration resistant prostate cancer (CRPC). In this stage the androgen receptor is transcriptionally active even at the low levels of circulating androgen resulting from androgen deprivation therapy (ADT), the primary treatment for patients presenting with metastatic prostate cancer (32). Numerous mechanisms have been reported to explain how prostate cancer cells become androgen independent. These include amplification or mutation of AR as well as altered expression of AR co-regulatory proteins. In addition, it has become apparent over the years that growth factors, for example IGF1, may activate the AR in a ligand independent manner, through activation of receptor tyrosine kinase pathways (33).

More recently, ACK was shown to bind and directly phosphorylate the androgen receptor on two sites, Tyr267 and Tyr363, both located in the transactivation domain (Figures 2 and 3) (34). Phosphorylation at these sites is required for optimal AR-dependent transcription and when mutated at Tyr267, the AR fails to promote castration-resistant growth of prostate xenograft tumours (34). Further analysis revealed a distinct transcriptional program directed by pTyr267-AR (35). pTyr267-AR binds ATM (Ataxia telangiectasia mutated) enhancer elements and upregulates transcription of ATM (Ataxia telangiectasia mutated), a key mediator of DNA repair. The AR recruits ACK to the nucleus as part of a larger AR transcriptional complex, upregulating transcription of androgen-dependent genes including ATM. As a DNA damage sensor, ATM is activated by double strand breaks (DSB) and initiates a signalling cascade to block cell cycle progression to promote DNA repair, ensuring genetic integrity and cell survival (36). In the absence of DNA repair pathways, cells that acquire DNA damage undergo apoptosis. The ACK mediated upregulation of ATM in prostate cancer cells increases resistance to DNA-damage inducing agents including, most notably, radiation therapy (35). Human prostate tissue microarray analysis showed that the levels of pTyr284-ACK and pTyr267-AR are elevated in prostate cancer cells and this correlated with disease progression to the CRPC stage (34). These data describe a signalling nexus involving ACK, the AR and ATM, which mediates prostate cancer progression (Figure 3).

As well as regulating transcriptional activity of the AR, ACK also promotes chromatin alterations to drive AR expression and subsequent CRPC progression (Figure 3). ACK directly phosphorylates Histone H4 on Tyr88 upstream of the AR transcriptional start site (37). pTyr88-H4 marks are recognized by the WDR5/MLL2 chromatin remodelling complex, which deposits transcriptionally activating H3K4-trimethylation marks. Via direct phosphorylation of Histone H4, ACK reprograms the AR locus to upregulate AR mRNA and subsequent protein levels in the androgen-deficient environment of CRPC. Tissue microarray analysis revealed H3K4me3 and AR levels increase with disease progression to the CRPC stage (37).

Combined, these studies have established a crucial role of ACK in the progression of CRPC, providing an additional mechanism by which prostate cancer cells can adapt to androgen deficiency. Deposition of pTyr88-H4 marks by ACK prompts enhanced AR expression to compensate for low androgen levels, while direct phosphorylation of the AR results in a distinct gene expression signature contributing to CRPC. Another feedback loop may also exist to upregulate ACK in a low androgen environment; following androgen deprivation, androgen-sensitive LAPC4 cells showed an upregulation of ACK levels (37). The signalling nexus involving the AR and ACK is therefore well characterized and describes crucial nuclear functions for ACK in prostate cancer progression (Figure 3).

ACK can also function as an epigenetic regulator in breast cancer, by directly phosphorylating KDM3A4, a histone demethylase that removes H3K9 marks, facilitating upregulation of oestrogen receptor (ER) target genes. Tyrosine phosphorylation of KDM3A promotes demethylation at ACK/ER bound promoters resulting in transcriptional upregulation of HOXA1, a critical ER-regulated mammary tumour oncogene (38). In fact ACK activation promotes ER activity even in the presence of tamoxifen (38), suggesting that the epigenetic functions of ACK in ER-positive breast cancers may underpin the mechanisms by which cells acquire tamoxifen resistance.

ACK has also been reported to directly target other transcription factors. STATs (signal transducers and activators of transcription) are cytoplasmic transcription factors and ACK promotes the phosphorylation of STAT1 and STAT3 on Tyr701 and Tyr705 respectively (39). Phosphorylation of STATs at this site is the major signal for their activation, resulting in nuclear accumulation and induction of STAT-dependent gene expression. Immunohistochemical staining of primary lung adenocarcinoma cells revealed a positive correlation between the levels of ACK and pSTAT3 (39).

Another mechanism through which ACK regulates transcriptional activity is by modulating the nuclear import of certain transcription factors. For example, ACK modulates early activation events in T-cells via the indirect regulation of transcription factors (40). ACK targets the adaptor protein SLP-76, to modulate nuclear import of certain transcription factors. ACK binds SLP-76 via its SAM domain and phosphorylates proximal tyrosine residues. SLP-76 has

been reported to bind RanGAP1 at the nuclear pore complex and regulate nuclear transport of transcription factors (41). ACK expression increased NFAT/AP1 promoter activity and enhanced calcium influx in T-cells in response to stimulation, processes dependent on SLP-76 (40).

Taken together these data suggest that ACK functions at may different levels and via several mechanisms, to alter the transcriptional activity of a variety of different transcription factors either indirectly or directly.

ACK modulates the PI3-Kinase signalling pathway at multiple nodes

The PI3-Kinase pathway is one of the most frequently activated pathways in cancer (Figure 4). Class 1a PI3-Kinases exist as heterodimers composed of a catalytic p110 subunit and regulatory p85 subunit. PI3-Kinase is recruited to activated RTKs by binding to pTyr residues in the C-terminal tail of receptors. The binding of p85 SH2 domains to these pTyr residues relieves the regulatory inhibition on the p110 subunit activating the lipid kinase activity. Catalytically active p110 phosphorylates PIP_2 resulting in accumulation of PIP_3 in the membrane. Akt, the key downstream signalling component of the PI3-Kinase pathway, is recruited to the membrane by binding PIP_3 via its PH domain. Once at the membrane Akt is activated by phosphorylation at Thr308 and Ser473, by PDK1 and PDK2/mTOR respectively, and can then phosphorylate its substrates to transduce various growth and survival signals. ACK has been described as the third PDK as it directly phosphorylates Akt on Tyr176 enhancing its membrane recruitment (Figure 2) (42). pTyr176 Akt preferentially binds to phosphatidic acid in the membrane dispensing with one of the PIP_3-dependent Akt activation steps (Figure 4) (9). Importantly, pTyr176-Akt and pTyr284-ACK were found to localize to the nucleus where they act to repress FoxO-responsive apoptotic gene expression.

Instances of breast and prostate cancer that exhibit Akt activation whilst retaining normal PTEN and PI3-Kinase activity have remained confounding, however direct activation of Akt by ACK may resolve this perplexity (43-45). An ACK transgenic mouse model displayed Akt activation and developed prostatic intraepithelial neoplasia (PIN). In addition, increased pTyr284-ACK and pTyr176-Akt levels were observed in progressive stages of breast cancer and inversely correlated with patient survival (9). These data highlight the role of ACK/PI3-Kinase signalling in breast and prostate cancer.

Work from our own group has recently uncovered a new role for ACK in the modulation of the PI3-Kinase pathway via a direct interaction with the PI3-Kinase regulatory subunits (Figures 2 and 4). ACK interacts with all five isoforms of the p85 regulatory subunit and directly phosphorylates four of them on a conserved tyrosine residue located in the iSH2 domain, equivalent to Tyr607 p85α (46). Phosphorylation at Tyr607 was shown to promote proliferation but not via an increase in PI3-Kinase signalling. Instead phosphorylation at this site led to increased stability of the regulatory subunits, via evasion of the ubiquitin-proteasome degradative pathway, so regulating

the cellular level of p110-free p85 subunits. p110-independent functions of p85 predominate in the nucleus and the ACK/p85 complex was detected in nuclear enriched fractions only. The phosphorylated p85 isoforms form homo- and hetero-dimers mediated by the C-terminal regions of p85. These newly identified C-terminally mediated dimers can form between any isoforms of the PI3-Kinase regulatory subunits, including the shorter splice variants of p85α; p50α and p55α but preferential binding to p85β was observed, suggesting p85β homodimers or heterodimers are favoured. C-terminally mediated p50α/p85β dimers were predominantly found in the nuclear enriched fractions, whereas standard N-terminally mediated p85α/p85β dimers were seen in cytoplasmic fractions. Therefore, via direct phosphorylation of the PI3-Kinase regulatory subunits, ACK promotes stabilization and formation of nuclear C-terminally mediated dimeric p85. The predicted configuration of these nuclear dimers suggests that SH3 or RhoGAP domain activities could drive the pro-proliferative functions of p85 phosphodimers.

Nuclear functions for p110-free p85 have been documented, predominantly in stress response pathways. Under conditions of stress, p53 mediates cell growth arrest and apoptosis, a process controlled by lysine acetylation, which leads to p53 activation. p85α has a role in mediating p53 acetylation at Lys370, regulating its promoter-specific transactivation (47). X-box binding protein-1 (XBP-1) is one of the main regulators of the unfolded protein response and both p85α and p85β have been found to interact with XBP-1 and upregulate its nuclear translocation (48). Nuclear functions for p50α and p55α have also been described. p50α and p55α were found to localize, bind chromatin and regulate transcription of a subset of genes that overlapped with STAT3 controlled genes (49). Whether a dimeric configuration of the PI3-Kinase regulatory subunits influences this growing list of nuclear functions remains to be determined. Changes in cellular levels of regulatory subunit can result in substantial changes in PI3-Kinase signalling (50). By direct phosphorylation, ACK may specifically target and stabilize one isoform of the PI3-Kinase regulatory subunit to further modify and direct PI3-Kinase signalling by perturbing the p85 isoform balance, so driving Cdc42/ACK mediated oncogenesis. N-terminal domain interactions have also been implicated in mediating p85 dimerization (51). These dimers have been reported to bind and directly enhance PTEN lipid phosphatase activity (51,52). Whether ACK mediated C-terminal dimers of p85 also retain this stimulatory activity on PTEN is unknown, but may account for the decrease in PIP_3 levels seen upon exogenous ACK expression (46).

The complexity of the PI3-Kinase/AKT/mTOR signalling network involves numerous feedback loops and extensive cross talk with other signalling pathways. We, and others, have demonstrated that ACK targets the PI3-Kinase pathway at multiple nodes providing additional layers of regulation. For example, exogenous ACK expression was shown to suppress EGF-induced stimulation of PI3-Kinase signalling as measured by the levels of PIP_3 in the cell (46), suggesting that ACK reduces PIP_3 signalling direct from the membrane. This seems discordant with a pro-proliferative function of ACK.

However this may be circumvented via direct phosphorylation and activation of Akt, independent of PIP_3, and formation of nuclear dimeric p85. Compensatory mechanisms in signalling pathways often provide opportunities for circumventing inhibitory effects (53). With such extensive regulation, the ACK/PI3-Kinase signalling network may underpin resistance to PI3-Kinase inhibitors and help drive ACK mediated oncogenesis (Figure 4) (54).

The cytoplasmic functions of ACK

Although many functions of ACK are localized to the nucleus, cytoplasmic roles and alternative layers of regulation for ACK have also been described. ACK forms a complex with HSP90 (Heat-shock protein 90), a chaperone protein that regulates the molecular structure and function of many signalling proteins, particularly kinases. HSP90 is required for the kinase activity of ACK and its association with Cdc42 (55). HSP90 has been shown to be required for ACK kinase activity towards STAT1 and STAT3 (39) as well as WWox (56). WWox (WW domain containing oxidoreductase) is a tumour suppressor protein that plays a role in DNA damage response pathways and genomic stability. Phosphorylation at Tyr33 activates WWox, enhancing its interactions with downstream binding partners, including p53. Following UV irradiation, pTyr33-WWox binds and stabilizes pSer46-p53 inducing apoptosis (57). A significant loss or reduction of WWox gene expression is observed in many cancer types, namely breast (58), prostate (59) and pancreatic (60). ACK phosphorylates WWox on Tyr287 targeting it for ubiquitination and subsequent degradation (Figure 2). Elevated levels of pTyr284-ACK and decreased WWox levels were observed in primary androgen-independent prostate tumours but not in benign prostate cells. It is becoming apparent that ACK often functions to regulate the stability of its substrates, as is the case for WWox and the PI3-Kinase regulatory subunits. By regulating the stability and degradation of distinct signalling proteins ACK can shift the equilibrium of these pathways to promote tumorigenesis as exemplified by ACK-mediated promotion of prostate cancer progression by negative regulation of the levels of proapoptotic Wwox.

ACK regulates receptor endocytosis and vesicular trafficking

ACK interacts with several components of vesicle dynamics including AP2, sorting nexin 9 (SNX9), synaptojanin-1 and clathrin (Figure 5). AP2 is an adaptor protein and is involved in receptor-mediated endocytosis (61), while SNX9 is an endocytic accessory protein involved in clathrin-mediated endocytosis (62). SNX9 is also described as a multifunctional scaffold due to its emerging role in membrane remodeling and trafficking (63). ACK binds and phosphorylates SNX9 on Tyr287 in mammalian cells. In doing so, ACK is able to regulate SNX9 endocytic functions. In fact, studies using ACK2 highlighted a role of SNX9 in EGF induced EGFR degradation, with cells expressing ACK2 and SNX9 displaying EGFR degradation following EGF treatment (64). SNX9 links synaptojanin-1 to ACK coated vesicles, further implicating the involvement of ACK in vesicle dynamics involving AP2, synaptojanin-1 and

clathrin (62). ACK also binds to clathrin heavy chains and is involved in clathrin-mediated endocytosis (61). Exogenous ACK expression induced reorganization of clathrin from a widespread distribution including distinct perinuclear and vesicular puncta to colocalize with ACK in large vesicular structures, which was associated with the loss of transferrin uptake. The over expression of ACK also inhibited clathrin-mediated endocytosis by functionally sequestering clathrin (61).

Endocytosis and trafficking of the Epidermal Growth Factor Receptor (EGFR) is crucial for the regulation of EGFR signalling. EGF binding results in EGFR dimerization, autophosphorylation and activation, initiating a signalling network mediating cell survival, proliferation and differentiation. Following activation, the EGFR is endocytosed from the cell surface and undergoes a regulated sorting process (Figure 5). Internalized EGFR is targeted to early endosomes for either membrane recycling or trafficked through the endosomal pathway to lysosomes for degradation (65).

In accordance with its role in clathrin-coated vesicle endocytosis, ACK interacts with the EGFR in response to EGF stimulation and plays a role in its degradation (66). The interaction with the EGFR is mediated by the region towards the C-terminus of ACK, designated the EBD (Figure 1). The interaction between ACK and EGFR is also dependent upon the kinase activity and tyrosine phosphorylation of the receptor (66). It has been postulated that the EGFR/ACK interaction is indirect and requires an SH3-domain containing EGFR adaptor protein (66). Grb2 is an adaptor protein that has been described for its role in EGF-stimulated EGFR internalization (21,67) and is a known partner of ACK (68). Grb2 is required for the interaction of ACK with other RTKs, such as Axl family kinases and insulin receptor-like kinases, LTK (leukocyte tyrosine kinase) and ALK (anaplastic lymphoma kinase) (21). ACK also regulates the degradation of these RTKs in response to stimuli, such as Axl receptor degradation following Gas6 treatment, however the adaptor protein mediating the interaction between ACK and the EGFR remains to be formally confirmed (21,66).

Mig-6, which is highly related to ACK, has also been identified as a negative regulator of EGF signalling. Studies have revealed that Mig-6 inhibits the catalytic activity of EGFR as well as directing EGFR endocytosis. Mig-6 knock out mice displayed hyperactivation of endogenous EGFR (69). Most recently, Mig-6 has been shown to regulate the ubiquitination and degradation of EGFR mutants in lung adenocarcinomas cells (70). Mig-6 deficiency accelerated the initiation and progression of mutant EGFR-driven tumourigenesis but in this case Mig-6 did not promote degradation of mutant EGFR (15). Thus, via their conserved EBD domain, both Mig-6 and ACK play roles in regulating EGFR endocytosis, trafficking and degradation. The tight interplay of both ACK and Mig-6 in coordinating EGFR signalling is potentially driven by competition for binding to the EGFR by their respective EBDs.

EGF stimulation also induces ACK degradation in parallel with EGFR degradation. The tight correlation between the degradation of ACK and the EGFR suggested co-transportation of the ACK/EGFR complex (71). Colocalization of ACK and EGFR on EEA-1 positive vesicles following EGF stimulation has been observed (66) and the EGFR/ACK complex is finally co-transported to lysosomes for degradation (Figure 5) (71).

The EGFR is ubiquitinated in response to EGF by recruitment of the E3 ligase Cbl (72). Ubiquitinated EGFR is transported through the endosome pathway to lysosomes for degradation, while non-ubiquitinated EGFR is recycled back to the membrane (72,73). ACK is ubiquitinated and degraded along with the EGFR in response to EGF stimulation and this is mediated by the Nedd4 proteins. Both Nedd4-1 (71) and Nedd4-2 (74) have been identified as E3 ligases targeting ACK but with contrasting outcomes. Co-expression with Nedd4-1 resulted in ubiquitination levels of ACK 10-fold higher than with Nedd4-2 and Nedd4-1 catalysed ubiquitination of ACK was required for EGF-induced lysosomal degradation of the EGFR. In contrast Nedd4-2 had a minimal effect (71) but was reported to trigger ACK turnover via proteasomal degradation (74). It is likely that both Nedd4 proteins target ACK for ubiquitination but the functional differences remain to be fully defined.

ACK has also been identified as a ubiquitin-binding protein, a property attributed to its UBA domain (Figure 1). Overexpression of a UBA deletion mutant of ACK displayed increased ubiquitination itself, attributed to enhanced binding to Nedd4-1. In contrast deletion of the ACK SAM domain reduced ubiquitination by Nedd4-1. It is speculated that the lysine-rich SAM domain contains ubiquitination target sites for Nedd4-1. When ubiquitinated, the UBA domain may interact with the SAM domain altering the conformation of ACK, blocking access to Nedd4-1 and preventing excessive ubiquitination (71). ACK ubiquitination therefore, may lead to conformation changes and regulate the catalytic activity of the kinase itself as well as EGFR endosomal trafficking.

The interaction between ACK and EGFR has been reported to occur at a late stage of EGFR internalization, following 30-60 minutes of EGF stimulation. These data suggest that the interaction might not occur on the plasma membrane (66). Alternatively, Grb2 has been shown to link ACK to multiple other receptor tyrosine kinases and ACK kinase activation has also been reported within 5 minutes of EGF treatment, suggesting ACK might be activated at an earlier stage in the process (21,67). It is possible that the EGFR/ACK complex forms on endosomes and the ubiquitination of ACK via Nedd4-1 may serve as a sorting signal for trafficking the EGFR/ACK complex to lysosomes (71). In fact, over expression of ACK led to the retention of the EGFR on early endosomes, inhibiting translocation to MVBs (75). EGFR signalling is not restricted to the plasma membrane and activated EGFR can continue to signal from endosomes. For example, endocytosis of EGFR is required for the sustained activation of Akt but not for activation of the MAPK pathway. Endocytosis of the EGFR is therefore crucial in maintaining normal signalling kinetics (65). In fact endosomal EGFR signalling is sufficient to

support cell survival and proliferation (76). Regulation of receptor endocytosis by ACK therefore functions to modulate distinct signalling networks. ACK senses EGF signalling and downregulates the EGFR receptor preventing excessive signalling from the membrane. ACK is recruited to the EGFR on endosomes and regulates endocytic trafficking, with ubiquitination serving as the sorting signal, which directs EGFR signalling. Ultimately, the role ACK plays in clathrin-mediated receptor endocytosis is key to regulating the normal kinetics of signalling pathways (Figure 5).

ACK regulates pathways involved in cell migration

Cell migration is orchestrated by the dynamic assembly and disassembly of actin filaments. Actin polymerization at the cell leading edge, in response to specific external cues, drives the plasma membrane forward using extensions known as filopodia and lamellipodia. Cell-cell and cell-extracellular matrix (ECM) adhesions are also rapidly modified to allow for cell movement through tissues. Formation of the appropriate actin network involves both nucleation of new filaments and polymerization of branched filaments by an activated Arp2/3 complex, driving filopodia and lamellipodia formation respectively. The Arp2/3 complex is activated by members of the Wiskott-Aldrich Syndrome Protein (WASP) family with WASP itself being one of the main activators (77). The VCA domain of WASP activates the Arp2/3 complex: the WASP V region interacts with actin monomers and couples them to Arp2/3, which associates with the CA region (78). Efficient migration and invasion requires coordination of these dynamic cellular processes and hence these pathways are tightly controlled.

Members of the Rho family of small GTPases, including Cdc42, play essential roles in coordination of pathways mediating cell migration both under normal physiological conditions and in disease (Figure 2) (79,80). In melanoma cells a signal transduction complex incorporating active Cdc42, ACK and phosphorylated $p130^{Cas}$ mediates cytoskeletal rearrangements permitting cell invasion (22). $p130^{Cas}$ is an adaptor protein involved in cell motility and integrin signalling (81) and has been implicated as a downstream target for ACK (22). ACK also binds and directly phosphorylates the Arp2/3 activator WASP on two distinct residues: Tyr256 and Ser242. In fact phosphorylation at WASP Ser242 was the first evidence of the dual kinase activity of ACK (82). Phosphorylation of WASP by ACK *in vitro* is enhanced by the presence of Cdc42 or PIP_2 and phosphorylation of WASP Tyr256 by Src-family kinases has been reported to enhance the ability of WASP to promote actin polymerization (83,84). Phosphorylation at both WASP Ser242 and WASP Tyr256 has been shown to stimulate actin polymerization *in vitro* (82). Ser242 is located in the Cdc42 binding region (CRIB region), which is bound to the VCA domain in the autoinhibited state. It is likely that phosphorylation at this site triggers a conformational change, releasing the VCA domain in WASP and allowing for activation of the Arp2/3 complex. Additional regulation of Arp2/3 dynamics is controlled by ACK via a direct interaction with cortactin, an Arp2/3 regulatory protein. ACK directly phosphorylates cortactin on Tyr421,

Tyr466 and Tyr482. Actin polymerization is vital in driving membrane dynamics necessary for receptor internalization and in fact cortactin localizes with ACK to vesicles containing ligand-bound EGFR. Cortactin forms a link between actin polymerization and EGFR internalization and degradation (85). The data described here highlight the involvement of ACK in multiple signal transduction complexes, coordinating actin dynamics necessary for cell migration and receptor downregulation. Signalling through these complexes may underpin the oncogenic properties of ACK in promoting cellular migration and invasion. Moreover, loss of ACK suppresses normal membrane dynamics and cell migration further highlighting the role ACK plays in these pathways (21).

Therapeutic AttACK in cancer

There are many lines of evidence suggesting that ACK plays an important role in tumourgenesis, several of which have already been discussed. The gene encoding ACK is located within a small amplicon on chromosome 3q and copy number gain as well as overexpression of ACK mRNA is frequently observed in advanced stage primary tumours as well as metastatic tumours, including lung, prostate, breast, pancreatic, hepatocellular and gastric carcinomas (Figure 6) (86). In fact although mutated variants of ACK are found in many forms of cancer, gene amplification (of up to 29%) seems to be the major change found for ACK in most cancers (Figure 6) (87,88). Despite this, mutated variants of ACK have also been reported but at lower levels (1-5% of cancers). A genomic study, seeking to identify cancer 'driver' mutations within the protein kinase family, identified four such mutations in ACK. In fact ACK was placed 28th out of 518 kinases ranked in order of carrying at least one driver mutation (7). Furthermore, the somatic missense mutations identified fall in several distinct domains of ACK and have been subsequently shown to stimulate ACK activity (7-9). These include two mutations (R34L and R99Q) in the N-terminus identified in lung and ovarian carcinoma, respectively; a mutation in the catalytic domain (E346K) identified in ovarian endometrioid carcinoma and a mutation (M409I) in the SH3 domain identified in lung adenoacarcinoma. More direct evidence supporting an oncogenic role for ACK came from studies in which LNCaP cells, stably expressing wild type (wt), constitutive active (ca) or kinase dead (kd) ACK, were injected subcutaneously into male nude mice. In contrast to wtACK or kdACK LNCaP cells which were poorly tumourigenic, LNCaP cells expressing caACK exhibited robust tumour formation in 23 days (56). In addition, transgenic mice expressing ACK, specifically in the prostate, developed prostatic intraepithelial hyperplasia by 22 weeks and mPINs by 44 weeks, further supporting a key role for ACK in tumour initiation (9). In a large-scale RNAi screen, ACK was also identified as one of 73 kinases that regulated cell survival and apoptosis in the HeLa cervical carcinoma cell line (89).

Due to the important role for ACK in numerous cancers it is not surprising that several efforts have been undertaken to identify small molecule inhibitors of

this tyrosine kinase. One of the first ACK inhibitors to be identified was 4-amino-5,6-biaryl-furo[2,3-d]pyrimidine, an ATP mimetic which inhibits ACK with an IC_{50} of 0.024 μM and shows selectivity over Lck (90). This inhibitor, more commonly known as AIM-100, has since been profiled against a panel of 30 serine/threonine and tyrosine kinases and showed >15-fold selectivity for all of the kinases tested, with the other major hits being Lyn, Lck, Abl1 and Btk with IC_{50}s of 0.35, 0.43, 0.71 and 0.87 μM respectively (35). Given its relative selectivity over other kinase inhibitors reported to target ACK, such as Dasatanib and Bosutinib, AIM-100 has been used extensively in *in vitro* studies to help reveal the role ACK plays in promoting the proliferation and survival of cancer cells. For example, increasing concentrations of AIM-100 inhibited Tyr267 phosphorylation on the AR and decreased the growth of two prostate cancer cell lines, LNCaP and LAPC4 (91). A similar effect was observed in pancreatic cells where AIM-100 abrogated ACK Tyr284 phosphorylation leading to a concomitant reduction in AKT Tyr176 phosphorylation and decreased cell growth, with GI_{50} values reported to be approximately 7-8 μM (60).

Whilst AIM-100 and other early probes are effective *in vitro* tool compounds they had poor pharmacokinetic (PK) properties, which prevented them from being utilized *in vivo*. More recently Jiao *et al.* reported the identification of a series of imidazo [1,5-a] pyrazine-derived molecules that are potent, selective inhibitors of ACK and display good *in vivo* PK properties, making them useful tools to explore ACK biology *in vivo* (92).

Conclusions

Studies to date have hugely expanded our understanding of the regulation and signalling functions of ACK in cells. However, despite the preclinical evidence suggesting ACK plays a role in the growth of many tumours and data from the clinic suggesting that expression levels of activated ACK correlate with disease progression, no ACK inhibitors have yet progressed into the clinic. This is possibly due to the plethora of roles reported for ACK, and lack of clarity around its primary function. The availability of more selective ACK inhibitors and the advancement of CRISPR technology to knockdown ACK will inevitably help uncover further new functions and aid understanding of the many different roles ACK plays in the cell. In particular, it is essential to understand how aberrant activation of ACK, through amplification, overexpression or constitutive activation, can lead to enhanced tumourigenesis and the development of resistance to standards of care such as radiation therapy. Further advances should establish ACK as an important therapeutic target and hopefully reveal novel avenues for attack.

Perspectives

Importance of the field: ACK is a non-receptor tyrosine kinase with a central role in a wide range of signalling pathways, controlling diverse processes

such as cell growth, cell migration and vesicular trafficking. A large body of data has emerged recently to suggest that ACK plays an important role in tumourigenesis with gene amplification, overexpression and activation being associated with many different cancers. This makes ACK an attractive therapeutic target, especially in prostate cancer, where its influence is particularly well-documented.

Current thinking: Despite its widely documented roles, the precise mechanisms by which ACK is activated, either as an effector protein of Cdc42 or downstream of receptor tyrosine kinases remains unclear. Several substrates of ACK have been identified and the molecular mechanisms by which these phosphorylation events impact on downstream signalling pathways are beginning to be uncovered. In particular, the emerging role for ACK as a regulator in the nucleus has been revealed, with ACK repurposing well-known proteins to new functions, regulating transcription factors and even acting as an epigenetic regulator controlling gene transcription through phosphorylation of proteins involved in chromatin remodelling.

Future directions: However, we are still a long way from understanding the full extent of ACK's roles in cell signalling pathways and how, when dysregulated, this can lead to disease pathologies such as cancer. In order to be able to develop safe and effective therapies, a full understanding of the complex signalling networks orchestrated by ACK will be imperative. Some studies also indicate possible kinase-independent functions for ACK and this remains a major gap in our understanding.

Acknowledgements
The authors would like Dr Helen Mott for helpful discussions and for critically reading this manuscript.

Declaration of Interests
The authors declare that they have no competing interests associated with this manuscript.

Funding Information
Work discussed in this manuscript was supported by an MRC CASE Studentship (MR/K017101/1) to D.O. and C.C.

Author Contribution
M.F., C.C. and D.O. wrote the paper and made all figures.

References

1. Yang, W. N., and Cerione, R. A. (1997) Cloning and characterization of a novel Cdc42-associated tyrosine kinase, ACK-2, from bovine brain. *J. Biol. Chem.* **272**, 24819-24824
2. Hoare, K., Hoare, S., Smith, O. M., Kalmaz, G., Small, D., and Stratford May, W. (2003) Kos1, a nonreceptor tyrosine kinase that suppresses Ras signaling. *Oncogene* **22**, 3562-3577
3. Abdallah, A. M., Zhou, X., Kim, C., Shah, K. K., Hogden, C., Schoenherr, J. A., Clemens, J. C., and Chang, H. C. (2013) Activated Cdc42 kinase regulates Dock localization in male germ cells during Drosophila spermatogenesis. *Dev. Biol.* **378**, 141-153
4. Sem, K. P., Zahedi, B., Tan, I., Deak, M., Lim, L., and Harden, N. (2002) ACK family tyrosine kinase activity is a component of Dcdc42 signaling during dorsal closure in Drosophila melanogaster. *Mol. Cell. Biol.* **22**, 3685-3697
5. Schoenherr, J. A., Drennan, J. M., Martinez, J. S., Chikka, M. R., Hall, M. C., Chang, H. C., and Clemens, J. C. (2012) Drosophila Activated Cdc42 Kinase Has an Anti-Apoptotic Function. *Plos Genetics* **8,** e1002725
6. Hopper, N. A., Lee, J. H., and Sternberg, P. W. (2000) ARK-1 inhibits EGFR signaling in C-elegans. *Mol. Cell* **6**, 65-75
7. Greenman, C., Stephens, P., Smith, R., Dalgliesh, G. L., Hunter, C., Bignell, G., Davies, H., Teague, J., Butler, A., Edkins, S., O'Meara, S., Vastrik, I., Schmidt, E. E., Avis, T., Barthorpe, S., Bhamra, G., Buck, G., Choudhury, B., Clements, J., Cole, J., Dicks, E., Forbes, S., Gray, K., Halliday, K., Harrison, R., Hills, K., Hinton, J., Jenkinson, A., Jones, D., Menzies, A., Mironenko, T., Perry, J., Raine, K., Richardson, D., Shepherd, R., Small, A., Tofts, C., Varian, J., Webb, T., West, S., Widaa, S., Yates, A., Cahill, D. P., Louis, D. N., Goldstraw, P., Nicholson, A. G., Brasseur, F., Looijenga, L., Weber, B. L., Chiew, Y. E., Defazio, A., Greaves, M. F., Green, A. R., Campbell, P., Birney, E., Easton, D. F., Chenevix-Trench, G., Tan, M. H., Khoo, S. K., Teh, B. T., Yuen, S. T., Leung, S. Y., Wooster, R., Futreal, P. A., and Stratton, M. R. (2007) Patterns of somatic mutation in human cancer genomes. *Nature* **446**, 153-158
8. Prieto-Echague, V., Gucwa, A., Craddock, B. P., Brown, D. A., and Miller, W. T. (2010) Cancer-associated Mutations Activate the Nonreceptor Tyrosine Kinase Ack1. *J. Biol. Chem.* **285**, 10605-10615
9. Mahajan, K., Coppola, D., Challa, S., Fang, B., Chen, Y. A., Zhu, W. W., Lopez, A. S., Koomen, J., Engelman, R. W., Rivera, C., Muraoka-Cook, R. S., Cheng, J. Q., Schonbrunn, E., Sebti, S. M., Earp, H. S., and Mahajan, N. P. (2010) Ack1 Mediated AKT/PKB Tyrosine 176 Phosphorylation Regulates Its Activation. *PLoS One* **5**, e9646
10. Hoare, S., Hoare, K., Reinhard, M. K., Lee, Y. J., Oh, S. P., and May, W. S., Jr. (2008) Tnk1/Kos1 knockout mice develop spontaneous tumors. *Cancer Res.* **68**, 8723-8732

11. Ferby, I., Reschke, M., Kudlacek, O., Knyazev, P., Pante, G., Amann, K., Sommergruber, W., Kraut, N., Ullrich, A., Fassler, R., and Klein, R. (2006) Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. *Nat. Med.* **12**, 568-573
12. Zhang, Y. W., Staal, B., Su, Y., Swiatek, P., Zhao, P., Cao, B., Resau, J., Sigler, R., Bronson, R., and Vande Woude, G. F. (2007) Evidence that MIG-6 is a tumor-suppressor gene. *Oncogene* **26**, 269-276
13. Reschke, M., Ferby, I., Stepniak, E., Seitzer, N., Horst, D., Wagner, E. F., and Ullrich, A. (2010) Mitogen-Inducible Gene-6 is a Negative Regulator of Epidermal Growth Factor Receptor Signaling in Hepatocytes and Human Hepatocellular Carcinoma. *Hepatology* **51**, 1383-1390
14. Kim, T. H., Lee, D. K., Cho, S. N., Orvis, G. D., Behringer, R. R., Lydon, J. P., Ku, B. J., McCampbell, A. S., Broaddus, R. R., and Jeong, J. W. (2013) Critical Tumor Suppressor Function Mediated by Epithelial Mig-6 in Endometrial Cancer. *Cancer Res.* **73**, 5090-5099
15. Maity, T. K., Venugopalan, A., Linnoila, I., Cultraro, C. M., Giannakou, A., Nemati, R., Zhang, X., Webster, J. D., Ritt, D., Ghosal, S., Hoschuetzky, H., Simpson, R. M., Biswas, R., Politi, K., Morrison, D. K., Varmus, H. E., and Guha, U. (2015) Loss of MIG6 Accelerates Initiation and Progression of Mutant Epidermal Growth Factor Receptor-Driven Lung Adenocarcinoma. *Cancer Discov.* **5**, 534-549
16. Yokoyama, N., and Miller, W. T. (2003) Biochemical properties of the Cdc42-associated tyrosine kinase ACK1 - Substrate specificity, autophosphorylation, and interaction with Hck. *J. Biol. Chem.* **278**, 47713-47723
17. Galisteo, M. L., Yang, Y., Urena, J., and Schlessinger, J. (2006) Activation of the nonreceptor protein tyrosine kinase Ack by multiple extracellular stimuli. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 9796-9801
18. Prieto-Echague, V., and Miller, W. T. (2011) Regulation of ack-family nonreceptor tyrosine kinases. *Journal of signal transduction* **2011**, 742372-742372
19. La Torre, A., del Mar Masdeu, M., Cotrufo, T., Moubarak, R. S., del Rio, J. A., Comella, J. X., Soriano, E., and Urena, J. M. (2013) A role for the tyrosine kinase ACK1 in neurotrophin signaling and neuronal extension and branching. *Cell Death Dis.* **4**, e602
20. Oda, T., Muramatsu, M. A., Isogai, T., Masuho, Y., Asano, S., and Yamashita, T. (2001) HSH2: a novel SH2 domain-containing adapter protein involved in tyrosine kinase signaling in hematopoietic cells. *Biochem. Biophys. Res. Commun.* **288**, 1078-1086
21. Pao-Chun, L., Chan, P. M., Chan, W., and Manser, E. (2009) Cytoplasmic ACK1 interaction with multiple receptor tyrosine kinases is mediated by Grb2: an analysis of ACK1 effects on Axl signaling. *J. Biol. Chem.* **284**, 34954-34963
22. Eisenmann, K. M., McCarthy, J. B., Simpson, M. A., Keely, P. J., Guan, J. L., Tachibana, K., Lim, L., Manser, E., Furcht, L. T., and Iida, J. (1999) Melanoma chondroitin sulphate proteoglycan regulates cell

spreading through Cdc42, Ack-1 and p130(cas). *Nat. Cell Biol.* **1**, 507-513
23. Yang, W. N., Lin, Q., Guan, J. L., and Cerione, R. A. (1999) Activation of the Cdc42-associated tyrosine kinase-2 (ACK-2) by cell adhesion via integrin beta(1). *J. Biol. Chem.* **274**, 8524-8530
24. Linseman, D. A., Heidenreich, K. A., and Fisher, S. K. (2001) Stimulation of M-3 muscarinic receptors induces phosphorylation of the Cdc42 effecter activated Cdc42Hs-associated kinase-1 via a Fyn tyrosine kinase signaling pathway. *J. Biol. Chem.* **276**, 5622-5628
25. Lougheed, J. C., Chen, R. H., Mak, P., and Stout, T. J. (2004) Crystal structures of the phosphorylated and unphosphorylated kinase domains of the Cdc42-associated tyrosine kinase ACK1. *J. Biol. Chem.* **279**, 44039-44045
26. Gajiwala, K. S., Maegley, K., Ferre, R., He, Y. A., and Yu, X. (2013) Ack1: Activation and Regulation by Allostery. *PLoS One* **8,** e5399
27. Prieto-Echague, V., Gucwa, A., Brown, D. A., and Miller, W. T. (2010) Regulation of Ack1 localization and activity by the amino-terminal SAM domain. *BMC Biochem.* **11,** 42-52
28. Kim, C. A., and Bowie, J. U. (2003) SAM domains: uniform structure, diversity of function. *Trends Biochem. Sci.* **28**, 625-628
29. Lin, Q., Wang, J., Childress, C., and Yang, W. N. (2012) The activation mechanism of ACK1 (activated Cdc42-associated tyrosine kinase 1). *Biochem. J.* **445**, 255-264
30. Chan, W., Sit, S. T., and Manser, E. (2011) The Cdc42-associated kinase ACK1 is not autoinhibited but requires Src for activation. *Biochem. J.* **435**, 355-364
31. Ahmed, I., Calle, Y., Sayed, M. A., Kamal, J. M., Rengaswamy, P., Manser, E., Meiners, S., and Nur-E-Kamal, A. (2004) Cdc42-dependent nuclear translocation of non-receptor tyrosine kinase, ACK. *Biochem. Biophys. Res. Commun.* **314**, 571-579
32. Eder, I. E., Culig, Z., Putz, T., Nessler-Menardi, C., Bartsch, G., and Klocker, H. (2001) Molecular biology of the androgen receptor: from molecular understanding to the clinic. *Eur. Urol.* **40**, 241-251
33. Taplin, M. E., and Balk, S. P. (2004) Androgen receptor: a key molecule in the progression of prostate cancer to hormone independence. *J. Cell. Biochem.* **91**, 483-490
34. Mahajan, N. P., Liu, Y., Majumder, S., Warren, M. R., Parker, C. E., Mohler, J. L., Earp, H. S., and Whang, Y. E. (2007) Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 8438-8443
35. Mahajan, K., Coppola, D., Rawal, B., Chen, Y. A., Lawrence, H. R., Engelman, R. W., Lawrence, N. J., and Mahajan, N. P. (2012) Ack1-mediated Androgen Receptor Phosphorylation Modulates Radiation Resistance in Castration-resistant Prostate Cancer. *J. Biol. Chem.* **287**, 22112-22122
36. Marechal, A., and Zou, L. (2013) DNA Damage Sensing by the ATM and ATR Kinases. *Cold Spring Harb. Perspect. Biol.* **5,** a012716

37. Mahajan, K., Malla, P., Lawrence, H. R., Chen, Z. H., Kumar-Sinha, C., Malik, R., Shukla, S., Kim, J., Coppola, D., Lawrence, N. J., and Mahajan, N. P. (2017) ACK1/TNK2 Regulates Histone H4 Tyr88-phosphorylation and AR Gene Expression in Castration-Resistant Prostate Cancer. *Cancer Cell* **31**, 790-803
38. Mahajan, K., Lawrence, H. R., Lawrence, N. J., and Mahajan, N. P. (2014) ACK1 Tyrosine Kinase Interacts with Histone Demethylase KDM3A to Regulate the Mammary Tumor Oncogene HOXA1. *J. Biol. Chem.* **289**, 28179-28191
39. Mahendrarajah, N., Borisova, M. E., Reichardt, S., Godmann, M., Sellmer, A., Mahboobi, S., Haitel, A., Schmid, K., Kenner, L., Heinzel, T., Beli, P., and Kramer, O. H. (2017) HSP90 is necessary for the ACK1-dependent phosphorylation of STAT1 and STAT3. *Cell. Signal.* **39**, 9-17
40. Thaker, Y. R., Recino, A., Raab, M., Jabeen, A., Wallberg, M., Fernandez, N., and Rudd, C. E. (2017) Activated Cdc42-associated kinase 1 (ACK1) binds the sterile alpha motif (SAM) domain of the adaptor SLP-76 and phosphorylates proximal tyrosines. *J. Biol. Chem.* **292**, 6281-6290
41. Liu, H. B., Schneider, H., Recino, A., Richardson, C., Goldberg, M. W., and Rudd, C. E. (2015) The Immune Adaptor SLP-76 Binds to SUMO-RANGAP1 at Nuclear Pore Complex Filaments to Regulate Nuclear Import of Transcription Factors in T Cells. *Mol. Cell* **59**, 840-849
42. Mahajan, K., and Mahajan, N. P. (2010) Shepherding AKT and Androgen Receptor by Ack1 Tyrosine Kinase. *J. Cell. Physiol.* **224**, 327-333
43. Sun, M., Wang, G., Paciga, J. E., Feldman, R. I., Yuan, Z. Q., Ma, X. L., Shelley, S. A., Jove, R., Tsichlis, P. N., Nicosia, S. V., and Cheng, J. Q. (2001) AKT1/PKB alpha kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. *Am. J. Pathol.* **159**, 431-437
44. Bose, S., Chandran, S., Mirocha, J. M., and Bose, N. (2006) The Akt pathway in human breast cancer: a tissue-array-based analysis. *Mod. Pathol.* **19**, 238-245
45. Mahajan, K., and Mahajan, N. P. (2012) PI3K-independent AKT activation in cancers: A treasure trove for novel therapeutics. *J. Cell. Physiol.* **227**, 3178-3184
46. Clayton, N. S., Fox, M., Vicente-Garcia, J. J., Schroeder, C. M., Littlewood, T. D., Wilde, J. I., Corry, J., Krishnan, K., Zhang, Q., Wakelam, M. J. O., Brown, M. J., Crafter, C., Mott, H. R., and Owen, D. (2019) Assembly of novel, nuclear dimers of the PI3-Kinase regulatory subunits underpins the pro-proliferative activity of the Cdc42-activated tyrosine kinase, ACK. *BioRxiv* 791277
47. Song, L., Gao, M., Dong, W., Hu, M., Li, J., Shi, X., Hao, Y., Li, Y., and Huang, C. (2011) p85alpha mediates p53 K370 acetylation by p300 and regulates its promoter-specific transactivity in the cellular UVB response. *Oncogene* **30**, 1360-1371

48. Park, S. W., Zhou, Y., Lee, J., Lu, A., Sun, C., Chung, J., Ueki, K., and Ozcan, U. (2010) The regulatory subunits of PI3K, p85 alpha and p85 beta, interact with XBP-1 and increase its nuclear translocation. *Nat. Med.* **16**, 429-U111
49. Pensa, S., Neoh, K., Resemann, H. K., Kreuzaler, P. A., Abell, K., Clarke, N. J., Reinheckel, T., Kahn, C. R., and Watson, C. J. (2014) The PI3K regulatory subunits p55alpha and p50alpha regulate cell death in vivo. *Cell Death Differ.* **21**, 1442-1450
50. Thorpe, L. M., Yuzugullu, H., and Zhao, J. J. (2015) PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nature Reviews Cancer* **15**, 7-24
51. Cheung, L. W. T., Walkiewicz, K. W., Besong, T. M. D., Guo, H., Hawke, D. H., Arold, S. T., and Mills, G. B. (2015) Regulation of the PI3K pathway through a p85 alpha monomer-homodimer equilibrium. *Elife* **4,** e06866
52. Chagpar, R. B., Links, P. H., Pastor, M. C., Furber, L. A., Hawrysh, A. D., Chamberlain, M. D., and Anderson, D. H. (2010) Direct positive regulation of PTEN by the p85 subunit of phosphatidylinositol 3-kinase. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 5471-5476
53. Yang, J., Nie, J., Ma, X. L., Wei, Y. Q., Peng, Y., and Wei, X. W. (2019) Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol. Cancer* **18**, 26-53
54. Le, X., Antony, R., Razavi, P., Treacy, D. J., Luo, F., Ghandi, M., Castel, P., Scaltriti, M., Baselga, J., and Garraway, L. A. (2016) Systematic Functional Characterization of Resistance to PI3K Inhibition in Breast Cancer. *Cancer Discov.* **6**, 1134-1147
55. Yang, W., Jansen, J. M., Lin, Q., Canova, S., Cerione, R. A., and Childress, C. (2004) Interaction of activated Cdc42-associated tyrosine kinase ACK2 with HSP90. *Biochem. J.* **382**, 199-204
56. Mahajan, N. P., Whang, Y. E., Mohler, J. L., and Earp, H. S. (2005) Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: Role of Ack1 in polyubiquitination of tumor suppressor Wwox. *Cancer Res.* **65**, 10514-10523
57. Chang, N. S., Doherty, J., Ensign, A., Schultz, L., Hsu, L. J., and Hong, Q. (2005) WOX1 is essential for tumor necrosis factor-, UV light-, staurosporine-, and p53-mediated cell death, and its tyrosine 33-phosphorylated form binds and stabilizes serine 46-phosphorylated p53. *J. Biol. Chem.* **280**, 43100-43108
58. Pospiech, K., Pluciennik, E., and Bednarek, A. K. (2018) WWOX Tumor Suppressor Gene in Breast Cancer, a Historical Perspective and Future Directions. *Front. Oncol.* **8**, 345
59. Watson, J. E. V., Doggett, N. A., Albertson, D. G., Andaya, A., Chinnaiyan, A., van Dekken, H., Ginzinger, D., Haqq, C., James, K., Kamkar, S., Kowbel, D., Pinkel, D., Schmitt, L., Simko, J. P., Volik, S., Weinberg, V. K., Paris, P. L., and Collins, C. (2004) Integration of high-resolution array comparative genomic hybridization analysis of chromosome 16q with expression array data refines common regions

of loss at16q23-qter and identifies underlying candidate tumor suppressor genes in prostate cancer. *Oncogene* **23**, 3487-3494
60. Mahajan, K., Coppola, D., Chen, Y. A., Zhu, W. W., Lawrence, H. R., Lawrence, N. J., and Mahajan, N. P. (2012) Ack1 Tyrosine Kinase Activation Correlates with Pancreatic Cancer Progression. *Am. J. Pathol.* **180**, 1386-1393
61. Teo, M., Tan, L., Lim, L., and Manser, E. (2001) The tyrosine kinase ACK1 associates with clathrin-coated vesicles through a binding motif shared by arrestin and other adaptors. *J. Biol. Chem.* **276**, 18392-18398
62. Yeow-Fong, L., Lim, L., and Manser, E. (2005) SNX9 as an adaptor for linking synaptojanin-1 to the Cdc42 effector ACK1. *FEBS Lett.* **579**, 5040-5048
63. Bendris, N., and Schmid, S. L. (2017) Endocytosis, Metastasis and Beyond: Multiple Facets of SNX9. *Trends Cell Biol.* **27**, 189-200
64. Lin, Q., Lo, C. G., Cerione, R. A., and Yang, W. (2002) The Cdc42 target ACK2 interacts with sorting nexin 9 (SH3PX1) to regulate epidermal growth factor receptor degradation. *J. Biol. Chem.* **277**, 10134-10138
65. Goh, L. K., Huang, F. T., Kim, W., Gygi, S., and Sorkin, A. (2010) Multiple mechanisms collectively regulate clathrin-mediated endocytosis of the epidermal growth factor receptor. *J. Cell Biol.* **189**, 871-883
66. Shen, F., Lin, Q., Gu, Y., Childress, C., and Yang, W. N. (2007) Activated Cdc42-associated kinase 1 is a component of EGF receptor signaling complex and regulates EGF receptor degradation. *Mol. Biol. Cell* **18**, 732-742
67. Yamazaki, T., Zaal, K., Hailey, D., Presley, J., Lippincott-Schwartz, J., and Samelson, L. E. (2002) Role of Grb2 in EGF-stimulated EGFR internalization. *J. Cell Sci.* **115**, 1791-1802
68. Satoh, T., Kato, J., Nishida, K., and Kaziro, Y. (1996) Tyrosine phosphorylation of ACK in response to temperature shift-down, hyperosmotic shock, and epidermal growth factor stimulation. *FEBS Lett.* **386**, 230-234
69. Jin, N., Cho, S. N., Raso, M. G., Wistuba, I., Smith, Y., Yang, Y., Kurie, J. M., Yen, R., Evans, C. M., Ludwig, T., Jeong, J. W., and DeMayo, F. J. (2009) Mig-6 is required for appropriate lung development and to ensure normal adult lung homeostasis. *Development* **136**, 3347-3356
70. Boopathy, G. T. K., Lynn, J. L. S., Wee, S., Gunaratne, J., and Hong, W. (2018) Phosphorylation of Mig6 negatively regulates the ubiquitination and degradation of EGFR mutants in lung adenocarcinoma cell lines. *Cell. Signal.* **43**, 21-31
71. Lin, Q., Wang, J., Childress, C., Sudol, M., Carey, D. J., and Yang, W. (2010) HECT E3 Ubiquitin Ligase Nedd4-1 Ubiquitinates ACK and Regulates Epidermal Growth Factor (EGF)-Induced Degradation of EGF Receptor and ACK. *Mol. Cell. Biol.* **30**, 1541-1554
72. Levkowitz, G., Waterman, H., Zamir, E., Kam, Z., Oved, S., Langdon, W. Y., Beguinot, L., Geiger, B., and Yarden, Y. (1998) c-Cbl/Sli-1

regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev.* **12**, 3663-3674
73. Levkowitz, G., Waterman, H., Ettenberg, S. A., Katz, M., Tsygankov, A. Y., Alroy, I., Lavi, S., Iwai, K., Reiss, Y., Ciechanover, A., Lipkowitz, S., and Yarden, Y. (1999) Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell* **4**, 1029-1040
74. Chan, W., Tian, R., Lee, Y. F., Sit, S. T., Lim, L., and Manser, E. (2009) Down-regulation of Active ACK1 Is Mediated by Association with the E3 Ubiquitin Ligase Nedd4-2. *J. Biol. Chem.* **284**, 8185-8194
75. Grovdal, L. M., Johannessen, L. E., Rodland, M. S., Madshus, I. H., and Stang, E. (2008) Dysregulation of Ack1 inhibits down-regulation of the EGF receptor. *Exp. Cell Res.* **314**, 1292-1300
76. Pennock, S., and Wang, Z. X. (2003) Stimulation of cell proliferation by endosomal epidermal growth factor receptor as revealed through two distinct phases of signaling. *Mol. Cell. Biol.* **23**, 5803-5815
77. Pollitt, A. Y., and Insall, R. H. (2009) WASP and SCAR/WAVE proteins: the drivers of actin assembly. *J. Cell Sci.* **122**, 2575-2578
78. Insall, R. H., and Machesky, L. M. (2009) Actin Dynamics at the Leading Edge: From Simple Machinery to Complex Networks. *Dev. Cell* **17**, 310-322
79. Keely, P. J., Westwick, J. K., Whitehead, I. P., Der, C. J., and Parise, L. V. (1997) Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. *Nature* **390**, 632-636
80. Lawson, C. D., and Ridley, A. J. (2018) Rho GTPase signaling complexes in cell migration and invasion. *J. Cell Biol.* **217**, 447-457
81. Defilippi, P., Di Stefano, P., and Cabodi, S. (2006) p130Cas: a versatile scaffold in signaling networks. *Trends Cell Biol.* **16**, 257-263
82. Yokoyama, N., Lougheed, J., and Miller, W. T. (2005) Phosphorylation of WASP by the Cdc42-associated kinase ACK1 - Dual hydroxyamino acid specificity in a tyrosine kinase. *J. Biol. Chem.* **280**, 42219-42226
83. Suetsugu, S., Hattori, M., Miki, H., Tezuka, T., Yamamoto, T., Mikoshiba, K., and Takenawa, T. (2002) Sustained activation of N-WASP through phosphorylation is essential for neurite extension. *Dev. Cell* **3**, 645-658
84. Cory, G. O., Garg, R., Cramer, R., and Ridley, A. J. (2002) Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. *J. Biol. Chem.* **277**, 45115-45121
85. Kelley, L. C., and Weed, S. A. (2012) Cortactin is a substrate of activated Cdc42-associated kinase 1 (ACK1) during ligand-induced epidermal growth factor receptor downregulation. *PLoS One* **7**, e44363
86. van der Horst, E. H., Degenhardt, Y. Y., Strelow, A., Slavin, A., Chinn, L., Orf, J., Rong, M. Q., Li, S. Y., See, L. H., Nguyen, K. Q. C., Hoey, T., Wesche, H., and Powers, S. (2005) Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 15901-15906

87. Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., Jacobsen, A., Byrne, C. J., Heuer, M. L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A. P., Sander, C., and Schultz, N. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2**, 401-404
88. Gao, J. J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., Sun, Y. C., Jacobsen, A., Sinha, R., Larsson, E., Cerami, E., Sander, C., and Schultz, N. (2013) Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. *Science Signaling* **6**, pl1
89. MacKeigan, J. P., Murphy, L. O., and Blenis, J. (2005) Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. *Nat. Cell Biol.* **7**, 591-600
90. DiMauro, E. F., Newcomb, J., Nunes, J. J., Bemis, J. E., Boucher, C., Buchanan, J. L., Buckner, W. H., Cheng, A., Faust, T., Hsieh, F., Huang, X., Lee, J. H., Marshall, T. L., Martin, M. W., McGowan, D. C., Schneider, S., Turci, S. M., White, R. D., and Zhu, X. (2007) 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.* **17**, 2305-2309
91. Mahajan, K., Challa, S., Coppola, D., Lawrence, H., Luo, Y. T., Gevariya, H., Zhu, W. W., Chen, Y. A., Lawrence, N. J., and Mahajan, N. P. (2010) Effect of Ack1 Tyrosine Kinase Inhibitor on Ligand-Independent Androgen Receptor Activity. *Prostate* **70**, 1274-1285
92. Jiao, X., Kopecky, D. J., Liu, J., Liu, J., Jaen, J. C., Cardozo, M. G., Sharma, R., Walker, N., Wesche, H., Li, S., Farrelly, E., Xiao, S. H., Wang, Z., and Kayser, F. (2012) Synthesis and optimization of substituted furo[2,3-d]-pyrimidin-4-amines and 7H-pyrrolo[2,3-d]pyrimidin-4-amines as ACK1 inhibitors. *Bioorg. Med. Chem. Lett.* **22**, 6212-6217

Figure Legends

Figure 1: Domain architecture of ACK related proteins and regulation of ACK

(A) Domain structure of ACK related proteins. SAM: Sterila Alpha Motif, NES: Nuclear Export Signal, SH3: Src Homology Domain 3, CRIB: Cdc42/Rac Interactive binding motif, Clath: Clathrin binding region, EBD: EGFR binding domain, UBA: Ubiquitin association region, PEST: PEST sequence, 14-3-3-BD: 14-3-3 binding domain, AH Region: ACK homology region. Numbers indicate amino acid positions. **(B) Activation of ACK.** Interactions between the PRR (proline rich region) within the C-terminus of the EBD region and the SH3 domain orientate the EBD permitting inhibitory interactions with the kinase domain. ACK is activated by dimerization mediated via the SAM domain. Full activation may require Src-mediated phosphorylation at position Tyr284 within the kinase domain activation loop. Membrane localization could be provided by interactions between the EBD and RTKs, the CRIB region and Cdc42 or the SAM domain with the membrane directly. Phosphorylation sites are shown as orange circles.

Figure 2: ACK regulates multiple targets

Cartoon summarizing the main targets of ACK kinase activity and the cellular processes they are involved in regulating. Phosphorylation sites are shown as orange circles. ACK: Activated Cdc42-Associated Kinase, Akt: AKR mouse thymoma, AR: Androgen Receptor, GPCR: G-Protein Coupled Receptor, RTK: Receptor Tyrosine Kinase, WASP, Wiskott-Aldrich Syndrome Protein, Wwox: WW Domain Containing Oxidoreductase, PI3K: Phosphatidylinositol-3-Kinase.

Figure 3: ACK modulates Androgen receptor signalling to drive castration resistant prostate cancer growth

Cartoon summarizing physiological AR activity and the known points of influence of ACK on both AR expression levels and activity. Phosphorylation sites are shown as orange circles. ACK: Activated Cdc42-associated kinase, AR: Androgen receptor, ARE: Androgen response elements, ATM: Ataxia telangiectasia mutated, DHT: Dihydrotestosterone, HSP90: Heat Shock Protein 90, H4: Histone H4, Pol II: Polymerase II, T: Testosterone, RTK: Receptor Tyrosine kinase

Figure 4: ACK regulation of the PI3K signalling pathway

Cartoon summarizing the points of influence of ACK on components of the PI3-Kinase signalling pathway. Phosphorylation sites are shown as orange circles. ACK: Activated Cdc42-associated Kinase, Akt: AKR mouse thymoma, FoxO transcription factor, AP2: adaptor protein 2 complex, PA: phosphatidic acid, PDK1: Phosphoinositide-dependent kinase-1, PDK2: Phosphoinositide-dependent kinase 2, PIP_2: Phosphatidylinositol 4,5-bisphosphate, PIP_3: Phosphatidylinositol (3,4,5)-trisphosphate, PTEN: Phosphatase and Tensin homologue, SNX9: Sorting Nexin 9, Ub: ubiquitin

Figure 5: ACK regulation of receptor endocytosis
Cartoon showing the main stages of receptor mediated endocytosis, the sorting of the EGFR after stimulation and the main points of influence of ACK in the process. ACK: Activated Cdc42-associated kinase, AP2: Adaptor protein 2, EGF: Epidermal Growth factor, EGFR: Epidermal Growth Factor Receptor, Nedd-4; Neural precursor cell expressed developmentally down-regulated protein 4, SNX9: sorting nexin 9, Ub: ubiquitin.

Figure 6: ACK in cancer

The frequency of changes in ACK by mutation, fusion, amplification, deep deletion or multiple alterations displayed across all types of cancers. Data taken from cBioportal (87,88).

Figure 1

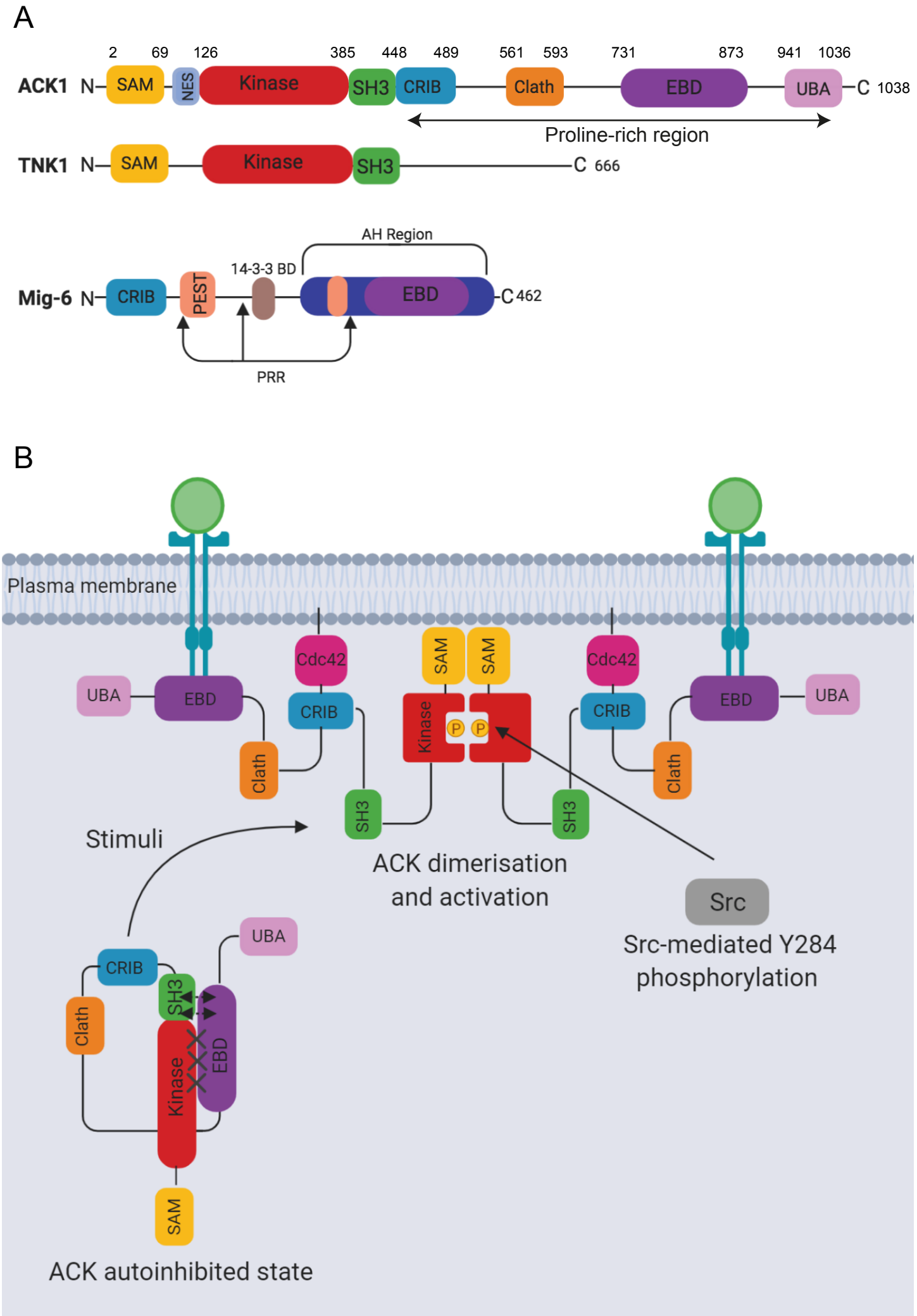

A
2
69
126
385
448
489
561
593
731
873
941
1036
ACK1
N
SAM
NES
Kinase
SH3
CRIB
Clath
EBD
UBA
C
1038
Proline-rich region
TNK1
N
SAM
Kinase
SH3
C
666
AH Region
14-3-3 BD
Mig-6
N
CRIB
PEST
EBD
C
462
PRR
B
Plasma membrane
UBA
EBD
Cdc42
CRIB
Clath
SH3
SAM
SAM
Kinase
P
P
Cdc42
CRIB
Clath
SH3
EBD
UBA
Stimuli
ACK dimerisation
and activation
Src
Src-mediated Y284
phosphorylation
UBA
CRIB
SH3
Clath
Kinase
EBD
SAM
ACK autoinhibited state

Figure 2

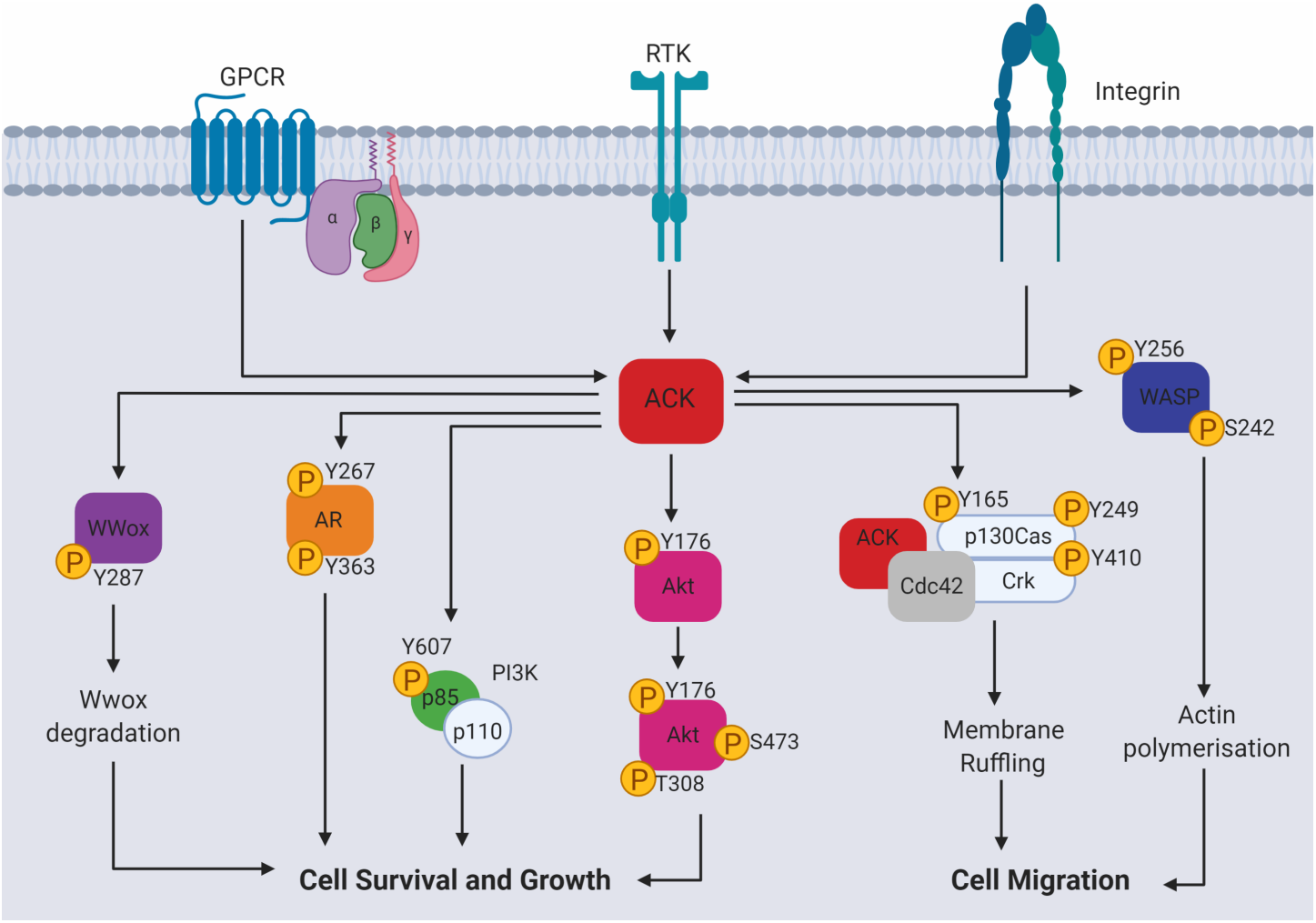

GPCR
RTK
Integrin
α
β
γ
ACK
WWox
P
Y287
Wwox
degradation
P
Y267
AR
P
Y363
Y607
P
PI3K
p85
p110
P
Y176
Akt
P
Y176
Akt
P
S473
P
T308
P
Y165
ACK
p130Cas
P
Y249
P
Y410
Cdc42
Crk
P
Y256
WASP
P
S242
Membrane
Ruffling
Actin
polymerisation
Cell Survival and Growth
Cell Migration

Figure 3

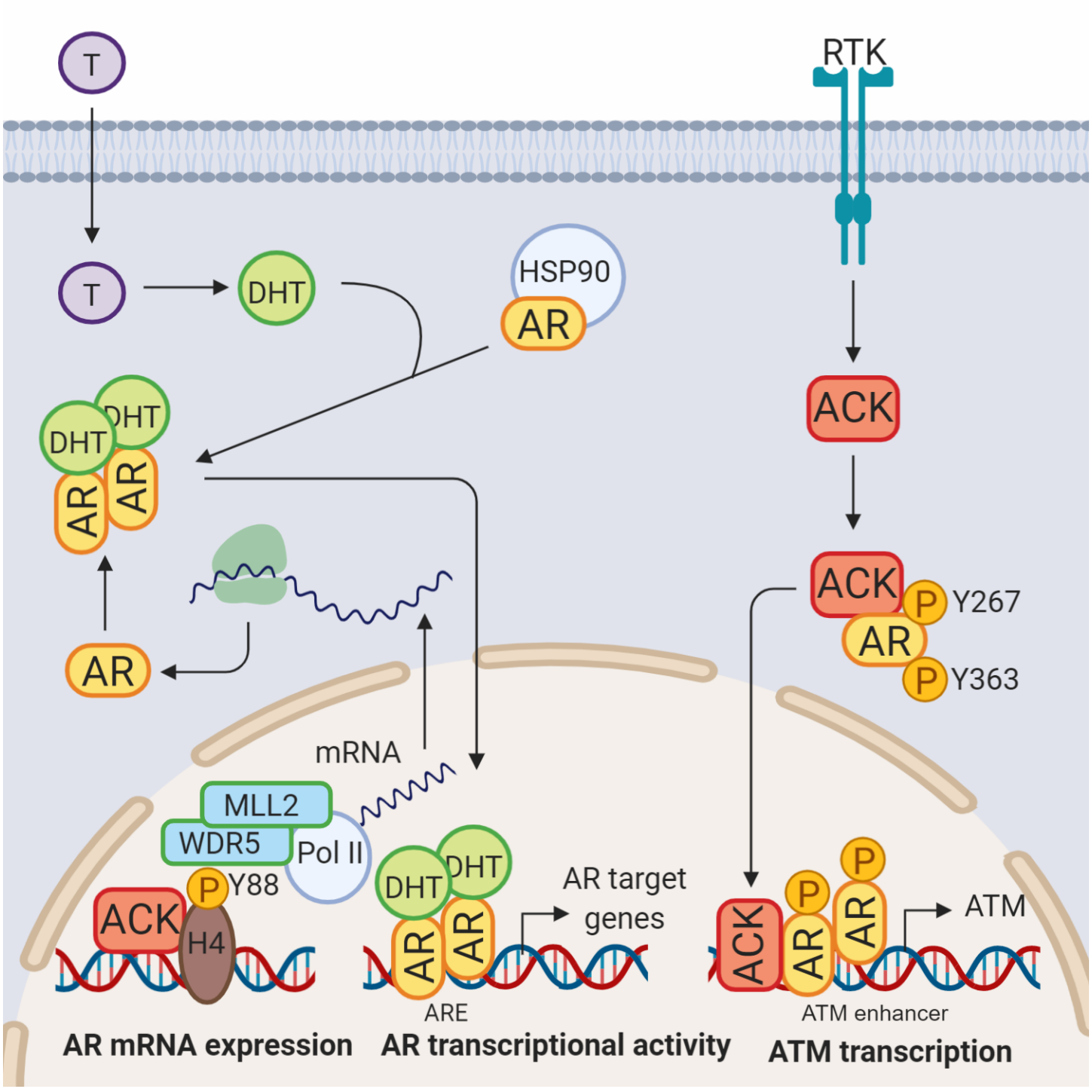

T
T
DHT
HSP90
AR
RTK
ACK
DHT
DHT
AR
AR
ACK
P
Y267
AR
P
Y363
AR
mRNA
MLL2
WDR5
Pol II
P
Y88
ACK
H4
DHT
DHT
AR
AR
AR target genes
ACK
P
AR
P
AR
ATM
ARE
ATM enhancer
AR mRNA expression
AR transcriptional activity
ATM transcription

Figure 4

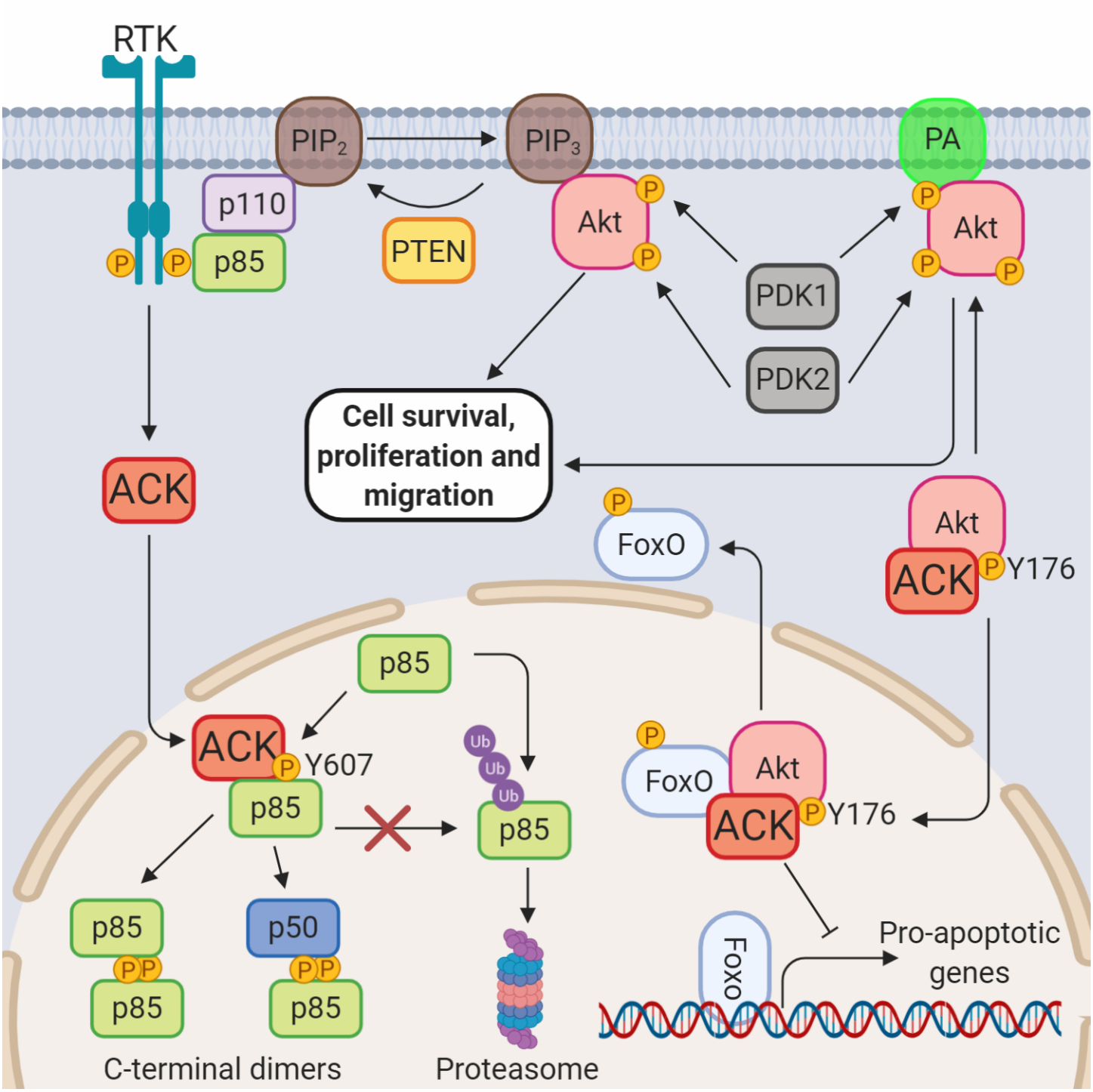

RTK
PIP2
PIP3
PA
p110
p85
PTEN
Akt
PDK1
PDK2
Cell survival, proliferation and migration
ACK
FoxO
Y176
Y607
Ub
p50
C-terminal dimers
Proteasome
Pro-apoptotic genes
Foxo

Figure 5

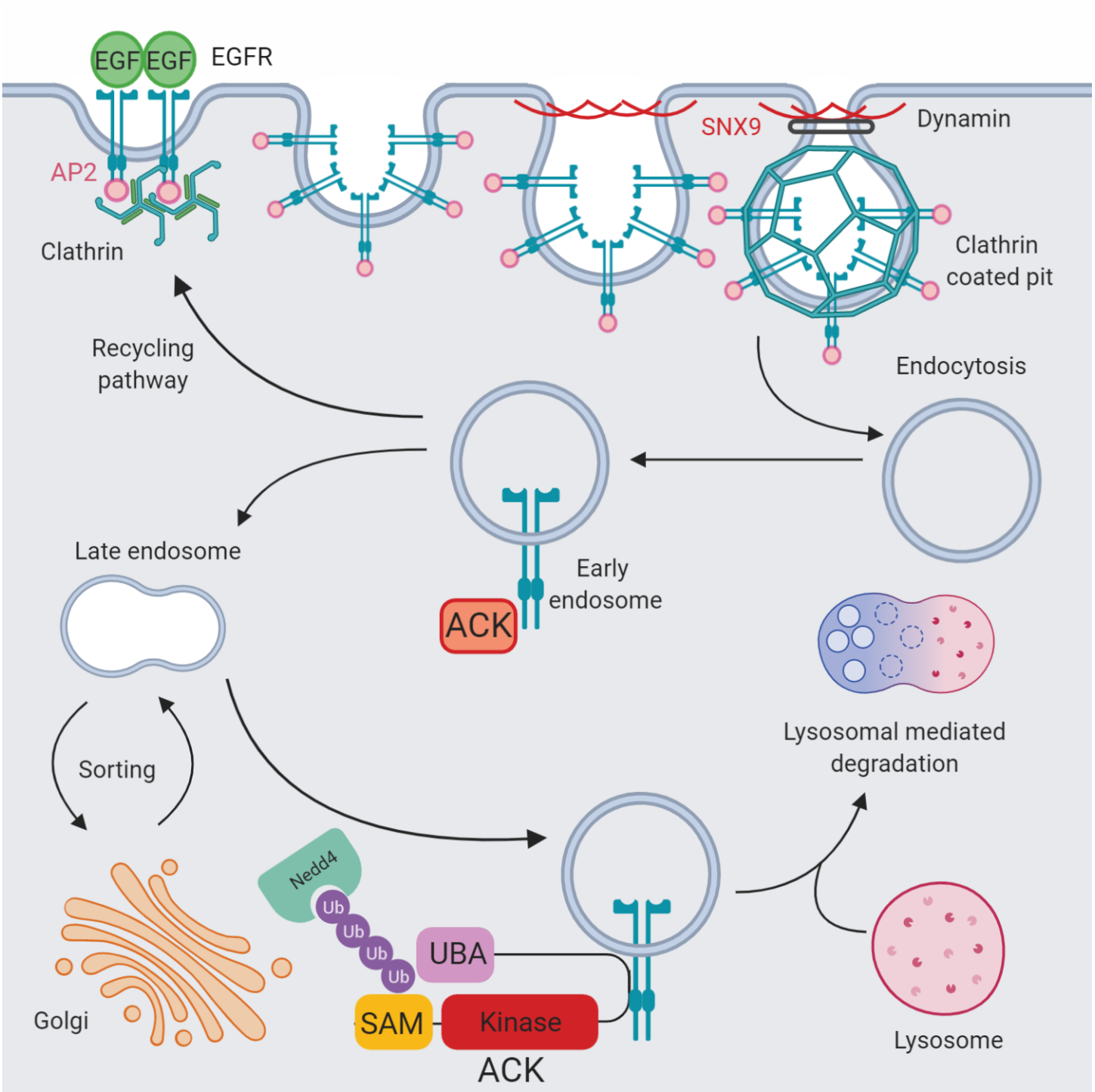
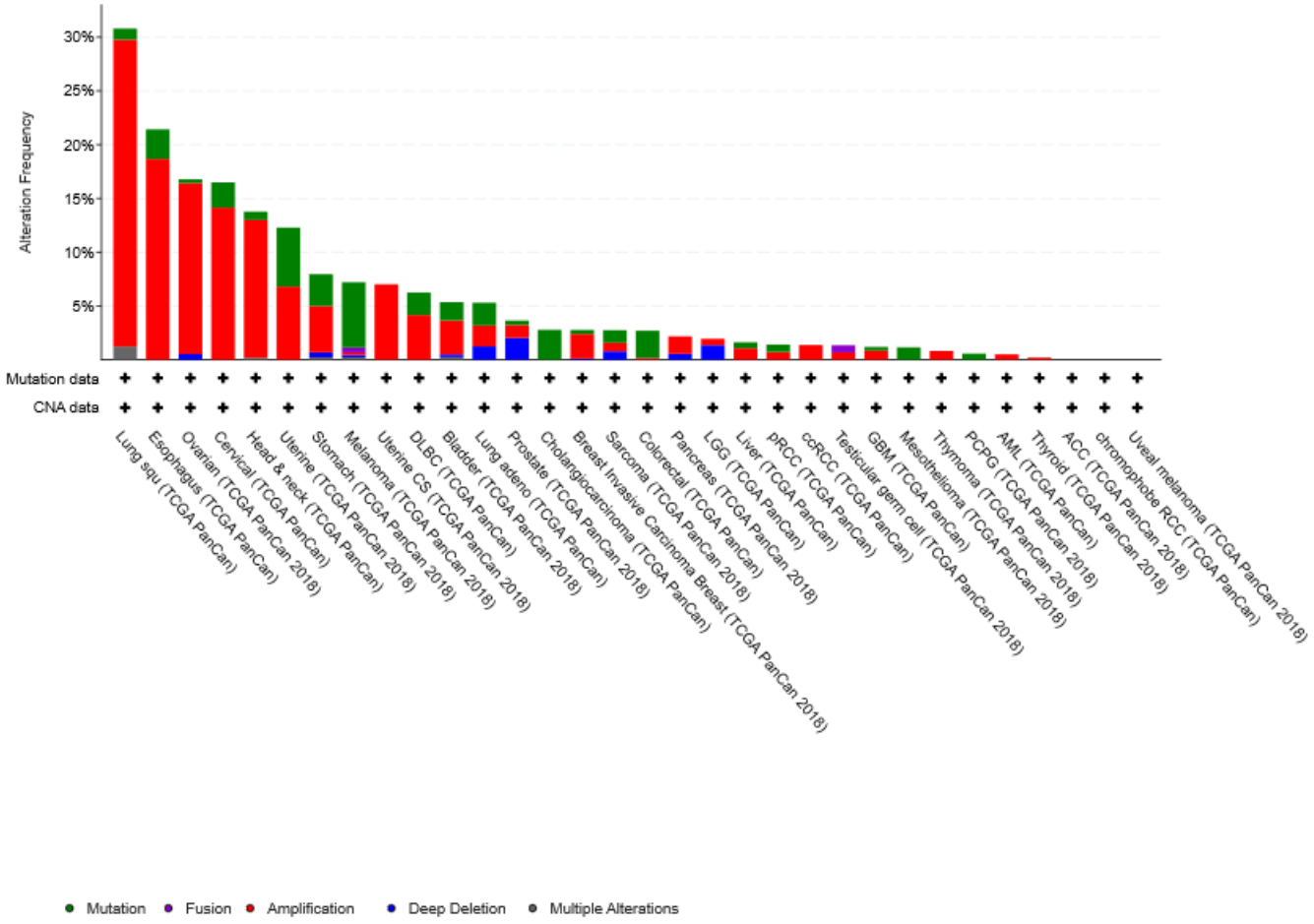

EGF
EGF
EGFR
AP2
Clathrin
SNX9
Dynamin
Clathrin
coated pit
Recycling
pathway
Endocytosis
Late endosome
Early
endosome
ACK
Lysosomal mediated
degradation
Sorting
Nedd4
Ub
Ub
Ub
Ub
UBA
Golgi
SAM
Kinase
ACK
Lysosome

Figure 6


Alteration Frequency
30%
25%
20%
15%
10%
5%
Mutation data
CNA data
Lung squ (TCGA PanCan)
Esophagus (TCGA PanCan)
Ovarian (TCGA PanCan 2018)
Cervical (TCGA PanCan)
Head & neck (TCGA PanCan)
Uterine (TCGA PanCan)
Stomach (TCGA PanCan 2018)
Melanoma (TCGA PanCan 2018)
Uterine CS (TCGA PanCan 2018)
DLBC (TCGA PanCan 2018)
Bladder (TCGA PanCan)
Lung adeno (TCGA PanCan 2018)
Prostate (TCGA PanCan 2018)
Cholangiocarcinoma (TCGA PanCan 2018)
Breast Invasive Carcinoma (TCGA PanCan)
Sarcoma (TCGA PanCan 2018)
Colorectal (TCGA PanCan 2018)
Pancreas (TCGA PanCan)
LGG (TCGA PanCan)
Liver (TCGA PanCan 2018)
pRCC (TCGA PanCan)
coRCC (TCGA PanCan)
Testicular germ cell (TCGA PanCan)
GBM (TCGA PanCan)
Mesothelioma (TCGA PanCan 2018)
Thymoma (TCGA PanCan 2018)
PCPG (TCGA PanCan 2018)
AML (TCGA PanCan 2018)
Thyroid (TCGA PanCan 2018)
ACC (TCGA PanCan 2018)
chromophobe RCC (TCGA PanCan)
Uveal melanoma (TCGA PanCan 2018)
Mutation
Fusion
Amplification
Deep Deletion
Multiple Alterations